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POSTERS

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POSTERS

DNA, RNA and nucleotides

P.01-001-Mon
Escherichia coli single-stranded DNA binding protein SSB binds to AlkB and promotes DNA repair

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Repair of alkylation damage in DNA is essential for maintaining genome integrity. *E. coli* DNA repair enzyme AlkB removes various alkyl adducts present in DNA by oxidative demethylation. Previous studies showed that AlkB preferentially repairs single-stranded DNA (ssDNA). However, ssDNA remains bound to *E. coli* single-stranded DNA binding protein SSB. It is unclear whether AlkB can repair alkyl adduct present in SSB-coated ssDNA. We have addressed this question by studying AlkB-mediated DNA repair using SSB-bound DNA containing alkyl-adduct as substrate. We have also found AlkB interacts with SSB. Yeast two-hybrid and in vitro pull-down experiments showed that SSB interacts with AlkB via structurally disordered 17 amino acid residues located in the disordered CTD. With reconstituted in vitro repair reaction we observed that AlkB efficiently remove alkyl-adducts from shorter oligonucleotide substrate but the efficiency of AlkB catalyzed reaction was less compared to SSB-bound DNA substrate. Using 70-mer oligonucleotide containing single alkyl adduct, we demonstrate that SSB-AlkB interaction was dispensable for the repair of terminally located methyl adducts but crucial for the faster repair of internally located adducts. When ssDNA was bound to truncated SSB lacking the interacting residues, stimulation of AlkB activity was partially abolished suggesting functional importance of SSB-AlkB interaction.

P.01-002-Tue
The effect of sperm DNA fragmentation on the pregnancy rate in Intra Uterine Cycles (IUI)

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The objective of this study was to detect the DNA damage ratio of sperm samples of infertile couples scheduled for intrauterine insemination (IUI) treatment with the method of terminal deoxynucleotidyl transferase-mediated dUTP- biotin end –labeling (TUNEL) test and to detect DNA damage with semen analysis and pregnancy rates. The study was designed as a prospective randomized controlled follow up study for 81 infertile couples. Assessment of sperm DNA damage prior to their use in IUI is essential for use in assisted conception. Semen samples analyzed with WHO laboratory manual. The percentage of each sperm with DNA fragmentation was determined using the method of TUNEL. The duration was 18 months after approval from of the ethics committee. Standard semen qualities are not sufficient for the outcome of IUI. However, sperm DNA quality has been emphasized on its clinical significance and its relationship with infertility. Written consent for use of the sperm for research was obtained from the patients who were selected for the IUI treatment. Questionary was performed to obtain smoking and alcohol consumption and days of abstinence prior to production of the semen sample. Gradient and swim-up preparation techniques were used in IUI. The most motile sperms in the supernatant after the swim-up test were used for IUI and the spare sperm suspension in the pellet was prepared for TUNEL staining. The staining was done according to the TUNEL staining kit procedure and the ratio of the defected DNA in the sperms were determined by fluorescent microscopy. A positive control was done on positive slides supplied in the kit. There was no statistical difference between the pregnancy and no pregnancy groups in terms of liquefaction time, volume, pH, sperm concentration, motility, and morphology. Although the DNA damage percentage measured by TUNEL test was less in the pregnancy achieved group compared to no pregnancy group, it did not reach statistically significant levels.

P.01-003-Wed
Novel backbone modified antisense oligonucleotides with improved potency effectively inactivate oncogenic miRNA-21 comparing to phosphorothioate oligonucleotides

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Vast amount of earlier studies confirms successful application of antisense oligonucleotides targeted to oncogenic miRNAs for the suppression of various events of carcinogenesis. Despite significant achievements in the field of antisense technology, development of new oligonucleotide structures and chemical modifications to increase the effectiveness of anticancer therapy remains an urgent and promising task nowadays. In the present work, the new effective antisense oligonucleotides containing the newest mesyl phosphoramidate modification of internucleotide bonds were developed and comprehensively studied. Functionality and biological potency of designed antisense oligonucleotides were assayed with respect to highly oncogenic miRNA-21. The study of biological properties of the engineered nucleic acids has shown a number of advantageous properties such as high binding affinity to the target, tremendous stability in the biological media and RNase H-activating ability. For these properties, the developed oligonucleotides significantly exceed the well-known phosphorothioate (PS) modification. In comparison with PS analogs it was shown that transfection of mesyl phosphoramidate oligonucleotides to the melanoma B16 cells is accompanied by low unspecific cytotoxicity and leads to the more significant dose-dependent decrease in the level of miR-21 and reduction in migration activity of cancer cells. Thus, a novel backbone modified antisense oligonucleotides were developed which are a worthy alternative to phosphorothioates and can become a new powerful tool in the development of antisense technology. This work is supported by Grants of Russian Science Foundation No. 14-44-00068 and No. 17-44-07003, Grant of Russian Foundation for Basic Research No. 16-03-01055 and State funded budget project (VI.62.1.3, 0309-2016-0005).

P.01-004-Mon
Analysis of microRNA at endometriosis and uterine endometrial cancer

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Oncological diseases of the female reproductive system are currently among most commonly occurring diseases and are worldwide the main cause of female mortality. Clinical diagnostics of many cancers are often successful in advanced stages of the tumour, therefore current research is still directed towards identification and characterization new biochemical and molecular markers. Molecules of miRNA can regulate cell proliferation, differentiation, apoptosis and many other biological processes in the cells. Due to properties, miRNA could be diagnostic marker. The main aim of our analysis was detection of molecular expression level of miR-17-5p and miR-99 in selected diseases of the female reproductive system. Experimental groups consisted from patients with histologically diagnosed endometriosis and carcinoma of *corpus uteri* (CU) (n = 15). The obtained material was used for isolation of miRNA, transcription into cDNA and determination expression levels of miR-17-5p and miR-99 in patients. Quantification of expression changes of selected miRNAs was detected by qRT-PCR. In the experimental group of patients with endometriosis, was observed a tendency to increase the miR-17-5p level by 132% compared to the control group. On the other side, was observed a rapid down – regulation of miR-99 up to 53%. At miR-17-5p in patients with CU were observed compared with the control group a significant increase of 207%. In the case of miR-99, in patients with CU was observed increased level expression by 136% versus the control group. The results correspond with the results of other scientific groups dealing with these diseases, which obtained different levels of relative expression of miR-17-5p and miR-99. The pilot study indicates the using of miR-17-5p and miR-99 as possible markers to differential diagnostics possibility of women cancer.

P.01-005-Tue
Vitamin C restores TET proteins activity in leukemic cells

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Epigenetic mechanisms that regulate gene expression (such as methylation and active demethylation of DNA) play a key role in the genomic imprinting, cells proliferation and differentiation and their abnormalities may be one of the early stages of carcinogenesis. Patients with acute myeloid leukemias frequently harbor mutations in genes involved in the DNA demethylation pathway. Loss-of-function mutations in *TET2* have been described in various hematological malignancies and also TET1 has contrasting roles in myeloid and lymphoid transformation. We have analyzed levels of epigenetic DNA modifications arising from TETs activity, namely 5-hydroxymethylcytosine – derivative of 5-methylcytosine, 5-formylcytosine and 5-carboxylcytosine, by using stable isotope dilution two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry in DNA isolated form peripheral blood nuclear cells of patients with acute myeloid leukemias. Our study showed significantly lower levels of 5-hmCyt and other 5-mCyt derivatives in afflicted individuals when compared with control group. In various studies vitamin C enhanced TET proteins activity, therefore we have developed in vitro model in which we cultured leukemic cells with different concentrations of vitamin C and observed dose-dependent increase of 5-hydroxymethylcytosine and 5-formylcytosine levels in cellular DNA. This suggests that vitamin C, by restoring TETs activity, normalize aberrant pattern of epigenetic modifications in leukemic cells and may be used as an adjuvant treatment of acute myeloid leukemias. This work was supported by the Polish National Science Centre (grant number UMO-2015/19/B/NZ5/02208).

P.01-006-Wed
Determination of epigenetic DNA modifications in *Drosophila melanogaster*

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The presence of 5-methylcytosine (5-mCyt) in the *Drosophila* genome has been subject to long-standing debates. It was demonstrated that cytosine DNA methylation could be detected and quantified in embryo development and in adult flies. Another epigenetic modification is 5-hydroxymethylcytosine (5-hmCyt), product of 5-methylcytosine oxidation by ten-eleven translocation (TET) enzymes. This mark was detectable in adult stages of *D. melanogaster*. Some evidence from experimental studies suggests that TET may be also involved in synthesis of 5-hydroxymethyluracil (5-hmUra), a compound with epigenetic function. To determine the levels of epigenetic DNA modifications in *D. melanogaster* we used 2 experimental models. In *in vitro* model S2 cells were cultured in control medium and in medium containing 1 mM ascorbic acid (AA) for 24 h, 48 h and 72 h. In *in vivo* model we measured levels of epigenetic modifications in DNA isolated from adult flies. Due to low content of analyzed compounds in *Drosophila* genome we optimized classical phenol extraction protocol to obtain large amount of high-polymerized DNA. Received genetic material were enzymatically hydrolysed to deoxynucleosides, spiked with stable-isotope labeled internal standards and analyzed using 2D-UPLC-MS/MS method. In S2 cells we were able to detected levels of uracil, 5-hmUra and 8-oxoguanine (8-oxoGua). In material isolated from adult *D. melanogaster* we identify and quantify levels of 5-mCyt, 5-hmCyt, uracil, 5-hmUra and 8-oxoGua. Incubation S2 cells with ascorbic acid caused time-dependent increase of 5-hmdC (~10 folds) and independent increase of 8-oxoGua (~1.7 folds) levels. This suggest that some portion of 5-hmUra observed in *Drosophila melanogaster* may be a product of enzymatic reaction. The work was supported by Polish National Science Centre grant No. 2016/21/N/NZ1/00563.

P.01-007-Mon
Evaluation of transcription factor 7-like 2 gene polymorphism in patients with prediabetes and type 2 diabetes mellitus

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Type 2 diabetes mellitus is an endemic chronic metabolic disease characterized by hyperglycemia. Prediabetes is the subclinical

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stage of T2DM with moderate hyperglycemia and blood glucose fluctuations. Transcription factor 7-like 2 (TCF7L2) polymorphisms are the most important single nucleotide polymorphisms associated with T2DM. However, the association of TCF7L2 polymorphisms with prediabetes remains to be clarified and must be thoroughly investigated for determination of its effects on metabolism. Therefore, the aim of this study were to investigate TCF7L2 rs7903146 polymorphisms in patients with prediabetes and type 2 diabetes mellitus. TCF7L2 rs7903146 SNPs were analyzed with DNA sequencing method with genetic analyzer in blood samples collected from prediabetes, T2DM patients and control subjects. These variants were examined by using BLAST 2 Sequences. Obtained results were evaluated together with clinical and laboratory findings and demographic features. Statistical analyses were performed by using SPSS 22.0 and GraphPad Prism 5, and $P < 0.05$ was considered significant. TCF7L2 rs7903146 SNPs, 7% of the prediabetes patients, 22% of the T2DM patients and 9% of the healthy control group had homozygote TT allele and 50% of the prediabetes patients, 50% of the T2DM patients and 52% of the control group had heterozygote CT allele. The T2DM patients had homozygote TT allele minimum two times as frequently as the prediabetes and healthy control groups. There was a significant positive correlation between TCF7L2 SNPs and waist circumference in the T2DM patients ($r = 0.479$, $P = 0.007$). In conclusion our results indicate that TCF7L2 rs7903146 variants were not significantly associated with prediabetes and T2DM but may be an important risk factor.

P.01-008-Tue
Transfer RNA-derived fragments target and regulate ribosome-associated aminoacyl-transfer RNA synthetases

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In the present study, we have functionally characterized molecular activity of *Saccharomyces cerevisiae* tRNA-derived fragments (tRFs) during protein biosynthesis. Ribosome-associated noncoding (ranc) RNAs are a novel class of short regulatory RNAs and the function as well as the trigger for the origin is not widely studied. In order to gain insight into the tRF binding sites with the ribosome we performed the polysome gradient analyses followed by northern blot assays as well as *in vitro* binding assays. To study the role of tRFs *in vivo*, we performed metabolic labeling approach using yeast spheroplasts. To corroborate on *in vivo* assays, *in vitro* translation reactions were performed using *S. cerevisiae* cell-free extracts. Furthermore we investigated tRF's influence on the earliest step needed for the proper protein biosynthesis, namely the tRNA aminoacylation. Finally, to gain more insight into the mechanism of tRNA aminoacylation regulation by yeast tRFs, we set up a series of electrophoretic mobility shift assays aiming at identification of interaction partners for tRNA-derived fragments. Our results indicate regulatory potential during translation of not only ranc-5′-tRFs (as reported previously) but also ranc-3′-tRFs. We have demonstrated the association of five ribosome-associated yeast 3′-tRFs (as well as one 5′-tRF) with small ribosomal subunit as well as aminoacyl-tRNA synthetases. Moreover, our proteomic analysis revealed that four yeast aa-tRNA synthetases directly interact with yeast ribosomes. The association of yeast aa-RSs to ribosomal particles, especially the small ribosomal subunit, is mediated by yeast tRFs. As a consequence, global tRNA aminoacylation is impaired.

P.01-009-Wed
Novel targets for the Orb2 CPEB protein in nervous system of *Drosophila*

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Gene expression is regulated at multiple levels. CPEB proteins bind special sequences in 3′UTR and can promote both activation or repression of translation through polyadenylation regulation. It helps to establish intracellular protein gradient and mediates cell polarization. Nervous system is characterized with high protein synthesis plasticity and specialization, that is why role of the CPEB proteins in such processes like postsynaptic potentiation is very important. One plausible model to study these phenomena is *Drosophila* CPEB protein Orb2 due to powerful genetic tools available for this model organism. Several Orb2 regulatory targets have been defined previously. Recently, based on cell culture model almost complete list of Orb2 regulatory targets have been obtained. We proposed that using of native material, namely fly heads extracts, we will be able to describe the transcripts specific for nervous system. The fly stock carrying GFP protein fused to Orb2 was used. Immunoprecipitation against GFP antibodies was performed, the isolated RNA from precipitated RNA-protein complex was sequenced with help of NGS approach. Obtained results for several genes were verified using real time PCR. We have obtained about 2000 transcripts enriched in our RIP-seq analysis. They were characterized with broad range of CPE- sequences. Transcripts with highest values of enrichment have elevated level in fly central nervous system. We identified near 100 RNA targets that previously were not described. Using RNA FISH, immunostaining and genetic tools we are investigating the role of novel Orb2-transcripts interactions in development and functioning of central nervous system. This study was supported by the Russian Foundation for Basic Research under Grant 16-34- 60214.

P.01-010-Mon
Experimental approaches for studying degraded DNA with the use of Next Generation Sequencing (NGS)

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The objectives of this study were to develop a protocol for sample preparation of archaeological DNA for NGS and a software pipeline for the data analysis and interpretation. The materials for the study were the skull teeth found in excavations of burial sites dating the XVIIth century near Radonezh, provided by the Zagorsk state historical and art museum-reserve. The conditions of burial were typical for Central Russia, thus experience with these remains can be a base for working with other samples from this important historical region. The sample preparation was carried in a specially designed unit, provided with an evacuation system and the possibility of filling with high-purity nitrogen, minimizing intra-laboratory contamination. Low DNA concentration after isolation led to the impossibility of preparation the sample for NGS by standard protocols. A library of fragments

suitable for sequencing was obtained only with the use of specially designed modified adapters, which did not allow the formation of adapter-dimeric structures. Sequencing was performed using the Illumina MiSeq platform. Identification of the organisms was carried out by aligning the individual reads, using the BLASTn program, to the NCBI Nucleotide Collection. About a third of all the reads could be identified. Overall, they were attributed to the 3,500 different species from 1,500 genera. About 22% of the identified reads belonged to organisms from the genus *Streptomyces* and *Bradyrhizobium*, which are soil bacteria. *Homo sapiens* took about 5% of raw data. The highest-quality alignment of the paired-end reads to the reference sequence of the human genome could be obtained using the algorithm BWA-backtrack. Sequencing of the negative control sample showed no contamination at the stage of sample preparation. Library enrichment application by DNA hybridization made it possible to significantly increase the concentration of individual fragments containing target loci for phylogenetic studies.

P.01-011-Tue
Defining the promoter sequence determinants for NAD+ -initiation by RNA polymerase

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The status of the 5′-end of RNA affects its stability, localization and translation efficiency, and it is considered as an epitranscriptomic regulatory element. A breakthrough discovery of the ubiquitous NAD^+ redox cofactor attached to the 5′-end of RNA in prokaryotic and eukaryotic cells revealed the existence of a new cap-like RNA modification. The biological significance of the NAD^+-cap and its consequences for the RNA and the cell are unclear, but a number of mechanistic aspects of the NAD^+-capping and its requirements are starting to be defined. It has been showed that NAD^+ can be used by RNA polymerase (RNAP) as a non-canonical transcription initiation (di)nucleotide (NCIN) at promoters encoding +1A (+1 is the transcription start site). The efficiency NAD^+-capping likely depends on promoter sequence. Here we present our study where we systematically investigated and defined the promoter sequence determinants for transcription initiation with NAD^+. To achieve this, we created a series of chimeric promoters composed of the sequences of promoters that encode +1A and display different NAD^+-initiating efficiencies, and used them as templates for *in vitro* transcription with *Escherichia coli* RNAP. These experiments identified a promoter region and its consensus sequence that maximizes utilization of NAD^+ as the transcription initiating substrate. The results presented here thus constitute a step forward in the characterization of this newly found prokaryotic RNA-capping phenomenon. This work is supported by grant No. 17-03419S, from the Czech Science Foundation.

P.01-012-Wed
Epigenetic aspect of function of a promising anticancer drug olivomycin A

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Olivomycin A is a promising anticancer agent that belongs to a family of aureolic acid antibiotics. However, the mechanism of its action is not completely understood. The drug binds to the DNA minor groove in GC-rich regions as Mg^{2+}-containing complexes. Given that minor groove ligands are known to disrupt a key epigenetic process of DNA methylation, we aimed to investigate the impact of olivomycin A and its synthetic derivative LCTA-1599 on the functioning of *de novo* murine DNA methyltransferase Dnmt3a. This enzyme establishes the DNA methylation pattern in eukaryotic cells. Using specially designed fluorescently labelled oligonucleotide substrates, we determined the optimal conditions for drug-DNA binding, including the concentration of Mg^{2+} ions that allowed for both olivomycin binding and Dnmt3a functioning. The impact of olivomycin on the Dnmt3a-DNA complex formation under these conditions turned out to be minimal. The binding of olivomycin A and LCTA-1599 to Dnmt3a was not observed. We then examined the inhibitory effect of olivomycin A and LCTA-1599 on DNA methylation by Dnmt3a. Employing the efficient method for *in vitro* quantification of DNA methylation that was recently developed in our laboratory, we have shown that both drugs can substantially inhibit the methylation reaction with IC_{50} values of 6 ± 1 μM and 7.1 ± 0.7 μM, respectively. Other minor groove ligands such as dimeric bisbenzimidazoles were found to have similar IC_{50} values. The inhibitory effect of olivomycin A and LCTA-1599 may be attributed to the disruption of movement of Dnmt3a catalytic loop toward DNA, which is necessary for the enzymatic activity of Dnmt3a and occurs via the DNA minor groove. Our results point at an epigenetic contribution to the mechanism of anticancer effect of aureolic acid family antibiotics. This work was supported by the RFBR grant 16-04-01087A.

P.01-013-Mon
Gre-like factors serve as lineage-specific transcriptional regulators in *Deinococcus* species

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Transcription is a major step in the regulation of gene expression in bacteria. Among various proteins that interact with RNA polymerase (RNAP) and modulate its activity, a prominent group of factors can bind in the secondary channel of RNAP and affect catalysis by direct interactions with the active centre. While GreA is a universal bacterial factor that stimulates RNA cleavage, which is important for transcriptional proofreading, the functions of its homologues may greatly differ. The famously stress-resistant bacterium *Deinococcus radiodurans* encodes three factors from the Gre-family: GreA, Gfh1 and Gfh2. We previously found that the Gfh factors from *D. radiodurans* strongly enhance site-specific pausing and intrinsic transcription termination by RNAP. The pause-stimulatory activity of Gfh is greatly enhanced by manganese ions, which are accumulated in *D. radiodurans* cells under stress conditions. Uniquely, another bacterium from the *Deinococcus* genus, *D. peraridilitoris* encodes four different Gfh factors. In this work, we analyzed their activities in various *in vitro* transcription assays. The results show that one of the four proteins significantly inhibits transcription initiation, prolongs transcriptional pausing and suppresses the RNA cleavage activity of RNAP. At the same time, the other three Gfh factors reveal much more moderate if any effects on different steps of transcription. We conclude that Gfh proteins serve as important transcriptional regulators in *Deinococcus* species and may potentially play a role in stress resistance, but the functions of their species-specific paralogues remain enigmatic. We speculate that in the case of *D. peraridilitoris* the Gfh proteins, though being homologous to transcription factors, may have additional functions in the cell that we will try to uncover in our future

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studies. This work was in part supported by Russian Science Foundation (grant 17-14-01393) and Russian Foundation for Basic Research (grant 18-34-00905).

P.01-014-Tue
Investigation of the argonaute protein from *Synechococcus elongatus*

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Argonaute proteins are key players in the diverse RNA interference pathways in eukaryotes. Eukaryotic argonautes bind short RNAs and use them as guides for target RNA recognition and cleavage. Argonaute proteins, often with unusual domain architectures, are also found in many bacterial and archaeal species but their functions in prokaryotes remain largely obscure. Recent studies revealed that prokaryotic argonautes can bind short RNA or DNA molecules and act as endonucleases to protect the cells against foreign genetic elements. However, their cellular roles and the molecular mechanisms of action are poorly understood. In this work, we studied argonaute protein SynAgo from the blue-green algae *Synechococcus elongatus*. We used purified SynAgo protein for *in vitro* experiments and demonstrated that it is able to bind short DNA guides and cleave target DNA substrates of various structures. We further constructed *S. elongatus* strain with tagged-argonaute expression and used it for purification of SynAgo and associated nucleic acids. It was found that in *S. elongatus* cells SynAgo is bound with short DNAs corresponding to the whole genomic sequence. Similarly, SynAgo also associates with short DNAs when expressed in the heterologic *Escherichia coli* system suggesting that no additional factors are required for short DNA processing. Thus, our experiments suggest that SynAgo is a DNA guided DNA nuclease that target various genomic sequences to perform its functions. To reveal these functions, we constructed a set of *S. elongatus* strains with various mutations in the argonaute gene and are now performing *in vivo* experiments aimed at understanding the roles of SynAgo in genomic defense and cell physiology. This work was supported in part by the Russian Science Foundation (grant 16-14-10377).

P.01-015-Wed
1,N^6-α-hydroxypropanoadenine and 3,N^4-α-hydroxypropanocytosine - acrolein adducts to adenine and cytosine, are substrates for AlkB dioxygenase

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1,N^6-α-hydroxypropanoadenine (HPA) and 3,N^4- α-hydroxypropanocytosine (HPC) are six-membered adducts formed in reaction of adenine and cytosine with acrolein (ACR). ACR is a mutagenic agent originated from different sources including cigarette smoke, exhaust fumes and overcooking. It is also generated endogenously during oxidative stress as a by-product of lipid peroxidation. *E. coli* AlkB dioxygenase (EcAlkB) is DNA repair enzyme that remove alkyl lesions from bases *via* an oxidative mechanism restoring native DNA structure. It belongs to the superfamily of 2-α-ketoglutarate (αKG) and Fe(II) dependent dioxygenases. AlkB is induced within *E. coli* system of adaptive response to alkylating agents (Ada response). Our *in vivo* data show that HPA and HPC show mutagenic properties and, generated in plasmids, causes (respectively) A→C and A→T transversions, C→T transitions followed by C→A transversions. We established the optimal pH, Fe(II) and αKG concentrations for enzymatic reaction. Our data proved that the protonated form of HPA is preferentially repaired by EcAlkB, though the reaction is stereoselective. Moreover, the number of reaction cycles carried out by an AlkB molecule remains limited and reached 38 ± 4 enzymatic cycles before its total inactivation. Molecular modeling of the AlkB/HPA complex demonstrated that the R stereoisomer in the equatorial conformation of the HPA hydroxyl group is strongly preferred, while the S one seems to be susceptible to AlkB-directed oxidative hydroxylation only when HPA adopts the *syn* conformation around the glycosidic bond. In addition to the biochemical activity assays, substrate binding to the protein was monitored by differential scanning fluorimetry allowing the identification of the active protein form with cofactor and cosubstrate bound and monitoring substrate binding.

P.01-016-Mon
The amount of humans with high or low ribosomal repeat copy number in population decreases with age

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Ribosomal RNA genes (rDNA) encode the rRNA species that form the ribosomes. The diploid human genome contains several hundred copies of 43-kb rDNA units tandemly arrayed on five acrocentric chromosomes. Dysregulation of rRNA biogenesis has been implicated in some human diseases. One of the factors affecting rRNA biogenesis is the ribosomal genes copy number (CN). Some authors have shown that the rDNA CN in human genome reduces with age. However, other authors deny this phenomenon. It can be explained with high degree of rDNA damage in aged cells and low efficiency of qPCR. We compared two methods: qPCR and nonradioactive quantitative hybridization (NQH) in quantitative analysis of human rDNA. We have shown that NQH is less sensitive to severe DNA damage than qPCR and thus is more appropriate for damaged rDNA detection. We have shown earlier that rDNA CN is higher in patients with schizophrenia compared to healthy people of the same age. We assessed blood leukocyte rDNA CN of 250 subjects (group I, 17 -71 years) in comparison to 126 subjects (group II, 72–91 years) using NQH. The leukocyte rDNA CN showed no significant difference in the median for group I and group II (median 419 vs 396, $P = 0.05$). However, the dispersion of CN for group I was much higher than for group II (200 -711 vs 252–541). In 80% of group II subjects CN of rDNA had a very narrow dispersion – from 300 to 450. Thus, the amount of humans with high or low rDNA CN decreases with age. This work was supported by RFBR grant No. 17-29-06017 ofi_m.

P.01-017-Tue
Ferritin2 RNA interference inhibits the formation of iron granules in honey bees (*Apis mellifera*)

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Iron granules containing superparamagnetic magnetite act as magnetoreceptor for magnetoreception in honey bees. Biomineralization of iron granules occurs in the iron deposition vesicles of trophocytes and requires the participation of actin, myosin, ferritin2,

and ATP synthase. If the formation of iron granules can be suppressed, it can help to understand the mechanism of magnetoreception in honey bees. Whether ferritin RNA interference can inhibit the formation of iron granules is unknown. In this study, double-stranded RNA of ferritin2 was injected into hemolymph from the abdomen of newly emerged worker bees to knock down *ferritin2*. The results showed that ferritin2 RNA interference declined the mRNA and protein expression of ferritin2 and the formation and accumulation of 7.5 nm iron particles leading to immature iron granules. Superparamagnetic magnetite is formed in the center of mature iron granules. These findings can be used to further study the magnetoreception of honey bees.

P.01-018-Wed
DNA aptamers against procalcitonin as emerging diagnostic molecules for sepsis

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Sepsis is the most common cause of death in Intensive Care Unit (ICU). Specific and rapid markers of bacterial infection have been sought for early diagnosis of sepsis. Procalcitonin (PCT), is the most promising sepsis markers, capable of complementing clinical signs and routine lab parameters suggestive of severe infection. The big issue in critical care patient is that lag time between sample collections and examining. Thus, real-time diagnostic on the bedside is an urgent need for clinicians. The fiber optic particle plasmon resonance (FOPPR) biosensor has high potential to meet these requirements. Our study would like to develop an advanced *in vitro* diagnostic platform based on FOPPR biosensor as scaffold with targeting aptamers. In this study, we screened a 62-mers single chain DNA aptamer library against human PCT by using a nitrocellulose filter systematic evolution of ligands by exponential enrichment (SELEX). We identified two human PCT-specific aptamer, PCTA1 and PCTA3, bound to PCT with high specificity and affinity. The use of an aptamer-based FOPPR biosensor for detecting PCT were carried out using different concentrations of PCT solutions and clinical serum samples. The FOPPR biosensor with PCT-aptamers can detect very low concentrations of PCT. High selectivity of the biosensor against PCT was also demonstrated in the presence of competitive proteins such as human serum albumin. This approach will further evaluation for clinical use, the combination of a high-affinity aptamers and FOPPR detection has some benefits compared with other bioanalytical methods. Upon success of the clinical validation, our aptamer-FOPPR biosensor might be translated from benchtop research to commercialization in the future.

P.01-019-Mon
The screening and application of DNA aptamers against neuropilin 1

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Aptamers are single-stranded DNA or RNA molecules which can bind to a target specifically by its special steric configuration. Because of its binding specificity, aptamers have a potential for diagnosis and treatment of cancers through binding to oncogenic molecules. Neuropilin 1 (NRP1) is a co-receptor for vascular endothelial growth factor 165 that promotes angiogenesis, tumor growth, tumor invasion and metastasis. High expression of NRP1 in non-small cell lung cancer (NSCLC) patients have been known to have low disease-free and overall survival rate. Silencing of NRP1 significantly suppressed lung cancer cell migration, metastasis, and tumor angiogenesis. Thus, development of specific aptamers against NRP1 would be a promising therapy for NSCLC patients. Here, we screened a DNA aptamer library against NRP1 protein by using a nitrocellulose filter systematic evolution of ligands by exponential enrichment (SELEX). Three NRP1-binding aptamers, AP5, AP15, and AP37, were isolated. We further screened a customized aptamer microarray, on which a short aptamer library was made based on the sequences of AP5, AP15, and AP37, and we got several short aptamers with high NRP1-binding affinity and specificity. We found that NRP1-specific aptamers decreased the migration ability of the highly invasive NSCLC cells, CL1-5, compared with that in cells treated with scramble-aptamers. Moreover, tube formation ability of HUVEC cells were also reduced while treating with NRP1-specific aptamers. These results showed that NRP1-specific aptamers decrease the migration ability of tumor cells and angiogenesis. This study revealed that NRP1-specific aptamers might be developed a useful therapeutic strategy to lung cancer in the future.

P.01-020-Tue
Six-stacked G-quadruplexes as a preferential target of p53

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Recently we have for the first time described more than four-stacked intramolecular G-quadruplex forming sequences. These sequences are very rare in human genome, but interestingly span important locations such promoters or sites of chromatin acetylation in most cases. Also preferential binding of tumor suppressor protein p53 to quadruplex DNA structures was demonstrated previously in general. In this work we have inspected the differences in p53 binding to the well known four-stacked intramolecular G-quadruplex and newly described six-stacked intramolecular G-quadruplex. Using combination of wet-lab methods (electrophoretic mobility assay and circular dichroism spectroscopy), we have proven the preferential binding of wild type p53 to the six-stacked intramolecular G-quadruplexes *in vitro* on oligonucleotide quadruplex forming sequences, further indicating their substantial role in biological regulatory processes. Also we have compared the binding ability of wild type p53 (amino acid residues 1 - 393) with the core domain of p53 (amino acid residues 94 - 312) to G-quadruplex DNA. According to widely accepted hypothesis, isolated core domain of p53 have shown minimal binding activity in comparison to wild type p53, which further confirms the major role of intrinsically unstructured C-terminal tail of p53 in preferential binding to non canonical DNA structures. Also we have observed temperature dependencies in p53 and six-stacked G-quadruplex interactions, indicating crucial role of physiological temperature achievement to the successful binding. We have also demonstrated some important features of six-stacked quadruplexes distribution in the human and other sequenced mammalian genomes as well. Interestingly, in human genome, occurrence of six-stacked G-quadruplexes is strongly enriched in neurodegenerative diseases related genes so in the future, six-stacked G-quadruplexes could be a great target in severe diseases treatment for their unique features.

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P.01-021-Wed
Prevalence of DNMT3B -149 C>>T and SYCP3 T657C polymorphisms among Russian women

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DNA methyltransferase 3b (DNMT3b) is an enzyme participating in epigenetic modification and mediating the transfer of the methyl group derived from folate to the 5′ position of a cytosine in embryonic stem cells and early post-implantation embryos. Synaptonemal complex protein 3 (SYCP3) is a DNA binding protein involved in the synaptonemal complex formation during meiosis I, and its activity leads to homologous chromosomes pairing and meiotic homologous recombination. Some studies reported variations in the frequency of both DNMT3b -149 (T>C) and SYCP3 T657C polymorphisms among populations and suggested that the mutations in the genes can be genetic risk factors causing reproductive disorders in women. To our knowledge, both gene polymorphisms have never been studied in Russia before, thus the aim of our study was to investigate the polymorphism prevalence of these genes among the healthy women in Russia. 60 peripheral blood samples were collected from the healthy Russian women living in Central Russia. The extracted DNA was subjected to DNA genotyping using restriction fragment length polymorphism-PCR for DNMT3b and Allele-specific PCR for SYCP3 followed by agarose gel electrophoresis. The C allele and T allele frequencies were 72.5% and 27.5% while the CC, CT and TT genotype frequencies were 50%, 45% and 5% respectively for DNMT3b polymorphism. For SYCP3 polymorphism the T and C allele frequencies were 90.85% and 9.15%, while the TT, TC and CC genotype frequencies were 86.7%, 8.3%, 5% respectively. Consequently, we recommend investigating both polymorphisms to determine whether there is a relationship between genetic changes in the DNMT3b and SYCP3 genes and reproductive problems in Russian women. The publication was prepared with the support of the "RUDN University Program 5-100".

P.01-022-Mon
The SEPS1 G-105A polymorphism is associated with risk of preterm birth in Russian women

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Inflammation plays an important role in the initiation of preterm birth. At the current moment many genes and their products are considered as markers of inflammation, most of them are directly involved in the infectious process. This group includes more than 200 cytokines (interleukins (IL), tumor necrosis factors (TNF-α), interferons, growth factors), C-reactive protein, transforming growth factor (TGF-β), etc. The SEPS1 gene is regarded as a new marker of inflammation. Several polymorphisms have been detected in the SEPS1 gene, but they are insufficiently studied. In this study the correlation of the SEPS1 G-105A polymorphism (rs28665122) with the risk of premature birth in Russian population from Moscow region is shown. The SEPS1 G-105A polymorphism was genotyped in 50 women with premature birth at terms from 23.5 to 37 weeks of pregnancy and 50 women with full-term pregnancy as a control group using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. According to our data, the frequency of allele A among healthy women is 14%. In the group of women with preterm birth the frequency of allele A is 20% which is significantly higher than in the control group ($\chi^2 = 5.0249$, $P = 0.02499$). The frequency of heterozygotes is also significantly higher in the group of women with preterm birth ($\chi^2 = 6.002$; $P = 0.01429$). Thus, our findings suggest that the SEPS1 G-105A polymorphism may be a potential gene marker for preterm birth. The publication was prepared with the support of the "RUDN University Program 5-100".

P.01-023-Tue
Upstream (-31/-24) box in RNA polymerase III promoters

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Most of the genes transcribed by RNA polymerase III (pol III) contain an internal promoter. Usually, such promotor consists of two 11-bp boxes (A and B) spaced by 30–40 bp. Apart from these boxes, tRNA genes of yeast, plants, and insects contain a TATA-box (TATAAAA or a similar heptamer) located in the upstream sequence between positions -24 and - 30. TATA-binding protein (TBP) binds this box, which promotes transcription initiation. However, tRNA genes of mammals lack typical TATA-boxes. We studied the genes of two noncoding mouse RNAs, 4.5SH and 4.5SI. These genes contain A and B boxes but have no canonical TATA-box. We replaced each nucleotide of 4.5SH RNA gene upstream sequence -31/-24 with three other ones. HeLa cells were transfected with 24 constructs and the efficiency of 4.5SH RNA gene transcription was estimated. The constructs containing substitutions T(-30) to G, C(-29) to A, A(-28) to C, and A(-27) to G or C demonstrated the strongest transcription decrease (up to 6-fold). The obtained data indicate that these nucleotides are important for the initiation of pol III transcription. In the case of 4.5SI RNA, the entire -31/-24 box (CTACATGA) was replaced with other nucleotide sequences. Certain sequences (for example, GCACTAGT) could successfully function as a -31/-24 box. On the contrary, GC-rich sequence totally repressed the gene transcription. Mouse SINEs B1 and B2, which are also transcribed by pol III, were studied likewise. Most of the studied B1 and B2 copies could be transcribed effectively; however, the replacement of the -31/-24 sequence with a TATA-box or the -31/-24 boxes of 4.5SH and 4.5SI RNA genes additionally increased B1 and B2 transcription. Thus, although various sequences can function as a -31/-24 box, the presence of certain nucleotides at critical positions is necessary for the maximum activity. This work was supported by the Program of Fundamental Research for State Academies for 2013–2020 years (no. 01201363821).

P.01-024-Wed
Affect of N6-methyladenosine mRNA modification on translation rate of bacteria

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N6-methylation of adenosine (m6A) is the most abundant mRNA modification in eukaryotes and viruses. Recently was shown the widespread occurrence of m6A modification in mRNA of bacteria. Modified adenosines consist of about 0.20% and 0.28% of all adenosines of Pseudomonas aeruginosa and *Escherichia coli* that comparable with eukaryotes. We used a

bioinformatics approach to assess the effect of adenosine modifications on mRNA abundance and protein production in bacteria. For every gene we collected published available data about its mRNA and protein levels in different conditions, absolute protein number, translation efficiency, and adenosine modifications. We correlated mRNA and protein abundances for genes in each growth condition and compared mean correlation coefficients among all conditions between modified and unmodified genes. Modified genes have statistically significant lower correlation (*P*-value Mann-Whitney test <0.01) between mRNA and protein abundances. Using ribosome profiling data we analyzed the impact of modifications on intermediate stages of cell information flow: from mRNA to ribosomes and from ribosomes to proteins. Modifications affect ribosome occupancy in such a way that translation efficiency only slightly correlates with mRNA level. Ribosome occupancy within modification sites is slightly lower than in other parts of the genes. We didn't find any difference in ribosome distribution upstream or downstream of modification sites. Was created the model that predicts protein abundance based on expression level, translation rate, and adenosine modification status. The model explains up to 63% of the variation in protein abundance, the model based on decision trees shows that m6A abundance has 15% contribution to protein level prediction. So, our calculations show statistically significant impact m6A modifications on information flow from mRNA to protein level. This work was supported by RSF grant No. 14-24-00159.

P.01-025-Mon
G-quadruplex RNA in Kirsten ras transcript as promising target for anticancer therapy

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Quadruplex-forming motifs of promoters of some oncogenes are considered as prospective targets for cancer chemotherapy. The human Kirsten ras (KRAS) transcript is capable to form several putative G-quadruplexes (G4s) as predicted by methods of bioinformatics. Among the three expected G4 motifs only one was recognised by BG4, an antibody specific for G-quadruplexes that confirms the initial assumption. Previously, it was demonstrated that heteroarene-fused anthraquinones have a strong affinity and selectivity to telomeric and Harley ras G-quadruplexes. Therefore, we investigated an ability of series ligands on heteroareneanthraquinones scaffolds to bind to G4s in the 5′-UTR of mRNA and suppress the KRAS oncogene in pancreatic cancer cells. Found, that 4,11-bis(2-aminoethylamino) anthra[2,3-*b*]furan-5,10-dione (ATFD) stabilizes G4 in the KRAS transcript (ΔT_M up to 32 °C). Moreover, synthesis and evaluation of biotinylated ATFD by means of biotin-streptavidin pulldown assay revealed that ATFD binds to RNA G4s in the KRAS transcript under low-abundance cellular conditions. Dual-luciferase assays showed that ATFD repress translation in a dose-dependent manner. The new G4-ligand efficiently penetrate into the Panc-1 cancer cells, suppressing protein p21KRAS to <10% of the control followed by apoptosis together with a dramatic reduction of cell growth at submicromolar concentrations. As the result one may conclude, a suppression of the KRAS oncogene via stabilization of G4s in the 5′-UTR of mRNA by low molecular weight ligands can be considered as a new strategy in G4-oriented anticancer therapy. Acknowledgment: this work has been carried out in part with the financial support of Italian association for cancer research (IG2013, project code 143, to Prof. L. Xodo) and the grant of the President of the Russian Federation for young Russian scientists (MK-2474.2018.3, to Dr. A. Tikhomirov).

P.01-026-Tue
C-reactive protein and renin-angiotensin system gene polymorphisms in patients with coronary artery stenosis

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The latest studies in the field of molecular cardiology demonstrate the important role of inflammation and renin-angiotensin system (RAS) in the pathogenesis of atherosclerosis. Gene polymorphisms of their components have been described to be associated with the development of coronary artery disease. Our work was aimed at studying the polymorphisms of the C-reactive protein (CRP), angiotensin II receptor type 1 (AGTR1), and angiotensin (AGT) genes in patients with coronary artery stenosis. 65 patients and 59 healthy individuals participated in the study. All of them were Russians. Total DNA was extracted from the blood and genotyped using the Real-time PCR. The CRP C1444T, AGT Thr174Met, AGT Met235Thr, and AGTR1 A1166C polymorphisms were analyzed. The Chi-square test and Fisher's exact test were used to estimate differences between groups. It was found that in patients the frequencies of most of minor alleles were significantly higher (AGT 174T 40.7% vs. 11%; AGTR1 1166C 39.2% vs. 19.5%; CRP 1444T 42.2% vs. 24.7%), which was accompanied by growth of the frequency of homozygous rare genotypes. Comparison of patients with multifocal atherosclerosis (n = 20) with other patients (n = 45) revealed that in this group the genotypic distributions of RAS genes were different due to a significant increase in the frequency of heterozygotes (AGT Thr174Met 15.8% vs. 4.3%; AGT Met235Thr 21% vs. 10.9%; AGTR1 A1166C 52.6% vs. 15.2%). The obtained results may be of interest for personalized medicine because heterozygosity for the given polymorphisms may predispose to the multifocal atherosclerosis. To confirm this idea it is necessary to continue research in larger samples. The publication was prepared with the support of the "RUDN University Program 5–100".

P.01-027-Wed
The expression of a nested alternative open reading frame may be a novel mechanism for intronless gene expression control

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The regulation of gene expression in animal and plant tissues at different stages of development and after exposure to external stimuli occurs at the transcriptional, post-transcriptional and post-translational levels. Recently, in the Solanaceae family of plants, we found an intronless gene for the Kunitz protease inhibitor-like protein (KPILP) with low mRNA levels in the leaves of an intact plant. However, these levels sharply increased after a 72-h incubation in the dark and under biotic stresses. To identify the mechanism for the regulation of

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Solanaceae KPILPs, we identified and examined the function of the KPILP nested alternative open reading frame (aORF) that controls the expression of maternal mRNA. Here, we investigated the regulation of KPILP under stress conditions at the level of transcription. We isolated a portion of *Nicotiana benthamiana* chromosomal DNA upstream of the KPILP coding sequence (proKPILP) and showed that proKPILP directed the expression of the *E. coli UidA* reporter gene encoding β-D-glucuronidase (GUS) in plant. A bioinformatic analysis revealed potential light-responsive elements in the proKPILP sequence. To understand if these elements influence proKPILP activity, agroinjected plants were isolated for 3 days in complete darkness. Then, the level of GUS and corresponding mRNA content were assessed in the leaves and compared with the constitutive CaMV 35S promoter. The results showed that, as in the case of the 35S promoter, darkness had a negligible effect on the activity of the proKPILP. We concluded that the stress-dependent content of the KPILP mRNA in the leaves is determined mainly by the aORF-mediated mechanism of maternal mRNA control. Thus, Solanaceae KPILP is an intronless matryoshka gene and is the first example of a gene expression regulation mechanism that is probably characteristic for intronless genes. This study was performed with financial support from the Russian Foundation for Basic Research (project No. 17-29-08012).

P.01-028-Mon
Transcriptome analysis reveals influence of RNases from toxin-antitoxin systems on gene expression in *Staphylococcus aureus*

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Toxin-antitoxin (TA) systems are widespread in bacteria. A typical TA system consists of a toxin, which is a stable protein, often with enzymatic activity, and an antitoxin, constituting a labile component with inhibitory properties towards the toxin. In case of plasmid-encoded TA systems a role in the maintenance of mobile genetic elements is attributed to these entities. However, localization of TA genes in chromosomal parts of bacterial genomes implies the existence of other functions. Staphylococcus aureus is a carrier of chromosomally-encoded MazEF TA system and less frequently of PemIK-Sa1, which is present in a number of staphylococcal plasmids. Toxins from these systems are sequence-specific RNases towards UACAU and UAUU, respectively. We hypothesize that the toxins, released from TA complexes upon activation, may degrade transcripts, and thus influence gene expression. To challenge this hypothesis we introduced plasmids carrying toxins' genes under inducible promoter to S. aureus null mutants for the respective TA systems. Upon induction of toxins' expression, RNA was isolated and RNA-seq was performed using Illumina platform. Interestingly, overexpression of MazF toxin resulted in rather moderate changes in gene expression. The number of genes with over two-fold difference was below twenty, however slightly increased over the time. Conversely, in case of PemK-Sa1 toxin over 160 gene transcripts were affected. This clearly indicates that the length of the recognized target sequence correlates negatively with the number of altered transcripts. Moreover, among transcripts down-regulated by PemK-Sa1 was RNAIII, which is an important molecule in the accessory gene regulator system, which suggests the existence of a possible regulatory cascade/coupling. The presented data demonstrate that TA RNase toxins may modulate gene expression in bacteria. The study was supported by the National Science Centre, Poland, grant no.: UMO-2014/13/B/NZ1/00043 (to B.W.).

P.01-029-Tue
Eukaryotic ribosome discriminates 3′ context and determines efficiency of stop codon readthrough

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Nucleotide context, surrounding stop codons, significantly affects the efficiency of translation termination. To study the mechanism of its influence, we assessed properties of several 3' contexts, unfavorable for translation termination, in the reconstituted mammalian translation system. Using pairs of stop codons divided by hexanucleotides we revealed, that the ribosome recognizes leaky and strong 3' stop codon contexts by itself. Stop codon readthrough depends only on the presence of suppressor / near-cognate tRNA and leaky 3' context. We estimated also the impact of 3' contexts on translation termination activity of eRF1. All tested contexts demonstrated approximately equal translation termination efficiency in the presence of release factors. However, comparison of basal readthrough levels showed the discriminate response among different weak context. It can reflect much more sophisticated mechanism of balancing between readthrough and termination, orchestrated by nucleotide surrounding of a stop codon that could be surmised on the first glance. The work was supported by the Russian Science Foundation (grant No. 14-14-00487) and by the Program of Fundamental Research for State Academies for the years 2013 to 2020 (No. 1201363822).

P.01-031-Mon
2′-Modified RNA aptamers specific to photoprotein obelin as a platform for new bioluminescent biosensors

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Aptamers are in vitro selected DNA or RNA molecules that can specifically bind a wide variety of targets from small molecules to proteins and living cells. Aptamers represent a high-potential alternative to monoclonal antibodies due to an opportunity to generate an aptamer to almost any desired target, longer shelf-life, cost-effective chemical synthesis, stability in a wide range of conditions, and low toxicity and immunogenicity. These unique properties determined aptamer applications as prospective therapeutic agents as well as biosensors for diagnostic purposes. In this study we proposed a new design of bioluminescent aptasensor made of two different aptamer modules: one is responsible for specific binding of analyte, the other provides bioluminescent reporting. We suggested that aptamers to Ca^{2+}-regulated photoprotein obelin could be used as universal reporting modules for bifunctional aptasensors. Due to the high quantum yield and low-level background, obelin established itself as a promising reporter molecule. We performed in vitro selection of 2'-F-modified RNA aptamers against His-tagged obelin immobilized on Ni-NTA sepharose beads. After high-throughput sequencing and bioinformatical analysis, we obtained a series of individual

aptamers. Full-length and truncated versions of candidate aptamers were synthesized and screened for their affinity. As a proof of concept, the most prominent truncated aptamer was used as a reporting module of the structure-switching bifunctional aptamer for bioluminescent detection of human hemoglobin. Therefore, obtained 2'-modified RNA aptamers specific to obelin could be used as a platform for diagnostic bioluminescent aptasensors. Proposed bifunctional design of bioluminescent aptasensors could be adapted for a detection of wide range of molecular targets by varying an analyte binding aptamer. The work is supported by Russian Science Foundation (Grant No. 16-14-10296).

P.01-032-Tue
Chemo-enzymatic transglycosylation of 2-aminopurine derivatives bearing bulky functional groups

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Biocatalytic method based on transglycosylation with purine nucleoside phosphorylases is an efficient approach to modified nucleosides. In the case of introduction of sugar residues into the purine position 9, the reaction, as a rule, proceeds stereo- and regioselectively. Typically, 2-aminopurine and purine derivatives without bulky groups at positions 2 and 6 are used as substrates. However, in cases where a further synthetic modification of substituted purines is expected, there is a need for preliminary glycosylation of purine derivatives with bulky substituents. The purpose of this work was to study the possibility of obtaining modified glycosides as a result of chemo-enzymatic transglycosylation of 2-aminopurine derivatives containing substituents at positions 2 and 6, including bulky groups. We carried out a comparative study of chemo-enzymatic transglycosylation of 2-aminopurine conjugates with amino acids and their protected derivatives in position 6. It has been shown that the 2-acetamidopurine derivatives containing *tert*-butyl glycinate, *S*-alaninate, *S*- or *R*-valinates, and *S*-phenylalaninate are good substrates for the genetically engineered recombinant *E. coli* purine nucleoside phosphorylase; the derivative containing *tert*-butyl prolinate was not subjected to chemo-enzymatic transglycosylation. Effectiveness of the transglycosylation in the synthesis of 2-deoxyribosides of unsubstituted 2-aminopurine conjugates was about the same as the aforementioned derivatives containing bulky substituents. In the case of ribosides and arabinosides, the reaction rate was decreased by approximately 3–4 times when purine bases containing bulky substituents were used as substrates. The advantages of chemo-enzymatic transglycosylation compared with chemical introduction of sugar residues were demonstrated. The work was financially supported by the Russian Science Foundation (grant 14-13-01077).

P.01-033-Wed
Murine lncRNA LL35 as a probable homolog of human lncRNA DEANR1

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Non-coding RNAs (ncRNA) play a significant role in the regulation of many cellular processes, including transcription, translation and cell differentiation. There are examples of ncRNA participation in the development of various diseases (C. Lin and L. Yang, Trends Cell. Biol. 2017), that allows to consider some ncRNAs as potential targets for therapy and diagnostics. It was demonstrated previously that DEANR1 ncRNA participates in the regulation of endoderm differentiation in human embryonic stem cells by cis-regulation of the Foxa2 transcription factor (W. Jiang et al., 2015). Foxa2 protein plays an important role in the glucose homeostasis in the liver, affects fatty acids catabolism under the stress conditions and regulates the biogenesis of high-density lipoproteins (M. Kanaki et al, 2017). Also DEANR1 influences on the epithelial-mesenchymal cell transition, which occurs not only during embryonic development but also in fibrosis and during progression of cancer tumors (Y. Fan et al, 2016). We found a homologue of the ncRNA DEANR1 in mouse - LL35 ncRNA by bioinformatics. We demonstrated LL35 expression in the mouse cell lines and liver with preferable localization in cell nucleus. We used antisense oligonucleotides for efficient downregulation of ncRNA LL35 (85–90%) that was confirmed by qPCR and FISH techniques. We propose ncRNA LL35 as a homolog of human lncRNA DEANR1 that can be involved in the transcription regulation of the Foxa2 factor in the liver and affect metabolic processes and various liver diseases in mice. This work is supported by the Russian Science Foundation under grant 17-74-10140.

P.01-034-Mon
LC/MS analysis of viral RNA

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Viruses are a major force that shapes the evolution of both pro- and eukaryotic organisms. The mechanism of action of various viruses has been the primary focus of many studies, yet, there are surprisingly scarce data on RNA modifications or RNA conjugates in any type of viruses. Development of vitally important methods for the sensitive analysis of RNA modification enabling detailed studies of the chemical structure of various RNA entities began only recently and they have never been applied to viral RNA. The simplicity of their genomes and well described structure and machinery of various viruses make them a perfect model system for searching for new RNA modifications as well as for understanding of the role of already known RNA modifications and RNA conjugates. In this particular project, we focus on HIV-1 (representative of *Retroviridae* family) and some eukaryotic viruses from order of *Picornavirales*. We isolated RNA from viral particles and analysed by LC/MS technique. The surprisingly high level of various methylations led us to the development of LC/MS quantification techniques. To reveal the exact position of methylation we had to apply the methylation profiling techniques in combination with next-generation sequencing. In next part of our studies, we will concentrate on understanding of the role of these methylated nucleotides in viral RNA. The Ministry of Education, Youth and Sports supported this work from the programme ERC CZ (LL1603).

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P.01-035-Tue
6S RNA as a transcription regulator in *Rhodobacter sphaeroides*

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E. coli 6S RNA was one of the first discovered non-coding RNAs. It forms a complex with the housekeeping RNA polymerase (RNAP) holoenzyme. The important finding was that RNAP utilizes 6S RNA as a template for the transcription of short product RNAs (pRNAs). We analyzed the expression and function of 6S RNA in α-proteobacterium *R. sphaeroides* that is a common model organism to study regulated formation of photosynthetic complexes and the response to (photo)oxidative stresses. *R. sphaeroides* 6S RNA was shown to have a unique expression pattern: it peaks at the end of exponential growth and strongly decreases during prolonged stationary phase. According to our Northern blot analyses, the levels of pRNAs are the highest in mid-to-late exponential phase and largely decrease toward prolonged stationary phase. This trend is also seen in the pRNA-Seq data; 12 to 16- meric pRNA are assumed to be long enough to remain bound to 6S RNA and to rearrange its structure which triggers RNAP release. It is shown that *E. coli* and *B. subtilis* cells lacking 6S RNA worse or better survive at some stress conditions. For *R. sphaeroides* we found the retarded growth phenotype of the 6S RNA deletion strains under high salt conditions, which can be explained by the dysregulated expression of SspA salt stress protein. The spatial vicinity of *ssrS* and *sspA* genes may also point to an interesting mechanistic scenario: newly transcribed 6S RNAs may bind to the same RNAP by which they were synthesized to reduce the frequency of incidences where an RNAP (after transcription of 6S RNA) reinitiates transcription at the nearby promoter that drives *sspA* expression. This work was supported by the Russian Science Foundation (project No. 14-24-00061).

P.01-037-Mon
The polymorphism of the COL1A1_1 gene as a risk factor of liver fibrosis development in patients abusing alcohol

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The main causes of liver fibrosis are hepatitis B and C, alcohol abuse, and genetic factors. Liver fibrosis is characterized by a significantly increased extracellular matrix which includes several types of collagen molecules. The most common is type I collagen. Mutations changing the structure of collagen fibers can lead to serious functional defects in connective tissue and the liver fibrosis development. The aim of the present study was to investigate the association between the *COL1 A1_1* C/A (rs1107946) polymorphism and liver fibrosis (F0-F4) in patients abusing alcohol. 47 patients (36 men and 11 women; the period of alcohol consumption was 15.6 ± 9.5 years) and 30 healthy individuals were examined. Patients with hereditary liver diseases or infected by hepatitis B and C viruses were excluded from the study. The polymorphism of the *COL1A1_1* gene was determined using the Real-Time PCR analysis. The stage of liver fibrosis was identified with the elastography method (FibroScan, Echosens, France). Liver fibrosis stages 0, 1, 3, and 4 were detected in 6 (12.7%), 3 (6.4%), 2 (4.3%), and 36 (76.6%) patients respectively. We did not find the patients with liver fibrosis stage 2. It was found that the frequency of allele A among patients was 38.3% which is significantly higher than in the control group (12.5%; $P = 0.005768$). The frequency of heterozygotes CA was not significantly different between the group of patients and the control group (38% and 25% respectively, $P = 0.4425$). Homozygous AA genotypes were revealed only in patients with liver fibrosis, the frequency was 19.1%. Thus, we suggest that the presence of allele A of the *COL1A1_1* gene, particularly in the homozygous state, may be one of the genetic factors involved in the development of liver fibrosis in patients abusing alcohol. The publication was prepared with the support of the "RUDN University Program 5–100".

P.01-038-Tue
The role of human Gle1 isoforms in translation termination

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Gle1 is a conservative essential and multifunctional protein involved in mRNA export from the nucleus into the cytosol. Yeast Gle1 also participates in initiation and termination of translation. Mutations of human Gle1 (hGle1) cause fetal motoneuron diseases (LCCS1 and LAAHD) and amyotrophic lateral sclerosis development. In human cells two isoforms (hGle1B and hGle1A) of this protein are formed as a result of alternative splicing. hGle1B is involved in mRNA export from the nucleus, and hGle1A is involved in stress granule (Sg) formation. In the present work, we studied the role of hGle1 isoforms in translation termination using a reconstituted mammalian translation system. We show that hGle1B binds to eRF1 and the eRF1-eRF3 complex (but not to eRF3) and activates translation termination. Conversely, hGle1A slightly inhibits translation termination. It effectively binds to pre-termination complexes (preTCs) and competes with eRF1 for binding to preTCs. We evaluated the levels of hGle1 isoform expression in Hek293 cells after different types of stress stimulation. Heat shock induced an increase of the mRNA expression of both hGle1 isoforms. Sodium arsenite treatment decreased the amount of hGle1A mRNA. We conclude that the two isoforms of hGle1 play different roles in translation termination: hGle1B enhances the efficiency of translation termination, while hGle1A binds preTCs and interferes with termination, possibly leading to Sg formation. The work was supported by the Russian Science Foundation (grant No. 14-14-00487).

P.01-039-Wed
Targeting ligand decorated exosome as a carrier of miRNA21 antisense oligonucleotide for brain cancer therapy

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Extracellular vesicles (EVs) are membrane-derived vesicles that secreted by various types of cells and involved in cell-cell signaling. Exosome is the smallest subtype among EVs with size of 30 to 100 nm in diameter. Since exosomes were small and native to the animals, they have several advantages as a drug delivery carrier. Rabies Virus Glycoprotein (RVG) is a small peptide that specifically bind to the acetylcholine receptor. It already has been reported that RVG decorated exosome can target brain by systemic injection. To generate a stable cell line that can secrete RVG-exosomes (RVG-exos), plasmid DNA encoding fusion protein of RVG and Lamp2b was transfected into 293T cell. RVG-exos and Unmodified-exosomes (Un-exos) were isolated and evaluated as a gene carrier. The result of SEM and Dynamic Light Scattering shows round shaped particles with a size of 30~100 nm. The miRNA21 antisense oligonucleotide (ASO) was loaded into the exosomes by electroporation. After transfection into C6 cell, cellular uptake of ASO loaded exosomes were measured by flow cytometer. In vivo therapeutic effect of RVG-exo-ASO was tested in an intracranial tumor rat model. Rats were bored 2 mm hole on the skull and C6 cells were implanted into the brain. A week later, ASO loaded RVG-exo and Un-exo were administrated by tail vein injection. The result of Nissl staining of the brain section showed that delivery of RVG-exo-ASO had anti-cancer effect on brain tumor. As programmed cell death 4 (PDCD4) and phosphatase and tensin homologue (PTEN) are pro-apoptotic gene that has known to be inhibited by miRNA21, the induction of them may be involved in the tumor volume in the animals. Immunohistochemistry (IHC) showed that the delivery of RVG-exo-ASO induced PTEN and PDCD4 more efficiently than that of Un-exo-ASO group, showing anti-tumor effect of the ASO delivery. In conclusion, RVG-exo delivered the ASO into the rat brain effectively and may be useful as a therapeutic agent for the brain tumor.

P.01-040-Mon
Differential expression profiling of the YABBY1 and YABBY3 genes in *Solanum* and *Capsicum* species

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The plant-specific bifunctional YABBY transcription factors have an important role in driving the evolution of the leaf and gynoecium. *YABBY* genes are involved in the initiation, growth and structural organization of almost all aboveground lateral organs, vegetative or reproductive, defining their adaxial-abaxial asymmetry, and in the control of shoot apical meristem organization and activity. The present study was focused on the identification and comparative characterization of *YABBY1* and *YABBY3* genes in species of tomato (*Solanum* section Lycopersicon) and pepper (*Capsicum*), their polymorphism, expression profiling in lateral organs, and evaluation of possible correlations between the expression pattern and the leaves and flowers size. The complete *YABBY1* and *YABBY3* genomic sequences were identified in 13 accessions of 11 tomato species and 6 accessions of 4 *Capsicum* species, cultivated and wild. The obtained genes encoded proteins, which contain conserved zinc finger and HMG-like YABBY domains and nuclear localization signals characteristic for YABBY transcription factors, and also, the conserved motifs specific for the YAB1/YAB3 subfamily. The *YABBY1* and *YABBY3* expression patterns were determined in the leaves and flowers of *S. lycopersicum*, *S. chmielewskii*, *S. peruvianum*, *S. habrochaites*, three *C. annuum* accessions, *C. chinense*, *C. frutescens*, and *C. pubescens*. In analyzed tomato species, the possible negative correlation between *YABBY1* and *YABBY3* co-expression level and the size of lateral organs was observed. The proposed evolutionary history suggests the more recent origin of *YABBY3* compared to *YABBY1*, as well as the emergence of these paralogous genes from the common ancestor before Dicots divergence on Rosids and Asterids. The study was supported by the Russian Science Foundation grant 16-16-10022.

P.01-041-Tue
Novel 2′-F-RNA aptamers specific to protein markers of glycemia – a basis for bioluminescent aptasensors

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Nucleic acid aptamers are now generally considered as the most prominent alternative to monoclonal antibodies thanks to their high target affinity and specificity, ease of chemical synthesis and a possibility to generate an aptamer with desired properties by in vitro evolution on a lab bench. In particular, aptamers are becoming increasingly popular as recognizing elements for biosensing platforms. In this study, we generated 2'-F-modified RNA aptamers specific to protein markers of blood glucose level – glycated and non-glycated human hemoglobin and serum albumin. All target proteins were covalently immobilized on magnetic beads. To select aptamers capable to bind only the glycated protein, each cycle comprised an incubation of the RNA pool with the non-glycated target (the negative selection step) followed by the incubation with the glycated one. In the case of the aptamers affine to the total hemoglobin or albumin, only the incubation with the 'normal' protein was applied. The enriched RNA pools were subjected to high-throughput sequencing and bioinformatic analysis. For each set of candidate aptamers we designed their truncated forms on the basis of RNAfold secondary structure prediction. Truncated aptamers were then chemically synthesized and screened for their affinity and specificity to the corresponding targets. The best aptamers to the total/glycated hemoglobin or albumin were then integrated as recognizing elements of bioluminescent aptasensors with the use of Ca^{2+}-dependent photoprotein obelin as a reporter. The investigation of their sensitivity and specificity revealed the optimal design of aptasensors. Novel aptamers and aptasensors developed in this work could be further used for the assessment and monitoring of the short-term (the percentage of glycated albumin) and long-term (the percentage action of glycated hemoglobin) blood glucose control. The work is supported by Russian Science Foundation (Grant No. 16-14-10296).

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P.01-042-Wed
A HMGB-1 siRNA delivery using brain-targeting ligand embedded exosomes for ischemic stroke therapy

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Exosomes, which are a member of extracellular vesicles (EVs), function as an intercellular messenger transferring biomolecules, including nucleic acids and proteins. Exosomes have emerged as a promising therapeutic delivery system because of their nature as cell-derived nanovesicles, which have low toxicity and high biocompatibility. Furthermore, exosomes have the smallest size (30–100 nm in diameter) out of EVs and were reported that exosomes can pass through blood brain barrier (BBB), implying a immense potential as biocarrier for therapy of brain related disease. To produce targeting ligand embedded exosomes, we delivered designed plasmid to HEK293T cells and isolated exosomes that involve Lamp2b, one of exosomal membrane proteins, fused to Rabies Virus Glycoprotein (RVG) peptide for acetylcholine receptor targeting in the brain. Activated after ischemic condition, immune cells secrete HMGB-1 as cytokine mediator of inflammation. For knockdown HMGB-1, HMGB-1 siRNA was loaded into RVG embedded exosomes (RVG-EXOs) by electroporation. In vitro characteristic test showed the morphology and the size of exosomes through SEM images and dynamic light scattering. RVG-EXOs showed highly efficient targeting and HMGB-1 siRNA delivery to Neuro2A neuron cells *in vitro* as demonstrated by flow cytometry. In vitro western blot experiment also demonstrated the knockdown effect of HMGB-1 siRNA loaded in RVG-EXOs. Additionally, intravenously injected HMGB-1 siRNA loaded RVG-EXOs notably reduced infarct volume in the ischemic rat brain generated by MCAO modeling. In conclusion, RVG-EXOs can efficiently deliver HMGB-1 siRNA to brain and reduce infarct volume by down-regulating HMGB-1 levels.

P.01-043-Mon
Identification of plant components in food samples using next-generation sequencing methods

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Next generation sequencing (NGS) methods offer new opportunities for food component identification using the DNA metabarcoding approach. The goal of the presented study was to demonstrate the applicability of NGS methods for the identification of plant components in food samples. The analysis was performed using the internal transcribed spacer (*ITS*) region, which is widely used as a barcode for both plants and fungi. Enhanced primers for amplification of *ITS1/2* of plant species only were designed and tested. The local database of reference sequences was created using Genbank data, which were previously filtered (misannotated and duplicate entries were discarded). Eighteen food samples were examined (6 common black teas, 6 herbal teas, 6 seasonings) on both the Ion S5 and Illumina MiSeq platforms. The obtained results confirmed the presence of components declared by the manufactures in most cases. However, the adulteration of several herbal tea samples manufactured in China was found: only two out of six declared components were detected (*Camellia sp*. and *Senna sp*.), the other components represented the different climbing plants, such as *Ipomoea sp*. Moreover, significant amount of weed species were found in most samples, which is likely caused by the violation of the manufacture technologies. Several contaminant components that may cause allergic reaction (*Cynodon sp*., *Ambrosia sp*.) were identified in three samples. Thereby, this work clearly demonstrates the applicability of NGS methods for plant species identification in food samples. The results obtained on the Ion S5 and MiSeq sequencing platforms were highly correlated and, moreover, outperformed the classic botanical analysis methods. Thus, NGS methods may become the "gold standard" in food quality control in the nearest future. This study was supported by Ministry of Education and Science of the Russian Federation, project # 14.609.21.0101 [grant number RFMEFI60917X0101].

P.01-044-Tue
Novel N^4-acyl-2′-deoxycytidine-5′-triphosphates for the enzymatic synthesis of modified DNA

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A huge diversity of modified nucleobases is used as a tool for studying DNA and RNA. The most suitable positions for modifications are C5 of pyrimidines and C7 of 7-deazapurines. This is mainly due to the excellent substrate properties shown by these analogues not only in primer extension but also in PCR. However, a little attention has been paid to altering other positions of nucleobases such as C6/C8 or O^4/N^4. Indeed, expanding a repertoire of the modified nucleotides would substantially improve development of aptamers, biosensors or therapeutics. Here, we report on the synthesis and enzymatic incorporation of novel N^4-acyl-2'-deoxycytidine-5'-triphosphates ($dC^{Acyl}TPs$). We find that a variety of family A and B DNA polymerases such as *Taq*, Klenow fragment (exo–), Bsm and KOD XL efficiently use $dC^{Acyl}TPs$ as substrates and incorporate the modified nucleotides at multiple positions. Moreover, 3'-exonuclease-deficient DNA polymerases pair N^4-acylated cytosine base not only with guanine but also with adenine to the great extent. In contrast, a proofreading phi29 DNA polymerase successfully performs primer extension reactions using $dC^{Acyl}TPs$ but is prone to form dC^{Acyl}•dA base pair under the same conditions. We also demonstrate that terminal deoxynucleotidyl transferase (TdT) efficiently utilizes $dC^{Acyl}TPs$ during 3'-elongation in a template-independent manner. In fact, TdT is able to incorporate as many as several hundred consecutive monomers bearing acetylbenzoyl or benzoylbenzoyl functional groups. We further propose that the 3′-tail of modified DNA containing acetylbenzoyl or benzoylbenzoyl groups folds into a specific tertiary structure. Overall, our results provide a set of novel N^4-acyl-deoxycytidine nucleotides that can significantly expand the toolbox for the enzymatic synthesis of modified DNAs. Novel modified nucleotides can be further applied for specific labelling of DNA, selection of aptamers or photoimmobilization.

P.01-045-Wed
Selection of DNA aptamers to odorous substances using Capture-SELEX protocol

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Food industry requires analysis of different odorous compounds to test product freshness, to discriminate between different products and to control the stages of foods and beverages manufacturing process. Aptamers, which are short ssDNA or RNA fragments displaying affinity to the target molecule, can be used for developing new odorant detection methods. Aptamers to a certain target can be selected from the randomized sequences library using systematic evolution of ligands by exponential enrichment (SELEX). In this research new aptamers with affinity to guaiacol, ethylguaiacol and homofuraneol were obtained. These substances contribute to coffee, wine and beer flavors. Capture-SELEX protocol was used for aptamer selection because it suits well for selection of aptamers to small organic molecules. Mix of few targets was used for selecting the aptamers from 98 nt ssDNA bank having 60 nt randomized region. After 12 rounds of selection, the aptamers were selected to each target separately in the final 13th round followed by cloning and Sanger sequencing of 96 clones for each target. 7-8 aptamer candidates were selected to each target. A novel isothermal DNA amplification based technique was used for affinity measurements. New aptamers for guaiacol, ethylguaiacol and homofuraneol are capable to detect at least 0.5 μM of the target in solution. This work was supported by the Russian Science Foundation (project #16-19-10697).

P.01-046-Mon
Single nucleotide loops stabilize intramolecular parallel G4 DNA

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Conformation of intramolecular G-quadruplex(G4) DNA depends on multiple factors including nucleotide sequence. Apparently the length of the loops inputs certain constrains on the resulting G4 DNA fold and affects its thermodynamic stability. To shed some light on this issue, we report here on effect of shortening of each of three loops on the resulting folding of the G4 DNA and its conformational stability. Telomeric DNA repeats $d(TTAGGG)_4$ have been chosen as a starting model of G4 structure. We considered all variants of potential quadruplex sequences in which one, two or all three TTA loops were replaced by a single thymine nucleotide. Circular dichroism, gel migration, chemical probing with DMS and ZnP1 (a porphyrin derivative sensitive to G4 conformation) were applied to monitor conformational changes occurred upon shortening each loop to single nucleotide. We demonstrate that all variants of sequences form intramolecular structures. Circular dichroism data of all DNA sequences confirm the formation of G4 structures of variable conformations. DNA sequences containing a single short loop formed a mixed quadruplex structure. More than two single thymine loops in quadruplex sequence lead to formation of parallel G4 structure with significant increase in thermodynamic stability. Our findings assist in understanding how nucleotide sequence governs the conformation and stability of the G4 DNA. This study was financially supported by the Russian Science Foundation (project 16-14-10396).

P.01-047-Tue
Benzothiazole derivatives for staining DNA structures: selectivity for G4/duplex junctions

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Non-canonical nucleic acid structures (ncDNA), such as G-quadruplexes (G4), are currently attracting much attention because of their diverse biological roles. Express methods for ncDNA visualization based on specific light-up probes are gaining popularity. We synthesized new benzothiazole derivatives – analogs of a known dye thiazole yellow – and evaluated their applicability as light-up probes for discriminating between ssDNA, dsDNA and ncDNA. Several new dyes exhibited specificity for G4s. Fluorescence quantum yields of the leading new dye in complexes with G4s from oncogene promoters were comparable to those of a "gold standard" light-up probe thioflavin T. Interestingly, both thiazole yellow and the new dyes demonstrated remarkable fluorescence increase upon complexation with a double-module DNA structure composed of G4 and duplex fragments. Molecular modeling (docking) results suggest that the pronounced sensitivity of the probes for the double-module structure is accounted for by stacking interactions with nucleic bases from the G4/duplex junction. We assumed that several benzothiazole-based ligands might recognize the ncDNA-dsDNA junctions. The assumption is being verified using an expanded set of DNA structures. The results have implications for visualizing ncDNA in duplex media. This work was supported by Russian Science Foundation [14-25-00013].

P.01-048-Wed
Synthesis and Degradation of Ms1 in *Mycobacterium smegmatis*

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Adaptation of microorganisms is necessary for their survival in changing environment. In this process, regulatory roles are played by small non-coding RNAs (sRNAs). Ms1 is an abundant sRNA (rivalling in amounts those of rRNA) found in *Mycobacterium smegmatis* and it has homologs in all mycobacteria including *Mycobacterium turberculosis*. Ms1 forms a complex with the RNA polymerase (RNAP) core and it is a pleiotropic regulator of gene expression, enhancing survival of the cell under various types of stress. Ms1 is highly expressed and stable in stationary phase and it is rapidly degraded when the cell is shifted into nutrient-rich medium. The accumulation of Ms1 in the cell depends both on its synthesis and degradation but the specific mechanisms involved are unknown. Here, we identify and characterize the Ms1 promoter, the dynamics of Ms1 expression, and reveal the presence of a transcription factor involved in regulation of its expression. Further, we identify an RNase, Polynucleotide phosphorylase (PNPase) to interact with Ms1. With recombinant PNPase we demonstrate that it is able to degrade Ms1 *in vitro* and identify Ms1 secondary structures that affect its stability. RNAseq data show that PNPase is expressed ~10x more in exponential than in stationary phase, inversely correlating with the accumulation dynamics of Ms1. In summary, we provide a comprehensive characterization of how the intracellular level of Ms1 is controlled, paving the way to potential future designs

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altering its expression in the case of pathogenic species. This work is supported by grant 946216 from the Grant Agency of Charles University.

P.01-049-Mon
In children with autism spectrum disorders, an increase in cell-free DNA content aggravates oxidative stress with decreasing activity of the antioxidant system

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Autism spectrum disorders (ASD) affect ~1% of the world's population and are characterized by impaired social interaction, communication, and behavior. We report here a new piece of evidence for elevated cell-free DNA (cfDNA) in ASD. Children with ASD (n = 112) aged 4–12 were tested with ABC, SRS-2, SCQ, AMSE scales and found to meet the F84.0, 84.1, 84.5 ICD-10 and DSM-IV-TR criteria. The controls were healthy children (n = 64) aged 4–12. We found that plasma cfDNA in ASD group was 2–4 times higher, while nuclease activity was 1.8-times lower than in controls ($P < 0.01$). The more severe disease, the higher was the cfDNA content in ASD children's plasma. Oxidative stress (OS) followed the autism progress. We supposed that the surplus amount of cfDNA acts as a pathogenetic factor that aggravates OS and potentiates apoptosis. Lymphocytes isolated from controls (n = 22) and ASD cases (n = 36) were exposed *in vitro* to cfDNA obtained from ASD cases at 50 ng/mL for 1–3 h. In control cells, the exposure to cfDNA induced a transient burst of reactive oxygen species (ROS) by a factor of 2–2.5 against the baseline, however, it was accompanied by a 2-fold increase in the expression of NRF2 and SOD1 transcription factors, which normalized the ROS level. In ASD cells, the exposure also provoked a 2–3-fold increase in ROS content, but no activation of the antioxidant system followed: NRF2 expression was 3.5–4 times and 2–3 times lower, and SOD1 expression was 2–2.5 times and 1.6–2 times lower than in healthy cells, respectively, after 3 and 24 h of the start of exposure ($P < 0.01$). Our findings could help to reveal novel prognostic markers of ASD, and develop new therapies. The study was supported by RFBR grant #17-04-01587_A.

P.01-050-Tue
Primer-extension stops at G4 DNA in oncogene promotors

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A number of oncogene promoters in the human genome contain G-rich nucleotide sequences that are able to form G-quadruplex (G4) structure. Folding of some of them was demonstrated in vitro and in vivo. Such G4 structures are considered potential molecular targets in treatment of cancers. Amplification of G-rich DNA sequences often considered problematic and potential G4 structures are referred a possible reason. As so, polymerase stops could be a convenient method for detecting G4 structures in the genomic DNA. In this work, we used double stranded DNA fragments of four oncogene promoters (C-MYC, C-KIT, KRAS and NRAS) which contained the potential quadruplex structure (PQS). Primer extension method allowed to detect the stops corresponding the beginning of PQS sequences. We compared several polymerases and solution conditions in synthesis of PQS containing sequences. Replacing of K+ ions by Cs+ ions in primer-extension reaction resulted in synthesis of full length DNA product as was observed by denaturing PAAG. Accordingly, the presence of K+ and not Cs+ ions led to inhibition of PCR when amplifying PQS containing DNA. The obtained results allow to amplify effectively G-rich DNA sequences by PCR and provide a new method for detection of stable cation-dependent alternative DNA structures. This work was supported by the Program of fundamental research for state academies for 2013–2020 years (№ 01201363818).

P.01-051-Wed
Changes in the transcriptome of coelomocytes from sea star Asterias rubens under endotoxic stress

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Echinoderms occupy a critical phylogenetic position in deuterostomes evolution. Coelomocytes are mobile cells present in the visceral coelom of the sea star and considered to be critically important for the host defense. We obtained 140.9 million of Illumina MiSeq pair reads of RNA from sea star coelomocytes and assembled them into 139 thousand contigs with N50 = 1911. After removing redundant contigs, the final assembly of 13002 contigs (unigenes) with N50 = 2913 was obtained. Comparison to similarly assembled transcriptomes of *A. rubens* oocytes and *S. purpuratus* coelomocytes demonstrated about 80% of common transcripts while 20% were cell type specific. Protein domain search identified a number of unigenes that may be related to evolution of the immune system. TIR domains and their flanking sequences defined 5 putative Toll-like receptors and 2 homologs of the adapter molecule MyD88. Unigenes encoding NACHT and DD domains suggested 8 potential NOD-like receptors expressed in coelomocytes. 17 unigenes encoded polypeptides with full length SRCR domain and were annotated as putative scavenger receptors. We found homologs of complement components B, C2 and C3, peroxidase and NO-synthase, cathepsins, granzymes, homologs of RAG-1/RAG-2 recombinase, transcriptional factor NFkB, as well as intracellular signaling and extracellular recognition domains of several cytokine receptors. Differential gene expression in response to stress and endotoxin was analyzed in silico, followed by analysis of selected candidate genes by quantitative real-time PCR. Of 14 genes activated by at least 5 fold, 6, including the top 2, appear to encode transcription factors of leucine zipper and zinc finger families domains related to stress responses and cell activation. The top 2 stress-induced unigenes (annotated as early growth response protein 1-B-like isoform X1 and fos-related antigen 1 isoform X1) also showed a significant increase of expression in response to *E. coli in vivo*.

P.01-052-Mon
Polyplex using tumor-specific aptamers, Wy-5a and AS1411 for targeting delivery of therapeutic gene to prostate cancer

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Prostate cancer (PCa) is one of the most frequently diagnosed cancers among western male population. So far, treatment of PCa mostly relies on chemotherapy and radiation therapy, which have high toxic to normal tissues and side effects. Therefore, new treatments for PCa have been developed to make up for non-specific therapy. Aptamers are short single-stranded oligonucleotides that can bind defined targets with high affinity and specificity. It is derived from Cell-SELEX (systematic evolution of ligands by exponential enrichment). Compared to antibodies, it has attractive characteristics such as low immunogenicity and higher stability. In this study, tumor specific aptamers, Wy-5a and AS1411, were applied on targeting delivery of nucleic acids. Wy-5a and AS1411 bind to membrane protein related to progression of PCa and nucleolin highly expressed on most cancer cell surfaces, respectively. Wy-5a and AS1411 were conjugated to polyethyleneimine (25 kDa, PEI25k) using maleimide polyethylene glycol succinimidyl ester (Mal-PEG-NHS, Mw 2000) as a linker. PEI-PEG-Wy-5a and PEI-PEG-AS1411 formed stable complexes with plasmid DNA (pDNA). The transfection assays showed that PEI-PEG-Wy-5a and PEI-PEG-AS1411 had higher transfection efficiencies to PC3 prostate cancer cells than their counter-parts without aptamers. In addition, the cytotoxicity of PEI-PEG-Wy-5A and PEI-PEG-AS1411 was lower than PEI25k. Therefore, PEI-PEG-Wy-5A and PEI-PEG-AS1411 may be useful for therapeutic gene delivery targeting to PCa.

P.01-053-Tue
Combination therapy of adiponectin gene and RAGE binding peptide for acute lung injury

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Acute lung injury (ALI) is a severe lung inflammatory disease, induced by various causes including infections, traumas, and ischemia-reperfusion damages. Previously, it was reported that RAGE binding peptide (RBP) reduced inflammation response in the ALI animal models by inhibition of RAGE-mediated signal pathway. Interestingly, RBP has high positive amino acids, suggesting that it may bind plasmid DNA (pDNA) and be useful as a carrier of pDNA. In the current study, RBP was characterized as a carrier of the adiponectin (APN) gene as well as therapeutic peptide. Our previous report showed that APN gene had an anti-inflammatory effect and protected the endothelial cells in the ALI animal models. Therefore, the delivery of the APN gene with RBP may synergistic effect in the treatment of ALI. A gel retardation assay and a heparin competition assay confirmed that RBP formed stable complexes with the APN plasmid (pAPN). In vitro transfection assays showed that the RBP/pDNA complex had higher transfection efficiency than naked pDNA in the L2 lung epithelial cells. In addition, the RBP/pDNA complex higher gene delivery efficiency than naked DNA in the LPS induced ALI animal models. Furthermore, the RBP/pAPN complex had higher anti-inflammatory effect in the ALI animal models, reducing the pro-inflammatory cytokine levels. The results suggested that RBP may be useful as a carrier of pDNA into the lungs as well as an anti-inflammatory therapeutic peptide. Therefore, the RBP/pAPN complex may have a synergistic effect for the treatment of ALI.

P.01-054-Wed
A promising biomarker for diagnosis of breast cancer: miRNA

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An important part of breast cancer studies focused on the discovery of biomarkers that will enable early diagnosis, because it can be completely cured with early diagnosis, MicroRNAs (miRNAs) are accepted as potential biomarkers for the early detection of breast cancer. MiRNAs posttranscriptionally regulating gene expression are known to affect tumor formation, growth and spread. We aimed to determine whether there is a statistically significant difference in serum cancer-specific miRNA levels among the patients diagnosed with breast cancer and healthy controls. The study included 40 breast cancer patients admitted to Gazi University Hospital and had not received any treatment yet, and 35 healthy women as a control group. 86 different miRNAs isolated from serum samples. Isolated miRNAs were transformed into cDNA using a reverse transcription kit, then pre-amplification reaction and exonuclease reaction is done. After PCR, analysis of the obtained RT-PCR results (Ct values) was calculated. For the intergroup comparisons Student T-test was used. Compared with the control group, in patients with breast cancer the levels of let-7a-5p, miR-125a-5p, miR-148a-3p, miR-21-5p, miR-100-5p, miR-150-5p, miR-27b-3p, let-7d-5p, miR-15b-5p, miR-125b-5p, miR-126-3p were found to be statistically significantly increased ($P < 0.05$); while the levels of miR-205-5p, miR-181a-5p, miR-301a-3p, miR-127-5p, miR-215-5p, miR-193a-5p, miR-372-3p, miR-149-5p, miR-181c-5p decreased ($P < 0.05$). MiRNAs which have a statistically significant difference between the two groups in the study have potential for early diagnosis of breast cancer. However, further studies are needed so that serum miRNAs can be validated as a noninvasive biomarker early in the diagnosis of breast cancer.

P.01-055-Mon
Detection of β-thalassemia Cd 39 mutation by using piezoelectric biosensor for non-invasive prenatal diagnosis

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β-Thalassemia is one of the most monogenic autosomal recessive disorder characterized by defective production of the β-chain of hemoglobin. Definition of the β-globin genotype is necessary for genetic counselling in the carriers, and for predicting prognosis and management options in the patients with thalassemia. DNA-based prenatal diagnosis of β-thalassemias routinely relies on polymerase chain reaction (PCR) and gel electrophoresis. The aim of this study is to develop a new procedure, a DNA-based piezoelectric biosensor, for the detection of β-thalassemia Cd 39 mutation on the fetus' cell free DNA from maternal blood, the common β-thalassemia mutation type in Turkey. Cell free fetal DNA taken from maternal whole blood. Bioactive layer was constituted by binding 2-Hydroxymethacrylate Methacryloamidoscysteine (HEMA-MAC) nanopolymers on the electrode's surface. Single oligonucleotide probes specific for Cd 39 mutation of β-thalassemia were attached to the nanopolymer. The measurements were executed by piezoelectric resonance frequency which is caused by binding of the cell free fetal DNA in media

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with the single oligonucleotide probe on the electrode surface. The results were confirmed by the conventional molecular method as ARMS. The piezoelectric resonance frequencies obtained by hybridization of the cell free fetal DNA on bioactive layer were found 221 ± 15, 279 ± 8, and 318 ± 9 Hz for the samples of normal β-globin, heterozygote, and homozygote of Cd 39 mutation, respectively. The developed biosensor serves as a specific result to Cd 39 mutation. It could accurately discriminate between normal and Cd 39 mutation samples. Because of low costs, fast results, specificity and high detection/information effectiveness as compared with conventional prenatal diagnosis methods, we can be offered this technique as an alternative to conventional molecular methods.

P.01-056-Tue
Preferential binding of p53 and BRCA1 proteins to naturally occurring G-quadruplexes forming sequences

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Aim of our work was to study preferential binding of BRCA1 and p53 proteins to different forms of G-quadruplexes found in promotor regions of *p21* and *ESR1* genes. Based on enhanced occurrence of sequences, that are able to form G-quadruplexes in promotor regions of large scale of genes including genes that are involved in cell cycle regulation, our hypothesis is, that formation of G-quadruplexes can be a part of regulation of gene expression and cell cycle progression/arrest. For both p53 and BRCA1 proteins binding to Non-B DNA motifs have been proven to be one of their modes of action. P53 protein is involved in regulation of expression of p21 and BRCA1 is involved in ESR1 regulation pathway. Understanding mechanisms of regulation is important for discovery of new anti-cancer therapeutic methods, for example possibility of using G-quadruplex stabilisation drugs. Using bioinformatic tools we have found sequences able to form G-quadruplexes in promotor regions of human *p21* and *ESR1* genes. Sequences of maximally 45 bp, with four repetitions of at least 3 guanines separated by loops of maximally 15 bp were searched. We have found 4 sequences able to form G-quadruplexes in p21 promoter and 3 sequences in ESR1 promoter. Using less strict criteria dozens of potential sequences were found. We have PCR amplified these promotor regions from HCT16 cell lines. Using B4G antibody we have tested ability of these sequences to form G-quadruplex structures in different conditions and tested preferential binding of p53 and BRCA1 proteins to these sequences using EMSA and immunochemical methods.

P.01-057-Wed
Functional analysis of novel compound heterozygous ACP4 mutations causing hypoplastic amelogenesis imperfecta

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Amelogenesis imperfecta (AI) is a rare hereditary condition affecting tooth enamel. The affected enamel can be hypomineralized and/or hypoplastic. Recently, recessive mutations in *ACP4* (acid phosphatase 4) have been identified to cause hypoplastic AI. In this study, we identified novel heterozygous compound *ACP4* mutations in a hypoplastic AI family using whole exome sequencing and Sanger sequencing: c.262C>A (p.Arg88Ser) and c.419C>T (p.Pro140Leu). Western blot revealed that the mutant proteins were expressed in a greatly reduced level compared to the wild type protein. To further explicate the effect of the altered protein sequence on ACP4 homodimerization, Co-IP was done. Moreover, to compare AP activity levels of wild type and mutant forms, acid phosphatase assay was performed. The aim of this study was to elucidate how the compound heterozygous ACP4 mutations, located in extracellular part of ACP4 protein, affect protein structure and function of ACP4.

P.01-058-Mon
Effects of paricalcitol on hydrogen peroxide-induced oxidative damage in human umbilical vein endothelial cells through vitamin D receptor signaling

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Oxidative DNA damage is caused by the imbalance between reactive oxygen species (ROS) and the antioxidants in cells. Accumulation of DNA damage might lead to a variety of mutations and consequently to genomic instability. Paricalcitol is a synthetic vitamin D analog that shows similar physiological properties with vitamin D, but has less hypercalcemic effects. Paricalcitol exerts most of its pleiotropic actions via binding to vitamin D receptor (VDR). The relation between vitamin D and non-calcemic analogs such as paricalcitol with oxidative DNA damage is noteworthy due to the high prevalence of vitamin deficiency and the importance of genomic instability. The aim of this study is to investigate the dose-dependent effects of paricalcitol on the formation of ROS, levels of oxidatively induced DNA damage and expressions of DNA repair enzyme APE1 and VDR in a model of hydrogen peroxide (H_2O_2)-generated oxidative stress in human umbilical vein endothelial cells (HUVEC). Three doses (0.1–10–100 nM) of paricalcitol and 100 nM of ZK159222 antagonist to evaluate the role of VDR were used in the study. The generation of ROS was assessed fluorometrically using the probe dichlorofluorescein diacetate (DCFH-DA). APE1 and VDR gene expressions were analyzed by qPCR. Treatment of 300 μM H_2O_2 significantly increased ROS as compared to control ($P < 0.001$). Paricalcitol pretreatments attenuated excessive ROS in HUVECs exposed to H_2O_2 ($P < 0.05$). Moreover, this effect was performed by VDR-mediated signaling. Treatment of HUVEC with H_2O_2 significantly decreased APE1 and VDR mRNA expressions ($P < 0.05$). But, preincubation with different doses of paricalcitol did not have a significant effect on the expressions of the APE1 and VDR. This study demonstrated that paricalcitol might protect endothelial cells from H_2O_2-generated oxidative stress. For further work, the levels of damaged DNA nucleosides will be analyzed using liquid chromatography-tandem mass spectrometry.

P.01-059-Tue
Copy number variations and damage level in ribosomal genes and mitochondrial DNA in patients with paranoid and hebephrenic forms of schizophrenia

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One of the most common concepts of the causes of schizophrenia is the influence of two groups of factors: the contribution of genetic variations and environmental influences. Copy number variations (CNVs) of genes make a significant contribution to human evolution, to normal phenotypic variability and morbidity in humans. Ribosomal genes (rDNA) encode RNA ribosomes and affect the level of protein synthesis by the cell. Mitochondria are responsible for the energy supply of the cell. The change in the copy of mtDNA occurs with oxidative stress. Oxidative stress is a component of the pathogenesis of schizophrenia. We researched the variability of CNVs and the level of damage of two components essential for the functioning of the cell - mtDNA and rDNA on blood samples of 108 men and 71 women aged 17 to 64 years with paranoid or hebephrenic schizophrenia (diagnosis F20.00 or F20.01). In the control group, mentally healthy 60 men and 62 women aged 17 to 78 years, not having a family burden of schizophrenia. Two independent methods - non-radioactive quantitative hybridization (NQH) and real-time PCR (qPCR) have shown that the genomes of schizophrenic patients are characterized by increased content of copies of 45S rDNA and mtDNA compared to the control. For the first time it was shown that the state of acute psychotic disorder of a patient in the absence of therapy is accompanied by an increase in the level of rDNA damage, especially in the region of the 28S rDNA gene. In response to oxidative stress, the cell begins to synthesize a large amount of mtDNA. The therapy leads to a decrease in the level of rDNA damage and to a decrease in the amount of mtDNA to the level of control, which is probably due to a decrease in the level of oxidative stress in the patient's body. Herewith, some of the mtDNA molecules are damaged. The data obtained by us confirm the contribution of the genetic component to the pathogenesis of schizophrenia. Support by RFBR grant № 17-29-06017 ofi_m.

P.01-060-Wed
miRNA and cytokine expression in active Behcet's patients

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Behcet's disease is a chronic multisystem inflammatory disorder characterized mainly by recurrent oral ulcers, ocular involvement, genital ulcers, and skin lesions, presenting with remissions and exacerbations. Environmental and genetic factors are thought to contribute to its onset and development. Although the etiology of Behcet's disease is not yet clear, new immunogenetic findings provide clues in the pathogenesis. miRNAs have created a new paradigm for gene regulation and pathways involved in the pathogenesis of autoimmune diseases. The goal of this study was to investigate the expression of miRNAs in patients with active Behcet's disease (BD). Peripheral blood mononuclear cells (PBMCs) were obtained from BD patients with exacerbation and from healthy controls. Expression level miRNAs were studied using Dynamic Array System. The levels of cytokines mRNAs were measured by qRT-PCR. Among 96 miRNAs, only levels of miR-145, miR-19b, miR-17 were significantly increased from BD patients with exacerbation patients as compared with healthy controls. Levels of IFN-γ, IFN-γR, IL-18, IL-8, IL-23R were lower in patients with active BD than in healthy controls. ($P < 0.001$). The present results suggest that miR-145, miR-19b, miR-17 expression are increased in active BD patients. Up regulated miR-145, miR-19b, miR-17 may be involved in BD pathogenesis by targeting cytokine signalling. Inflammatory cytokines are also closely implicated in the evolution of BD. Consequently, diagnostic and therapeutic implications of miRNAs and cytokines involved in BD will contribute to providing information for clinical effects.

P.01-061-Mon
NAD-RNA and its function in virus-infected cells

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In recent years, only a few new RNA modifications have been discovered, one of them is nicotinamide-adenine dinucleotide (NAD) found in prokaryotes, Saccharomyces cerevisiae, and human cells. It was proved that NAD is covalently bound to the small regulatory RNA and short fragments of processed mRNA in prokaryotic cells. In Saccharomyces and human cells, NAD was found to be attached to a broad spectrum of mRNA and snoRNA. Nevertheless, its function remains unknown. Thought the experiments showed that the presence of NAD at the 5' end of the RNA increased its stability in prokaryotes, in human cells the NAD-RNA was degraded more rapidly compared to RNA without a classical cap. We envisaged that the viruses, thanks to their simple intrinsic structure, can serve as suitable model systems for the understanding of the roles of various RNA modifications such as NAD. To reveal the role of NAD-RNA, we focus on small RNA isolated from the cells infected by two types of viruses: HBV and HIV-1. Infected and non-infected cells were harvested and fractions of short RNA were isolated. Afterwards, we applied the NAD captureSeq protocol on isolated RNA to identify the RNA molecules with attached NAD.

P.01-062-Tue
mRNA 5′ UTR modulates positive effect of incorporated m5C and N1 mΨ nucleotides on mRNA expression

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mRNA transfection is considered to be a promising approach for gene delivery due to minimal hazards of genome modification and no dependence on cell proliferation status. It may be successfully applied for stem cell reprogramming, vaccination and substitutional therapy. However, *in vitro* transcribed mRNAs

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activate the innate immune response, complicating transfection of primary cells. Incorporation of modified nucleotides into a transcript can both decrease the immune response and improve mRNA stability. However, published data on the effects of nucleotide modifications on protein production are controversial. Here, we examined the effects of two modified nucleosides, N^1-methylpseudouridine and 5-methylcytidine, within a luciferase transcript, as well as three 5' cap variants, on reporter mRNA expression in several *in vitro* and *in vivo* systems. We compared efficiency and kinetics of regular and modified transcript translation in cell extracts, immortalized cultured cells, and mouse primary hepatocytes and fibroblasts. All mRNAs bearing m^7G-cap, both with and without m^5C and N^1 mΨ, efficiently produced luciferase for up to 10–12 h in living cells and for 1–2 h in cell-free systems. The effects of mRNA modifications on translation efficiency differed for the systems used. For modified mRNAs, we documented a delay in product appearance, indicating a decrease in elongation rate that was especially pronounced in the case of m^5C containing transcripts. By using reporter mRNAs with various 5' untranslated regions, we showed that the modified nucleotides within mRNA leader negatively affect translation initiation, presumably due to interference with ribosomal scanning. Thus, 5' leaders with poor CU content may be preferred for therapeutic applications. The work was supported by the grant of the Russian Federation government № 14.W03.31.0012.

P.01-063-Wed
Various G-quadruplex structures of the human telomere sequence studied by circular dichroism and Raman spectroscopies

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In this work, we combine conventional CD measurements with Raman spectroscopy (RS) to probe solution structures adopted by the 22-mer DNA sequence of $AG_3(TTAG_3)_3$ (wild type, referred to as wt hereinafter) derived from the human telomere repeat region as well as its abasic modification, in which adenine in one loop at the position 19 (ap19) or three loop adenines at the positions 7, 13 and 19 (ap7,13,19) were removed. As shown by us, CD spectra unambiguously differentiate between quadruplex structures adopted by wt, ap19 and ap7,13,19 in the presence of different stabilizing cations (Na^+ or K^+), at different ionic strengths and temperatures. Here we aim to provide additional and complementary information to the earlier CD studies by means of RS. As demonstrated by several researchers over the last five decades, conventional non-resonant RS is highly sensitive to DNA structure and its conformational transitions induced by various physicochemical parameters. We have already shown that RS can be used to monitor transition from antiparallel to parallel G-quadruplex folding induced by increasing oligonucleotide concentration. In the present study, Raman spectra of wt, ap19 and ap7,13,19 quadruplexes stabilized by Na^+ and K^+ cations at rather high oligonucleotide concentrations (~ 1 mM) have been compared with those measured in the absence of stabilizing cations. The greatest difference was observed between the Raman spectra of ap7,13,19 and those of the other two sequences, wt and ap19, reflecting the fact that ap7,13,19 forms a predominantly parallel quadruplex, whereas wt and ap19 seem to adopt mixture of parallel/antiparallel alignment. Specific Raman markers also reflect further increase in the extent of parallel folding of ap7,13,19 with increasing temperature (max. at ~50°C), as well as thermal denaturation of all three quadruplex structures above this temperature. This work was supported the project SYMBIT reg. number: CZ.02.1.01/0.0/0.0/15 003/0000477

P.01-065-Tue
Identification of RNA secondary structure in *Mycoplasma gallisepticum* living cells

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Mycoplasma gallisepticum is a representative of the class Mollicutes. It is characterized by lack of cell wall and many metabolic pathways, reduced genome size and loss of many of the known regulators. Due to this, genome-wide profiling of RNA secondary structure may be a useful tool for studying the transcriptome of the cell and its regulation. To determine the RNA secondary structure *in vivo*, the method of chemical probing with dimethyl sulphate (DMS) with further detection of modification sites by high-throughput sequencing was chosen. We optimized conditions for modification of *M. gallisepticum* RNA by DMS. Then we developed a method of obtaining strand-specific cDNA library for further high-throughput sequencing. Samples of interest were sequenced and analyzed using a number of bioinformatics tools. Quality of identified RNA secondary structures was checked by comparing our data with the predicted conservative structure of RNase P. Genome-wide RNA secondary structure map at nucleotide resolution with quantitative structural information was created. Differences in structure for genes and antisense RNA and for coding sequence, intergenic regions, 5'UTR and 3'UTR were determined. This work was supported by Russian Science Foundation 14–24–00159 "Systems research of minimal cell on a *Mycoplasma gallisepticum* model".

P.01-066-Wed
Mutation m14441T>C in ND6 gene of mitochondrial DNA inhibits antioxidant response in fibroblasts of patients with Leigh-like syndrome

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Mitochondria have the key role in energy production. Mutations in mitochondrial DNA (mtDNA) can lead to hereditary mitochondrial disorders connected to disturbances in oxidative phosphorylation and metabolic pathways. Dying cells emit DNA that becomes part of the oxidized cell-free DNA (cfDNAoxy) pool. CfDNAoxy as well as irradiation dose of 10 cGy can induce oxidative stress in healthy donors' fibroblasts by increasing ROS production, activating NRF2-signaling pathway. NRF2 activation is an adaptive response to endogenous and exogenous oxidative stress and it leads to resistance of cells towards exposure to damaging and toxic agents. The significance of mutations m14441T>C in ND6 and m.3945C>A in ND1 in mtDNA of two patients with Leigh-like syndrome (in both cases heteroplasmy was 100%) on the mitochondrial function under cfDNAoxy treatment or irradiation with 10 cGy was assessed. It was shown that mitochondrial potential is decreased 60–80% in fibroblasts with mutation in the ND6 gene compared to healthy donors. It

decreases 2–4 times ($P < 0.01$) when cells are treated with cfDNAoxy or irradiated with 10 cGy. In fibroblasts of healthy donors and in case of mutation in ND1 gene mitochondrial potential increases when cells are treated with cfDNAoxy or irradiated with 10 cGy. In fibroblasts of healthy donors and in case of mutation in ND1 gene expression of NRF2 is decreased when cells are treated with cfDNAoxy or irradiated with 10 cGy. Cells with mutation in ND1 gene, however have a 50% decrease in NRF2 level. Mutation in m14441T>C causes a decrease in NRF2 levels 5–8-fold and NRF2 protein 3–4-fold ($P < 0.01$). Thus, m14441T>C in ND6 of mtDNA significantly influences signaling pathways involved in the response of cells to oxidative stress. This work was supported by RFBR grant No. 16-04-01099_A.

P.01-067-Mon
Single-stranded DNA aptamers to *Mycobacterium tuberculosis* RNA polymerase

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Mycobacterium tuberculosis is a human pathogen with a highly regulated transcription machinery which is composed of the RNA polymerase core enzyme (RNAP), the major sigma subunit (sigmaA) and 12 alternative sigma subunits. RbpA is an essential transcription regulator, which is found only in Actinobacteria and is absent in other species including *Escherichia coli*. Alternative sigma subunits and RbpA are implicated in stress response and antibiotics resistance. The mechanisms of promoter recognition by alternative forms of RNAP holoenzyme, and the role of RbpA in this process remain only partially understood, in part due to the low growth rate and high pathogenicity of *M. tuberculosis*. To uncover the mechanisms of specific transcription initiation we used an *in vitro* transcription system with purified RNAP and transcription factors of *M. tuberculosis* in combination with analysis of specific single-stranded DNA aptamers obtained by *in vitro* selection. We obtained ssDNA aptamers to RNAPs containing either sigmaA or sigmaB subunits in the presence and in the absence of the RbpA factor. We showed that the aptamers are specifically recognized by RNAP, with apparent dissociation constants of the aptamer-holoenzyme complexes being in the nanomolar range. We identified extended -10-like promoter elements in aptamers to both sigmaA or sigmaB holoenzyme forms. Some aptamers did not contain known promoter elements suggesting that additional as yet unidentified elements may contribute to promoter recognition by mycobacterial RNAP. Finally, we demonstrated that the aptamers sense RNAP interactions with the sigma subunits or RbpA, and RbpA can strongly stimulate the binding of some of them to the RNAP holoenzyme. Thus, the aptamers may provide a useful tool for analysis of RNAP interactions with promoters, transcription factors and antibiotics. The work was supported in part by the Russian Foundation for Basic Research (grant 17-54-150009) and PRC Russie CNRS/RFBR.

P.01-068-Tue
Guanine quadruplexes in the oct4 gene promoter

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Oct4 (POU5f1) gene codes the key regulator of cellular pluripotency. Recently, we have described a guanine quadruplex motif in close proximity to the *oct4* transcription start site (TSS) and we confirmed its influence on the regulation of the *oct4* gene expression. Guanine quadruplexes (G4) are non-canonical secondary structures of nucleic acids, based on several stacked square-shaped planar tetrads; each formed by four Hoogsteen-hydrogen bonded guanines, and stabilized by monovalent cations in central cavity. Our recent bioinformatics analysis revealed seven other potential quadruplex-forming motifs (PQS) within the -5000 to 500 bp range around the mouse oct4 TSS, some of them located within the highly conserved regulatory regions of the *oct-4* gene, including the proximal and distal enhancers. We characterized these PQS in terms of overall topology and thermal stability by circular dichroism and UV absorption spectroscopy and gel methods. Out of the eight selected sequences, six form monomolecular, i.e. relevant G4 with melting temperature higher than 50°C, two exceeding 80°C, under physiological salt concentrations. Interestingly, we observed that the folding of the quadruplexes ranged from pure antiparallel G4 through hybrid forms to pure parallel ones; as shown before, each quadruplex type might have significantly distinct interacting partners and this variability might be broadened by the variability of the loops. Our results indicate potential biological regulatory role of some of the quadruplexes and offer solid base for consecutive in cell studies involving functional assays as well as detection of G4 within the cells. This work was supported by the CSF (17-19170Y) and by the project SYMBIT reg. nr. CZ.02.1.01/0.0/0.0/15 003/0000477 financed by the ERDF.

P.01-069-Wed
Oxidized cell-free DNA causes a short-term activation of TLR9-signaling pathway in MSCs

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Low-dose ionizing radiation (LDIR) causes cell death of a fraction of population in mesenchymal stem cells in vitro, which leads to release of oxidized cell-free DNA (cfDNA). Oxidized cfDNA can cause adaptive response to LDIR in MSC. We have shown that oxidized cfDNA like LDIR induces ROS production, DNA oxidation, DNA damage, cell-cycle arrest, activation of reparation processes, antioxidant response and apoptosis inhibition in MSCs. Could TLR9-signaling pathway play a significant role in protection of MSCs from apoptosis? To answer this question, cells were treated with oxidized DNA (300 mM H_2O_2/Fe^{2+}/EDTA) in concentration 50 ng/mL. The level of oxidation marker 8-oxodG in the obtained DNA was measured with ESI-MS/MS and was ~ 400 8-oxodG per 10^6 nucleotides. This oxidized DNA causes a 3–4-fold increase in TLR9 mRNA level within an hour after addition. Three hours later the mRNA level returns to control levels. Protein TLR9 levels increase 2–3-fold 1 h after start of incubation and decrease to control levels 3 h after.

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Unoxidized DNA doesn't affect TLR9 mRNA and protein levels. Gene expression levels of adaptors and effectors of TLR9-signaling pathway change in response to addition of oxidized DNA: after 1 h mRNA levels of MYD88, TIRAP, HSPD1, MAP2K3 increase 3- 4 -fold, levels of TICAM2, SARM1, TOLLIP, HRAS, HSPA1A and MAPK8IP3 increase 2–3-fold, there is a 1.5-fold increase in the level of expression of PELI1 and RIPK2. Expression of genes EIF2AK2, PPARA increases 3- 4-fold, genes TRAF6, FADD, IRAK1 and IRAK2 2-fold. 3 h after start of incubation levels of these genes return to control. Blocking TLR9-signaling pathway with ODN 2088 (TCCTGGCGGGG AAGT) and chloroquine in combination with oxidized DNA did not lead to increase in the expression of these genes. Thus, oxidized cfDNA causes a short-term activation of TLR9-signaling pathway in MSCs. This work was supported by RFBR grant No. 16-04-01099 _ A.

P.01-070-Mon
Folding rules of intercalated cytosine tetraplexes based on genome-derived C-rich DNA fragments

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Intercalated cytosine tetraplex (i-motif, iM) is a four stranded DNA structure which is adopted by cytosine-rich regions in DNA at slightly acidic pH. This structure is formed by two parallel duplexes bound by hemi-protonated cytosine base pairs ($C{\cdot}C^+$), which are mutually intercalated in antiparallel fashion. iM structure formation and stability depends on: 1) the number and lengths of cytosine stretches, 2) number and sequence of non-C nucleotides between cytosine stretches forming iM loops and 3) pH value. Using electronic circular dichroism (CD), UV absorption spectroscopy and electrophoretic methods, we have examined iM formation and stability of the four C_3TAA repeats of the human telomere DNA sequence and of its modified analogues with substituted cytosines at selected positions. The obtained results have enabled us to formulate simple rules for the manner of iM folding: At least four alternating $C{\cdot}C^+$ pairs are needed to form intramolecular iM structure; one or two missing terminal Cs in different repeats decrease the iM thermal stability, but the intramolecular folding is preserved. Loss of the middle C in the repeat hinders the intramolecular iM folding and instead a bimolecular iM is formed. The bimolecular iM consists of two iM parts. In this case, even mere three $C{\cdot}C^+$ pairs in one part, probably stacked on the intact six $C{\cdot}C^+$ pairs of the second part, suffice to maintain bimolecular iM stable. To test the robustness of these rules they were applied to C-rich sequences derived from the promoter of mouse *oct4* gene that have irregular numbers of cytosines in the block, as well as, variable primary sequence and length of the loop. The behaviour of iMs formed by such a complex sequences seem to be more complicated and cannot be fully followed by these simple rules. This work was supported by the CSF (17-19170Y) and by the project SYMBIT reg. nr. CZ.02.1.01/0.0/0.0/15 003/0000477 financed by the ERDF.

P.01-071-Tue
Catalytically active Argonaute proteins from mesophilic bacteria

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Argonaute proteins are an integral part of eukaryotic RNA interference machinery. They bind small noncoding RNAs and utilize them for guided cleavage of complementary RNA targets or indirect gene silencing by recruiting additional factors. Argonaute proteins are also encoded in many bacterial and archaeal genomes (pAgos). pAgos from thermophilic bacteria were initially studied to gain structural insight into eukaryotic RNA interference. They were later shown to cleave DNA substrates in a guided manner employing small RNAs or DNAs, which appear to be generated autonomously by pAgos. Thus, pAgos might be considered as means of prokaryotic defense against invasive genetic elements. Here we characterize pAgos from non-cultivable or pathogenic mesophilic bacteria. Candidate proteins were selected through bioinformatic screening of genomic databases. Corresponding pAgo genes were chemically synthesized and used for expression in a heterologous system. Upon expression in *E. coli* these proteins have been shown to associate with short (14–25 nt) 5'-phosphorylated DNA molecules. Such short DNA loading relies on the catalytic activity of pAgos and is abolished in catalytically dead protein variants, which bear amino acid substitutions in the DEDX catalytic tetrad. Further *in vitro* assays have shown that purified pAgos cleave various DNA substrates in a guide-dependent manner. They display high activity at temperatures ranging from 30 to 45 °C, with the efficiency of cleavage being greatly affected by ionic strength, supplied divalent cations and guide molecules. This suggests that all studied pAgos act as DNA-dependent DNA nucleases which may subsequently be used as means of targeted genome editing in eukaryotic organisms. This work was supported in part by the Grant of the Ministry of Education and Science of Russian Federation 14.W03.31.0007.

P.01-072-Wed
The role of *Drosophila* CPEB protein Orb2 in regulation of localized translation

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mRNA localization is a phenomenon important for development and functioning of polarized cells. Multiple localized mRNAs carry motifs at their 3' UTRs called cytoplasmic polyadenylation elements (CPEs). These elements are recognized by a highly conserved family of RNA binding proteins, cytoplasmic polyadenylation element binding proteins (CPEBs). The CPEBs belong to regulatory switch proteins that have dual functions either to repress or to activate translation. Orb2 is one of the CPEB proteins in *Drosophila*. Previous studies have revealed its functions in asymmetric cell division, viability, motor function, learning, and memory in *Drosophila*. Recent experiments indicated that Orb2 regulates its own expression in at least two different contexts, in the nervous system in the processes of learning and memory formation and in the testis in the spermatogenesis. To

study Orb2 functions in more details we have generated novel allele *orb2*^{R} using CRISPR/Cas9 approach. *orb2*^{R} carries 3'-UTR deletion, thus it is lacking CPE elements what disrupts its autoregulation. *orb2*^{R} has little effect on viability; however, *orb2*^{R} males are sterile. Confocal studies of testis suggest that malfunction of spermatogenesis is a consequence of sperm differentiation defects. The defects are due to the failure of spermatid nuclei bundling and condensation at the final stages of sperm maturation. Orb2 could regulate expression of genes involved in assembly of the individualization complex. Preliminary experiments indicated that *orb2*^{R} flies has CNS malfunction as well. A plausible interpretation of our current results is that Orb2 negatively regulates its own expression in the CNS while it positively regulates its own expression in testes. Using different approaches, we are testing these hypotheses. This study was supported by the Russian Foundation for Basic Research under Grant 16-34-60214.

P.01-073-Mon
Mega-Stokes system of fluorophores for contrast imaging of non-coding RNA in living cells

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Previously we developed novel light-up molecular beacon probes labeled with excimer-forming pyrene pairs. We demonstrated a possibility of fine-tuning fluorescent properties of excimer by varying the composition of multi-pyrene labels, e.g., excimer-to-monomer ratio, maximum of excimer fluorescence and intensity of fluorescence. These valuable features pave the way for a design of excimer molecular beacons with a tuned fluorescence, acting as a donor in various FRET applications in hybridization assays. We propose a system of fluorescent DNA probes with improved photophysical characteristics for visualization of endogenous non-coding RNA-targets. The contrast of fluorescent images is realized by increasing the distance between the excitation maximum and the emission of fluorescence. Mega-Stokes fluorescence shift (200–250 nm) is realized by using a developed previously pyrene excimers as Förster donors and Cy3 as an acceptor. Complementary sequence of targeted RNA is divided into two DNA-probes: excimer molecular beacon and adjacent linear probe with the acceptor-Cy3. The current set of probes with extra-large Stokes shift allows to reduce background of cell autofluorescence and the residual fluorescence of the unhybridized probes by separation of excitation and emission windows in the spectral range. We successfully applied designed set of probes to detect intracellular lncRNA in living cells which demonstrated increased signal-to-background ratio compare to linear probes labeled with a single fluorophore as well as molecular beacons. In this manner, we reached decreasing of a false-positive signal, which in common caused by unspecific binding and denaturation of molecular beacons as well as insufficient wash steps. This scientific work was supported by Grant RFBR no. 17-32-80061.

P.01-074-Tue
Functional activities of DNA-guided and RNA-guided bacterial Argonaute proteins

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Specific targeting of nucleic acids by Argonaute (Ago) proteins lies at the heart of RNA interference. Eukaryotic Ago's bind small RNAs and use them as guides for target RNA recognition and cleavage. Argonaute proteins are also found in bacterial and archaeal genomes where their roles remain unclear. Structural and biochemical studies of a few prokaryotic Ago proteins showed that they can function as endonucleases *in vitro* and may provide cell defense against foreign genetic elements *in vivo*. However, most prokaryotic Ago's are predicted to lack endonuclease activity; they also often have unusual domain architectures and are associated in the same operons with putative nucleases or helicases. In this study, we focused on prokaryotic Ago's from several cultivable bacterial species. We showed that although eukaryotic Ago's work only with RNA, prokaryotic proteins included in our analysis can use either RNA or DNA guides to recognize DNA targets. To define the specificity of bacterial Ago's we expressed them in *Escherichia coli* and analyzed associated short nucleic acids. Furthermore, we tested nucleolytic activity of three proteins *in vitro* using different guide and target molecules, and also measured the affinity of Ago's to guides and targets. The slicer activity of Ago's is known to depend on four conserved amino acid residues in the catalytic center. We showed that, in agreement with bioinformatic predictions, two of the three proteins possess the endonuclease activity. Our results indicate that bacterial Ago proteins can cleave target nucleic acids with high specificity *in vitro* and can function in the heterologous *E. coli* system *in vivo*. These properties likely underlie the process of DNA/RNA interference in bacterial cells and may promote horizontal transfer of the Ago genes. This work was supported by the Grant of the Ministry of Education and Science of Russian Federation 14.W03.31.0007.

P.01-075-Wed
Roles of sigma finger and DksA in transcription initiation by *Escherichia coli* RNA polymerase containing alternative sigma factors

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In bacteria, the process of transcription is conducted by a single RNA polymerase (RNAP) that relies on specialized sigma factors for specific transcription initiation. *Escherichia coli* contains one principal (sigma 70) and six alternative sigma factors, which are responsible for gene expression under various growth conditions. We previously demonstrated that a flexible "sigma finger" formed by conserved region 3.2 in sigma 70 is essential for transcription initiation; in promoter complexes, it directly binds the template DNA strand upstream of the RNAP active site, thus promoting the binding of initiating NTPs and short RNA transcripts. However, the role of sigma finger during transcription initiation by

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RNAP holoenzymes containing alternative sigma factors remains unknown. In this work, we studied transcription by RNAPs containing wild-type or mutant sigma 38, 32 and 28 subunits. We showed that deletion of region 3.2 in sigma 38 has relatively mild effects on transcription initiation, while corresponding deletions in sigma 32 and 28 significantly increase the stability of RNAP-promoter complexes. Thus, sigma finger likely plays an important role in the regulation of transcription activities of these holoenzyme forms. During stringent response, transcription factor DksA cooperates with the small alarmone ppGpp to suppress the activity of many bacterial promoters recognized by the sigma 70 holoenzyme, by decreasing promoter complex stability. Surprisingly, DksA was shown to enhance the stability of promoter complexes formed by sigma 32, 28 and 24 RNAP holoenzymes. The effects of DksA on the structure of promoter complexes formed by these RNAPs were analyzed by footprinting methods. The results suggest that DksA may play an important and unexpected role in the regulation of activities of alternative forms of RNAP holoenzyme under stress conditions. This work was supported in part by the Russian Foundation for Basic Research (grants 17-04-02133 and 16-34-60237).

P.01-076-Mon
Highly specific target recognition by an Argonaute protein from *Rhodobacter sphaeroides*

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Small noncoding RNAs play essential roles in genetic regulation in eukaryotic cells. In particular, several classes of small RNAs associate with proteins from the Argonaute (Ago) family to suppress the expression of complementary mRNA targets. Many bacteria also have Ago proteins, whose functions and the mechanism of action are poorly understood. Homology of prokaryotic Ago's with eukaryotic proteins makes them a promising model for structural and functional studies of the interference mechanisms. In our work we focused on the Ago protein from *Rhodobacter sphaeroides* (RsAgo) that was recently shown to use small RNA guides for targeting DNA. We demonstrated that RsAgo binds guide RNA (gRNA) with high affinity and found a single amino acid residue that participates in the specific recognition of the guide 5'-nucleotide (U). We also measured the affinity of the RsAgo-gRNA complex to single-stranded target DNAs (tDNAs) with either full complementarity to the guide or with mismatches at each position. We found that fully complementary tDNA is bound with very high affinity, while mismatches both in the seed region close to the guide 5' end and in the downstream guide part significantly decrease target binding. Complexes of RsAgo with only gRNA or with gRNA and complementary tDNA are stable and reveal very limited exchange of nucleic acids over time. At the same time, we found that mismatches between gRNA and tDNA lead to dissociation of the guide-target duplex from the RsAgo protein. We propose that this may serve as a mechanism for nucleic acid exchange in the cell, promoted by Ago interactions with non cognate targets. Our results reveal how complementarity between the guide and target strands affect the efficiency of target recognition and suggest a potential mechanism for guide release and Ago recycling. This work was supported in part by the Russian Science Foundation (grant 16-14-10377).

P.01-077-Tue
Interaction of mismatch repair proteins and G-quadruplexes in DNA

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G-quadruplexes in DNA (G4) have recently gravitated the attention of researches. Being widespread in genomes, especially in promoter regions and telomeres, they perform regulatory functions supporting vital cell processes. However, G4 DNA is supposed to contribute to genome instability alongside. Mismatch repair system (MMR) is among mechanisms summoned to cope with DNA lesions. Latter evidence of interaction between G4 and one of key MMR proteins MutS has revealed significant differences in binding mode as compared to specific MutS substrate – DNA with a mismatch. Nevertheless, additional information is required to comprehend the actual role and mechanism of interaction between MutS and G4. Moreover, there is no available data on the interaction between G4 and other MMR proteins – MutL and MutH. In order to investigate the influence of G4 on the initial steps of MMR, we suggested DNA model system that included the motif of parallel intramolecular G4 flanked with duplex regions carrying MutH recognition site and GT-mismatch. The presence of G4 structure in conditions employed in further experiments was confirmed by dimethylsulphate footprinting and ^{1}H NMR. Applying gel mobility shift assay we investigated the binding of MutS from *E. coli* to G4 DNA in conditions providing different MutS conformations. Not significant in the presence of ADP, the difference between MutS affinity to G4 and other DNA molecules greatly increased upon ATP or ATPγS addition. Thereafter the proposed G4 DNA were subjected to hydrolysis induced by *E. coli* MutH. The hydrolysis efficiency in the presence of protein cofactors (*E. coli* MutS and MutL) was demonstrated to be not dependent on G4 presence in DNA duplex. Hence, in this work for the first time the interaction between G4 and MMR as a whole was investigated, yet the influence of G4 DNA on MMR functioning is still not obvious. *This work was supported by RFBR grants (N 18-34-00768, N 16-04-00575).*

P.01-078-Wed
Stringent mutations in *Escherichia coli* RNA polymerase do not affect translesion RNA synthesis

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Bacterial RNA polymerase (RNAP) is the central enzyme in gene expression that is not only involved in RNA synthesis but also coordinates transcription with translation, DNA replication and repair. Accordingly, many mutations in RNAP were shown to have pleiotropic phenotypes and to have profound effects on other genetic processes. In particular, one class of RNAP mutations suppress defects in the stringent response system responsible for changes in gene expression during stationary phase or starvation. Interestingly, the stringent RNAP mutations also suppress mutations in many factors involved in DNA repair. It was therefore suggested that these mutations may affect the ability of RNAP to transcribe damaged DNA, and may also decrease the

stability of transcription complexes and thus facilitate their dislodging by DNA repair and replication factors. We obtained a series of mutations of this class in *Escherichia coli* RNAP and analyzed their transcription properties *in vitro*. We found that most of the mutations destabilize promoter complexes of RNAP. At the same time, they do not greatly affect the rate of transcription elongation, although certain amino acid substitutions significantly stimulate site-specific transcriptional pauses. Interestingly, none of the mutations changed the ability of RNAP to transcribe DNA templates containing various types of damaged nucleotides, including AP-sites, oxidized and alkylated bases, and thymine-thymine dimers. Furthermore, the mutant RNAPs did not reveal increased sensitivity to the Mfd protein that couples transcription to DNA repair. Thus, we propose that the stringent phenotypes of the mutations are likely explained by their effects on transcription complex stability. Since most of the mutated residues flank the DNA binding channel of RNAP, these effects may likely result from changes in RNAP-DNA contacts at various steps of RNA synthesis. This work was supported in part by the Russian Science Foundation (grant 17-14-01393).

P.01-079-Mon
Regulation of the activity of ribosomal RNA promoters by region 3.2 of the RNA polymerase sigma subunit and DksA

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Ribosomal RNA promoters are the most active bacterial promoters during exponential phase of growth but their activity is silenced during stationary phase. The rRNA promoters form intrinsically unstable complexes with RNA polymerase, which is required for efficient transcription initiation from these promoters. In *Escherichia coli*, regulation of the rRNA synthesis was shown to depend on the action of transcription factor DksA, which binds RNA polymerase in the secondary channel and together with ppGpp dramatically decreases the stability of rRNA promoter complexes. We demonstrate that the low stability of rRNA promoter complexes depends on region 3.2 of the RNA polymerase sigma subunit that directly interacts with the template DNA strand in the open promoter complex. Mutations in region 3.2 significantly increase promoter complex stability, thus making transcription initiation less sensitive to the action of DksA and ppGpp both *in vitro* and *in vivo*. Not only the stability of promoter complexes is increased but also the apparent affinity of DksA is decreased, suggesting the existence of an allosteric pathway connecting sigma region 3.2 with the binding site of DksA. Together, our data reveal an unexpected negative role of the sigma subunit in promoter complex formation. The unstable complexes thus become a target for highly sensitive regulation during bacterial stress response. This work was supported in part by the Russian Science Foundation (16-14-10377) and Russian Foundation for Basic Research (17-54-150009).

P.01-080-Tue
Evaluation of DNA isolation to the sensitivity of molecular diagnostics of Cms bacteria

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Molecular diagnostics of bacteria isolated from an environment is often difficult because of different factors and contaminants presented in the environmental samples. Diagnostics of *Clavibacter michiganensis* ssp. *sepedonicus,* the causal agent of quarantine disease called as ring rot of potato is particularly difficult. In the latent infection Cms bacteria occurs at low concentration in the potato tissue and often without causing symptoms on plants. In addition the gram-positive bacteria it is characterized by the presence of a cell wall with exopolysaccharide coat, which more or less surrounds the surface of the tested bacterial cells and can make the identification difficult. One of the most important factors enabling effective molecular diagnostics of Cms bacteria is the selection of an effective method of DNA isolation that allows to obtain a high sensitivity of the detection method. Therefore, the aim of the presented research was to develop and evaluate the effectiveness of the DNA isolation method used in the diagnosis of Cms strains with different degree of mucous and evaluation of the influence of bacterial mucous on the sensitivity of the PCR assay. The studies were carried out using different mucoid Cms strains. In the first step, the bacterial suspensions were standardized spectrophotometrically and after obtaining the bacterial colonies growing on the microbiological medium, the working suspensions 10^8 cfu/mL (colony forming units per ml) for each strain were prepared. Than the suspensions series with a concentration from 10^6 to 10^0 cfu/mL were prepared and used to isolation of bacterial DNA. Because of the high sensitivity of the classical molecular diagnosis methods on the environmental contaminants, the efficiency of the developed isolation method was evaluated using the classical PCR assay.

P.01-081-Wed
Heat shock proteins as a part of response to stress in Chironomidae midges

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Chironomids (non-biting midges) are among the most stress-resistant insects in the world that makes them an interesting model for studying various aspects of response to different kinds of abiotic stress. Acting like molecular chaperons, heat shock proteins play an important role in protection of proteins and the whole cell from adverse abiotic factors, and besides, they are quite conservative in structure and sequence, especially among closely related organisms. In this study we compare the number and activity of HSP-coding genes in larvae of several *Chironomidae* species from different habitats, being exposed to desiccation, heat shock and other kinds of severe stress. Methodology of our study is based on high-throughput DNA and RNA sequencing, *de-novo* genome assembly and further accurate, transcriptome-based prediction of protein-coding regions, therefore the obtained results present reliable platform for understanding molecular evolution of stress-resistance in insects. Detailed analysis of expansion and contraction of HSP-coding genes shows that high ability to withstand stress is not always necessary linked to changes in number of such genes in a genome, while sometimes accommodation to abiotic stress goes on the way of regulation. Together with revealing common functional patterns of response to abiotic stress in different species (i.e. upregulation of HSP20, HSP70 and HSP90-coding genes), we also detect some species-specific stress-induced genes, which, in many cases, do not have any orthologous genes in other species. This work was supported by Russian Science Foundation grant No. 14-44-00022P.

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P.01-082-Mon
Conserved sequences in the Drosophila mod (mdg4) intron promote transcription termination and trans-splicing

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Alternative splicing is a major mechanism for the enhancement of transcriptome and proteome diversity. The most common type of splicing is known as "cis-splicing" which involves the ligation of exons within one pre-mRNA. Unlike typical cis-splicing, trans-splicing joins exons from two separate transcripts to produce chimeric mRNA, which represents a unique case of functional diversification. Trans-splicing is the rare example of splicing. Among higher eukaryotes it was found in insects, at the unphysiological conditions and some pathological processes. The *mod(mdg4)* locus of diptera and lepidoptera is the only evolutionary conserved case of trans-splicing between protein coding exons. In *Drosophila melanogaster mod(mdg4)* produces at least 31 splicing isoforms that share four common 5′-exons and differ in their 3′-ends. Alternative 3′-pre-mRNAs transcribed independently from both DNA strands. We identified conserved RNA sequence critical for trans-splicing using *in vivo* model and confirmed it with CRISPR/Cas9 mediated mutants. We showed that upstream sequences of alternative 3′-exons and distance to them are also critical for efficient trans-splicing. We identified polyadenylation-independent transcription termination after common exons and revealed highly conserved RNA secondary structure required for this. Analysis of common RNA distribution throughout locus reveals enrichment of donor pre-mRNA at the alternative promoters and depletion downstream of termination point. Thus our findings suggest co-transcription mechanism of trans-splicing. The work was supported by the Russian Scientific Foundation (grant No 14-24-00166).

Chromosome structure and chromatin

P.02-001-Mon
Live cell visualization of extra-chromosomal telomeric repeats in human ALT cancer cells

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In about 10% of all cancer cells, telomeric DNA is maintained through a telomerase-independent homologous recombination mechanism known as ALT (for 'Alternative Lengthening of Telomeres'). In these cells, DNA fragments containing the telomeric 5'-TTAGGG-3' repetitive sequence have been described and termed extra-chromosomal telomeric repeats (ECTRs). Although they may provide insight concerning telomere maintenance in telomerase-inactive cancer cells, ECTR functions remain poorly understood. To develop a methodology for visualization of ECTRs in living cancer cells, we have generated a human ALT cell line where telomeric repeats and condensed chromosomes are fluorescently labelled. To label telomeric repeats, a catalytically inactive mutant of the RNA-guided DNA endonuclease CRISPR-associated protein 9 (Cas9) fused to green fluorescent protein (GFP) was stably expressed. Chromosomes were labelled by stable expression of a fusion protein containing the histone H2B and the red fluorescent protein mCherry. Cells simultaneously exhibiting green and red fluorescence were selected by flow cytometry. A guide RNA targeting Cas9 to telomeric repeats was then integrated. Living cells undergoing mitosis were identified under a spinning-disk confocal microscope through their chromosome condensation state and telomeric repeats were visualized as diffraction-limited spots. While most telomeric signals co-localized with chromosome ends, some were extra-chromosomal and possibly corresponded to ECTRs. We have analyzed a preliminary dataset of 51 mitotic cells with telomeric repeats labelled. At least one putative ECTR was found in about 73% of all labelled cells and about 47% had two or more putative ECTRs labelled. Our new methodology opens new avenues towards understanding ECTR behavior and functions. This work was supported by Fundacão para a Ciência e a Tecnologia through the fellowship SFRH/BPD/102323/2014 to J.P.

P.02-002-Tue
OCT4 and NANOG chromatin structure modifications via AhR-regulated Alu transposon modulates developmental and oncogenic processes

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Local chromatin accessibility (CA) defines spatial and temporal gene expression patterns during cell differentiation. This chromatin conformation is modulated by transposable elements (TE), likely because of their ability to bind specific transcription factors (TF) and other associated proteins, becoming insulators and boundaries elements. How these three elements, CA, TEs and TFs are interacting at the molecular level in differentiation processes is largely unknown. By using enhancer blocking assays (EBAs) we report that, in the human genome, three *Alu(s)* retrotransposon located in the flanking regions of pluripotency genes *NANOG* and *OCT4* have potent insulation activity conferred by binding the transcription factor dioxin receptor (AhR) to consensus elements present in the transposon sequence. The analyses of histone marks revealed different methylation patterns of me3H3K4, me3H3K9 and me3H3K27 upon AhR expression and differentiation with retinoic acid (RA). We have used chromosome conformation capture (3C) technique to address long-range physical interactions between the *Alu(s)* flanking *NANOG*. The results obtained showed that the interaction frequency changes drastically with RA differentiation, suggesting the formation of a new chromatin loop. In addition, to extract the protein complex contained in the *NANOG* loop, we used a modification of CRISPR-Cas9 protocol known as enChIP-Cas9, where specific genomic regions are immunoprecipitated with antibody against a tag (FLAG) fused to a catalytically inactive form of Cas9 (dCas9), which is co-expressed with a guide RNA (gRNA) and recognizes endogenous DNA sequence in the genomic region. Then, enChIP-mass spectrometry (enChIP-MS) targeting endogenous loci identified associated proteins. We propose that AhR-regulated *Alu(s)* elements represent evolutionary conserved genome-wide insulators that control developmental, oncogenic or toxicological-dependent processes via physical heterochromatin modifications.

P.02-003-Wed
Active rDNA clusters in human genome possess the hot spots of DSBs and are involved in contacts with different chromosomes

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DNA double-strand breaks (DSBs) are tightly linked with transcription patterns and cancer genomics. Using deep sequencing approach we detected nine hot spots of DSBs (denoted Pleiades) located in human rDNA units that are located inside intergenic spacer (IGS). The profiles of these hot spots coincide with the profiles of γ-H2AX or H2AX, strongly suggesting a high level of *in vivo* breakage inside rDNA genes. Microscopic observations strongly suggest in metaphase chromosomes only some portion of rDNA clusters possess γ-H2AX foci and that all γ-H2AX foci co-localize with UBF-1 binding sites/ The fact strongly suggests that only active rDNA units possess the hot spots of DSBs. Both γ-H2AX and UBF-1 are epigenetically inherited and thus indicate the rDNA units that were active in the previous cell cycle. The profiles of DSBs and γ -H2AX in CD4+ T lymphocytes practically coincide. CD4+ T lymphocytes are resting cells, and thus are not prone to replication stress. It follows that the observed pattern of H2AX and γ -H2AX should be associated only with active transcription inside rDNA genes. The data strongly suggest that the Pleiades are the result of *in vivo* DNA breakage. The 4C (circular chromosome conformation capture) data indicate that the regions of rDNA units, possessing Pleiades, often contact with specific set of chromosomal regions. These regions often also often possess hot spots of DSBs. We assume that the contacts of rDNA clusters with genomic regions which are enriched with DSBs provide a potential for Robertsonian and oncogenic translocations. The study was supported by the grant from Russian Science Foundation No. 18-14-00122. Computer analysis of the ChIP-Seq data was performed under support by the Program of fundamental research for states academics for 2013–2010 years (No. 0103-2014-0005).

P.02-004-Mon
Chromosome number in Ixodes tick cell lines

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The tendency to gain or lose chromosomes is typical for the individual cells within a tick cell lines, similarly to cell lines of another origin. It was shown that *I. scapularis* cell lines IDE1, 2, 8, and 12, as well as ISE5 and 18, had a diploid number of chromosomes, while coincidently some cell subpopulation had the tendency to be aneuploid or tetraploid. The karyotype of *I. ricinus* cell lines for a long time remained unstudied. The aim of our work was to analyze and compare the karyotypes of *I. scapularis* and *I. ricinus* tick cell lines. Methods. Mitotic chromosomes of cell lines ISE6, 18, IRE11 and IRE/CTVM19, 20 were prepared using colchicine treatment and methanol-acetic acid fixation. Flow cytometry analysis of tick cells was performed after ethanol fixation and propidium iodide staining. In the ISE6 line, cells were predominantly diploid (87.4%) with the modal chromosome number 22, 12.6% of cell population were aneuploid. In the ISE18 line, most of the cells were tetraploid (75.0%), while 18.3% and 6.7% of cells were octoploid and aneuploid, respectively. IRE11 cells with the modal chromosome number 22 were diploid (65.5%), with 24.6% being tetraploid, 7.8% being triploid, and 2.1% being aneuploid. In the IRE/CTVM19 line, most of the cells were tetraploid (55.5%), while 34.2% of cell population were aneuploid and 10.3% were octoploid. IRE/CTVM20 cells were predominantly aneuploid (48.8%) with the modal chromosome number 23, part of the cell population were diploid (27.9%) and tetraploid (23.3 %). We verified the results of karyotype analysis by flow cytometry measurements. We found the doubled amount of DNA in predominantly tetraploid tick cell lines in comparison to the cell lines that were predominantly diploid and aneuploid. Passaging of tick cells resulted in karyotype changes in their populations. ISE6 and IRE11 cells were predominantly diploid, ISE18 and IRE/CTVM19 were tetraploid, and IRE/CTVM20 cells were aneuploid.

P.02-005-Tue
One signal stimulates different transcriptional activation mechanisms

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Transcriptional activation is often represented as a "one-step process" that involves the simultaneous recruitment of co-activator proteins, leading to a change in gene status. Using *Drosophila* developmental ecdysone-dependent genes as a model, we demonstrated that activation of transcription is instead a continuous process that consists of a number of steps at which different phases of transcription (initiation or elongation) are stimulated. Thorough evaluation of the behaviour of multiple transcriptional complexes during the early activation process has shown that the pathways by which activation proceeds for different genes may vary considerably, even in response to the same induction signal. RNA polymerase II recruitment is an important step that is involved in one of the pathways. RNA polymerase II recruitment is accompanied by the recruitment of a significant number of transcriptional coactivators as well as slight changes in the chromatin structure. The second pathway involves the stimulation of transcriptional elongation as its key step. The level of coactivator binding to the promoter in this case shows almost no increase, whereas chromatin modification levels change significantly.

P.02-006-Wed
Analysis of protein complexes binding at termini of 50–250-kb forum domains in human cells

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We developed a new approach for the genome-wide profiling of double-strand breaks (DSBs) with 1-bp precision. Using this approach we detected large chromosomal domains (50–250 kb), denoted forum domains, that are delimited by hot spots of DSBs, and contain genes that are expressed much more coordinately than in random domains of the same size (-z->4, *P*-value < 0.0001). The data suggested a strong link between chromosomal breakage and transcription. Previously we detected that PARP1 and HNRNPA2B1 specifically bind at hot spots of DSBs in human cells at termini of forum domains. It was supposed that PARP1 binding might be connected with the suggested mechanism of coordinated expression of genes located in domains delimited by hot spots of DSBs. It is known that PARP1 behaves as a strong regulator of chromatin structure and transcription and is even capable of somatic cell reprogramming. To elucidate whether PARP1 recognizes forum domains termini and recruits different proteins that are also involved in the coordinated

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regulation of genes in a forum domain we used the isolation of such complexes using genome-wide preparation representing the forum domains termini that were immobilize on paramagnetic particles. Our data indicated that the complexes possessing PARP1, HSP90, HSP70, TBP5, EF1A1 and some other proteins bind specifically with forum domains termini. The data are important for understanding the mechanisms of coordinated expression of genes in human chromosomes. The study was supported by the grant from Russian Foundation for Basic Research (No. 18-04-00680). Computer analysis of the proteomics data was performed under support by the Program of fundamental research for states academics for 2013–2010 years (No. 0103-2014-0005).

P.02-007-Mon
PARP1 modulates nucleosome structure in the absence of NAD+

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The mechanisms that guarantee access of transcription factors, polymerases and other effectors to genomic DNA, which is tightly packed into chromatin remain poorly understood. Human poly-ADP-ribose polymerase 1 (PARP1) is a protein sensor of DNA breaks, transcriptional regulator and an enzyme responsible for poly-ADP-ribosylation of numerous proteins, including histones and chromatin-associated proteins. To study the interaction of PARP1 with chromatin, we used three mononucleosomal templates, each containing two fluorescent labels placed into different parts of nucleosomal DNA: either near the entrance/exit of DNA in/from nucleosome or near the nucleosomal dyad. Each nucleosomal template contained a short DNA fragment extending from the nucleosomes and supporting PARP1 binding. spFRET microscopy analysis of these nucleosomes in solution revealed two subpopulations: compact high-FRET nucleosomes (major subpopulation) and low-FRET nucleosomes containing partially uncoiled DNA (minor subpopulation). Binding of PARP1 to nucleosomes in the absence of NAD+ results in formation of a uniform population of complexes that have an intermediate FRET efficiency, indicating global structural reorganization of the entire nucleosomal DNA. Addition of NAD+ results in dissociation of PARP1 from the nucleosomes and the recovery of the nucleosomal structure. The data suggest that PARP1 can increase accessibility of nucleosomal DNA for external probes by reversibly reorganizing nucleosomes. This newly discovered nucleosome structure-modulating activity of PARP1 is independent of its catalytic function and likely plays an important role in PARP1-dependent DNA repair. This study was supported by RFBR grant 17-54-33045.

P.02-008-Tue
Mapping of functional elements in Fab-7 boundary involved in regulation of *Drosophila* hox gene *Abd-B*

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The regulatory region of the *Drosophila* Hox gene *Abd-B* is one of the best models to study mechanisms and proteins involved in organization and regulation of the specific distance enhancer-promoter interaction. Expression of the Abd-B gene in the appropriate parasegment-specific pattern is controlled by *cis*-regulatory domains: *iab*-5 to *iab*-9. Each of these regulatory domains is bracketed by boundary elements. In particular the *Fab-7* boundary is located between the *iab-6* and *iab-7* domains and display two important functions: to block crosstalk between the regulatory domains and to facilitate specific distance interactions between the *iab-6* enhancer and the promoter in order to proper activate *Abd-B* in PS11. The 1.6 kb *Fab-7* boundary includes four chromatin-specific nuclease-hypersensitive sites: HS*, HS1, HS2 and HS3. HS3 is Polycomb Responsible Element (PRE) and responsible for repression of premature activation of the *iab-7* enhancer. Using *attP*-mediated replacement strategy we found that only distal 240 bp part of the HS1 region (dHS1) in combination with PRE can form functional element. The dHS1 or HS3 alone had no activity, while dHS1 in combination with five sites of the architectural protein Pita restored both boundary functions. The Pita sites alone functioned as an insulator that block crosstalk between the *iab-6* and *iab-7* domain / *Abd-B* promoter. Probably that dHS1 functions as the *Abd-B* promoter tethering element that facilitates the specific enhancer-promoter interaction across the insulator. It was shown previously that dHS1 is bound by Late Boundary Complex (LBC) that is essential for boundary functions. ChIP in embryos showed that the Clamp protein, component of LBC, bound to dHS1 only in combination with Pita sites or HS3. These results are in agreement with a model that Pita or HS3 facilitate recruiting of protein complex to dHS1 that can fulfill all boundary functions. The work was supported by the Russian Science Foundation grant N 14-24-00166.

P.02-009-Wed
Antibody analysis supports the G4/IM-synaptic complex structures

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G-quadruplexes (G4s) are noncanonical nucleic acid structures consisting of planar guanine tetrad arrangements. They play important roles in regulation of key cellular processes, such as DNA recombination, replication, transcription and others. Wherever there is a G-quadruplex forming sequence in one strand the complimentary strand contains a C-rich sequence capable of forming an I-motif (IM). Although structures of G4s and IMs themselves are well-characterized, little is known about their behavior in dsDNA. In our previous work we reported AFM studies of DNA duplexes containing $(G_3T)_nG_3$ sequences ($n = 1 \div 5$) in the middle regions. In the case of $n \geq 3$, the middle regions in theory could fold into G4/IM; while in the case of $n = 1, 2$, no intrastrand noncanonical structures are possible. Surprisingly enough, AFM scanning of the duplexes revealed intermolecular G4/IM-synaptic complexes in all cases (the images contained cruciforms and higher order structures). No signs of such complexes were visible in AFM images of the control duplex that lacks the $(G_3T)_nG_3$ middle region. These data confirm our hypotheses about several ways of self-association of non-canonical structures (Varizhuk et al. Biochemistry-Moscow 2017; Protopopova et al. PCCP 2018). Here we used anti-G4-DNA antibody (clone 1H6) to prove existence of G4 structure in our synaptic complex. AFM images show interaction of the antibody with the junctions of cruciforms and higher order structures. These results confirm possible structures of the G4/IM-synaptic complexes elucidated by molecular modeling. Not every DNA-cruciform was recognized by antibody molecules due to possible covering of G4 in the synaptic complex structure.

Collectively, our findings demonstrate that G-rich DNA sites can form G4/IM-synaptic complexes - potentially important factors of chromatin remodeling, recombination, replication, and transcription processes. This work was supported by Russian Science Foundation [14-25-00013].

P.02-010-Mon
The structural and functional analysis of the orc6 protein – Drosophila model of the Meyer-Gorlin syndrome

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The origin recognition complex (ORC) is important for DNA replication in eukaryotic cells. ORC is also involved in other cell functions. The smallest ORC subunit, Orc6, has an active role in both DNA replication and cytokinesis. The structural and functional analyses revealed that Orc6 in eukaryotes has a homology with transcription factor TFIIB. Orc6 has a DNA binding activity on its own and is important for DNA binding of whole six-subunit ORC. We propose that Orc6 may position the whole complex at the origins of DNA replication similar to the role of TFIIB in positioning transcription pre-initiation complex at the promoter. The cytokinesis function of *Drosophila* Orc6 is achieved through its interaction with the septin protein Pnut, which together with Sep1 and Sep2 forms a septin complex, a highly conserved polymerizing protein assembly that is critical for cytokinesis in many species. Septins are weak GTPases that can assemble into large filaments. The binding of Orc6 to Pnut stimulates septin complex filament formation. Mutational analysis revealed that both N-terminal and C-terminal domains of Orc6 are important for this activity of the protein. The C-terminus of Orc6 contains a highly conserved motif important for the interaction of the protein with the core ORC. A mutation found in patients with Meier-Gorlin Syndrome (MGS) maps to the same segment in Orc6, destabilizing the interaction of *Drosophila* and human Orc6 with the core Orc1-5 sub-complex. We have shown that mutations in this region result in reduced DNA replication. In order to study the effects of MGS mutation in animal model system we introduced MGS mutation in Orc6 and established *Drosophila* model of the disease. Obtained flies are unable to fly and display planar cell polarity defects. The availability of the *Drosophila* model of MGS will allow thorough studies revealing crucial and conserved mechanisms of organism development and disease pathogenesis.

P.02-011-Tue
Role of Kaiso in homeostasis regulation

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Kaiso is a member of the BTB/POZ zinc finger family, which is involved in cancer progression, cell cycle regulation, apoptosis and WNT signaling. It regulates methyl-dependent repression of gene transcription by recruiting corepressors N-CoR and SMRT. We showed early that transcriptional properties of Kaiso may depend on its posttranslational modification- SUMOylation, that is changed with the hyperosmotic stress. SUMOylation reverts Kaiso from repressors to activators. Here, we demonstrated how Kaiso modification influences on setting and maintenance of epigenetic marks. By genomic DNA editing, we generated cell line with Kaiso that cannot be modified by SUMO. The presence of non-modified Kaiso in cells results in changes of epigenetic marks on promoters of target genes. Target regions was characterized by depletion of active marks and enrichment with repression marks. On the other hand depletion of Kaiso also led to alteration in epigenetic environment of target gene promoters. We hypothesize that in cellular processes, such as an inflammation, where hyperosmotic conditions are observed, deSUMOylation of Kaiso may lead to stable inactivation of target genes by generating repressive chromatin structures, while Kaiso knockout will have different consequences. Thus, we propose a novel biological role for Kaiso in the regulation of homeostasis. The work was supported by RNF 14-14-01202

P.02-012-Wed
Multiple roles for insulator complex LBC in regulating chromosome architecture in *Drosophila*

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Chromatin structure influences gene expression on a local level by regulating expression of individual genes, and on a global level by changing the activity of large gene domains or entire chromosomes. Examples of global regulation include repression of the fly homeotic gene complexes by PcG silencing and the upregulation X-chromosome gene expression by MSL complexes. Like the formation of local chromatin domains, these global mechanisms depend upon chromosome architectural elements. MSL complexes exploit the pre-existing X-chromosome topological organization. This organization depends special elements called Chromatin Entry Sites (CES) that are distributed along the X-chromosome. CES are boundaries of topological domains. Likewise, a large network of contacts between Polycomb response elements (PREs) in different PcG target loci has been discovered in genome-wide studies. Thus, the MSL and PcG chromatin modifying complexes would appear to share a similar dependence upon chromosome architecture and architectural elements. We find that a large complex, LBC, is responsible for the architectural functions of CES and PREs. The LBC has previously been shown to be required for the architectural functions of insulators. The LBC binds to CES and to PREs. Mutations which disrupt LBC binding *in vitro* abrogate PcG/CES functions *in vivo*. We propose that the architectural functions of LBC provide a scaffold for the efficient recruitment and subsequent spreading of the MSL and PcG complexes, facilitating the epigenetic regulation of transcription. This work was supported by Russian Scientific Foundation grant 16-14-10346 and Russian Foundation for Basic Research grant 16-34-00509 mol_a.

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P.02-013-Mon
Changes in dynamics and kinetics of chromatin binding of proteins from the replication machinery during licensing for DNA replication

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DNA replication is a process that is strictly controlled. One of its most important stages is the licensing of the genetic material for initiation of DNA synthesis, when every origin of replication is "tagged", so that a single activation event can take place per round of the cell division cycle. Some of the key proteins of the replication apparatus in the eukaryotic cells are Claspin, Tim and Tipin, which form a triple complex, that controlled the activity of the MCM helicase, and And1, that stabilizes the DNA polymerase α / primase complex to the Replisome. The evaluation of the kinetic changes of chromatin binding of these harmonization factors during early and late G1-phase, where the chromatin is licensed for replication and the formation of the Initiation complex takes place, can elucidate the intimate mechanisms of control, that aim to execute the dynamic program of DNA synthesis. In our study, by means of modern microscopic techniques, we study the kinetic changes of chromatin binding of key, GFP-tagged, replication proteins - MCM6, PolA2, Claspin and And1 during the early and late G1-phase of the cell cycle.

P.02-014-Tue
Drosophila repression protein Insv is involved in activity of the Fab7 boundary from the bithorax complex

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Insensitive protein (Insv) was first describe as a nuclear corepressor for the Notch transcription factor Suppressor of Hairless (Su (H)). Insv lacks domains of known biochemical function but contains a single BEN domain and negatively regulate some E(spl) complex genes in nervous system of *Drosophila*. The genome-wide mapping of Insv binding sites in embryo have shown that most Insv sites overlap with known fly architectural proteins, including CP190 and CTCF. In particular, Insv binding sites were found in well-studied boundaries Fab-7 and Fab-8 in the bithorax complex. Using in vitro approaches and the yeast two-hybrid assay, we found that Insv directly interacts with CP190. By the series of deletion, we mapped a region in the N-terminus of Insv [135–150] that interacts with the BTB domain of CP190. To determine whether Insv is boundary factor, we used mutations in the Fab-7 boundary that weakly inactivate its function. Combination of the null insv ($insv^{23B}$) mutation with the *Fab-7* mutation dramatically disrupted boundary function. Expression of the wild type Insv protein rescued boundary activity in $insv^{23B}$; *Fab-7* mutant flies. The Insv mutant lacking CP190-interacting domain also completely restored the boundary activity. While our results suggest that the CP190-interacting domain is not essential for Insv function in Fab-7, we found that it is important for recruiting Insv to many sites in polytene chromosomes. Taken together our results suggest that Insv is a new architectural/insulator protein. The work is supported by Russian Science Foundation (grant 14-24-00166).

P.02-015-Wed
Biochemical and structural analysis of Msh4/Msh5 protein complex involved in meiotic crossover formation

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Meiosis is a form of cell division that occurs in sexually reproducing organisms and results in the production of haploid gametes from diploid parental cells. A key process in meiosis is reciprocal homologous recombination, also known as crossing-over. This process not only promotes genome diversification but is also essential to ensure faithful segregation of chromosomes. While it has been established that crossovers are required for error-free gametogenesis, little is known about their formation and regulation. Crossovers are derived from programmed double-strand DNA breaks, but most breaks do not result in crossovers. It has been previously shown that the meiosis-specific ZMM proteins (Zip, Msh, Mer) promote crossover formation in budding yeast, with homologs of these proteins found in many other eukaryotes. In the following poster, I focus on the investigation of the role of two ZMM proteins Msh4 and Msh5. These two proteins form a DNA binding complex, which recognizes double Holliday junctions in an ATP dependent manner and further slides onto the homologous arms of a four-way junction. The Msh4/Msh5 heterodimer potentially stabilizes strand invasion during meiotic homologous recombination. In order to investigate the function of the complex in meiotic recombination process I produced and purified the *Saccharomyces cerevisiae* Msh4/Msh5 complex from insect cells, and then I explored their potential interactions with different proteins and different DNA substrates. Interactions were analyzed *in vitro*, with a view to the high-resolution structural analysis of large complexes. Understanding the mechanisms underlying the regulation of the crossovers formation will further our understanding of the critical process of meiosis and its regulation.

P.02-016-Mon
Control of DNA double-strand break formation in meiosis

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Meiosis is a key step in sexual reproduction, which leads to the formation of haploid gametes from a diploid cell. During genome reduction, the homologous chromosomes are segregated into daughter cells, therefore they have to be physically linked via homologous recombination. The linkage is enabled by repair of programmed double-stranded DNA breaks (DSBs) using the homologous chromosome instead of the sister chromatid. Formation of DSB has to be strictly controlled - too little breaks would not be sufficient to hold homologs together, too many breaks would lead to chromosome destabilization. It is known that DSB formation in *S. cerevisiae* involves regulation by phosphorylation and the assembly and disassembly of the Hop1-Red1-Mek1- and Rec114-Mer2-Mei4-complexes together with the nucleosome binding protein Spp1. However, mechanistic detail of these processes are lacking. We use an *in vitro* reconstitution approach combining biochemistry and structural biology to provide insight into the mechanisms underlying DSB control; both in time and in space. Unravelling DSB control would represent a significant step forward in our understanding of break positioning, genome stability and, ultimately, the critical process of meiosis as a whole.

P.02-017-Tue
Kinetics of DNA repair proteins at complex DNA damage sites

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The integrity of DNA of every cell is constantly challenged by numerous mutagens which generate a variety of DNA lesions, which must be promptly repaired in order to prevent cell cycle arrest, genomic instability, cancer transformation, or apoptosis. Most mutagens are able to generate different types of DNA lesions which are repaired by means of different DNA repair pathways. This imposes the need for precise coordination between different repair pathways which act at the same place in the nucleus. In order to investigate the interplay between DNA repair pathways we measured the kinetics of recruitment and removal of 70 fluorescently-tagged DNA repair proteins to complex DNA damage sites in living HeLa Kyoto cells. We generated DNA damage via UV laser micro-irradiation. A thorough analysis of our data revealed that PCNA and the error-free DNA replication machinery (POLD1, POLD2) were recruited approximately 60 s earlier compared to RAD18 and translesion DNA polymerases DNA polymerase η (POLH) and DNA polymerase κ (POLK). In order for TLS polymerases to bind to PCNA at damage sites it must be monoubiquitinated at K164 by RAD18 E3 ubiquitin ligase. To reveal the reason behind this delay, we investigated the mechanism for direct loading of RAD18 to damage sites and show that its loading is dependent on DSB-dependent polyubiquitination of chromatin. Moreover, suppressing polyubiquitin chain formation strongly precluded the recruitment of POLH to damage sites. This ensures a window of opportunity for error-free DNA repair synthesis before the initiation of error-prone translesion synthesis (TLS). Surprisingly, PARPi treatment abolished the time benefit of error-free over error-prone DNA repair synthesis which could lead to increased mutagenesis during complex DNA damage repair.

P.02-018-Wed
Three-dimensional organization of genome of sleeping chironomid *Polypedilum vanderplanki*

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The most complex and largest anhydrobiotic animal known today – larvae of *Polypedilum vanderplanki*. Fully dehydrated state is a natural phase in the lifecycle of larvae. Such extreme water loss in the body can mean significant changes in chromatin conformation during desiccation. Pv11, a cell line derived from the embryos of *P. vanderplanki*, is also able to endure water loss as larvae. The aim of this work is determining peculiarities of spatial chromatin organization in chironomids and possible alteration of chromatin packaging driven by desiccation. For understanding the character of chromatin interactions during anhydrobiosis, we applied Hi-C method on intact Pv11 cells and cells in transition to anhydrobiotic state by trehalose pretreatment, which has a similar effect as desiccation on larvae. Libraries were prepared according in situ Hi-C protocol, from intact Pv11 cells and cells after 48 h of incubation with trehalose. High level of reproducibility was demonstrated between both replicates for each condition. Two methods were applied to assembly scaffolding using contact frequency data and leads to reduction in the number of scaffolds from hundreds to 4 chromosome-size scaffolds, covered more than 80 percent of genome. Such remarkable improvement of the assembly was demonstrated, and important that it can be achieved, not only in well characterized model insect species, but also in non-model ones, with only partial known genomic data. Comparison between contact matrices for trehalose incubation and control condition shows minor changes in chromatin compaction and TADs boundaries in this locus, especially in regions with high level of expression. Further research of the 3D chromatin organization on whole genome level and its response to influence of adverse factors in stress resistant organisms will be the next step to understanding the anhydrobiosis-survival machinery. This work was supported by Russian Science Foundation grant No. 14-44-00022.

P.02-019-Mon
Analysis of the mechanism of specific recruitment of the Su(Hw)/Mod(mdg4)67.2/CP190 complex to Su(Hw) sites

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The Drosophila Su(Hw) is an example of multifunctional transcription factor, first identified in gypsy retrotransposon. Properties of this DNA binding protein depend on two BTB/POZ domain proteins, Mod(mdg4)-67.2 and CP190. Su(Hw) was first identified for its insulator function, since it is responsible for enhancer blocking. Recent studies revealed that Su(Hw) has a transcriptional activation function, and finally, a repressive function was discovered as well. Su(Hw) complexes interact with each other over a significant distances, as it was demonstrated in the insulator bypass experiments. However, the molecular mechanism of this phenomenon is still poorly understood. We developed a new model, which allows us to reveal particular Su(Hw) complex proteins responsible for this phenomenon. Existing su(Hw) alleles have been identified based on screens for reversal of gypsyinduced mutant phenotypes. su(Hw)E8 mutation produces a full lenghth Su(Hw) protein that is unable to bind DNA. A combination of su(Hw)v and su(Hw)2 produces no Su(Hw) protein. We generated FLAG-tagged Su(Hw) protein with deletion of its N-terminal part. Su(Hw) antibody does not recognize this transgene, since it was generated against the N-terminal part. Such transgene moderately reduced gypsy-induced insulation in su(Hw)v/su(Hw)2 mutant background, while completely restoring it in su(Hw)v/su(Hw)E8 background. This effect of su(Hw)v/su(Hw)E8 was abolished by an additional introduction of mod(mdg4)u1 mutation. Using ChiP assay and polytene chromosome staining, we demonstrated that Su(Hw)E8 protein occupied Su(Hw) binding sites in the presence of Su(Hw)ΔN, and disappeared from them in mod(mdg4)u1 mutation background. We identified selfinteracting domains of Su(Hw), which along with Mod(mdg4)-67.2 protein, may stabilize such interactions. Thus, our model can recapitulate interactions within the Su(Hw) complexes and allow us to verify proteins and their particular domains involved in this phenomenon.

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P.02-020-Tue
A whole-genome map of chromatin-bound RNAs

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The recent studies show that the major part of the eukaryotic genome is transcribed to produce a broad range of RNAs, including both protein-coding and noncoding RNAs (ncRNAs). ncRNAs participate in various biological processes from regulating enzymatic activities to sustaining genome imprinting and nuclear body biogenesis, but the mechanism of action is still unclear for most ncRNAs. Several studies reveal the role of ncRNAs in establishing the spatial organization of chromosomes. The further progress in studying ncRNAs and their functions in chromatin will depend on the availability of the genome wide spectrum of RNA associations with chromosomes, the RNA-DNA interactome. An objective of our work is to develop a new wholegenome method to simultaneously identify the sites of associations with chromosomes for all RNA molecules present in the cell nucleus. The gist of the method, designated Red-C (RNA ends on DNA capture), is adaptermediated RNADNA ligation in fixed nuclei followed by the analysis of the chimeric RNA-DNA molecules using high-throughput sequencing. In pilot experiments we applied Red-C protocol to uncover RNA-DNA interactome of the cultured human erythroleukemia cells (line K562). We studied interaction of various RNA biotypes (lincRNA, antisense RNA, snoRNA, snRNA, piRNA, eRNAs, protein-coding RNA, miRNA, etc.) with different DNA elements such as promoters, enhancers, insulators, gene bodies, transcription factor binding sites, as well as with different types of active and repressed chromatin that can be distinguished based on distribution of epigenetic marks. We also compared the obtained RNA-DNA contact maps with the profiles of genome partitioning into topologically associating domains and A/B spatial compartments. The results of our study demonstrate a possible role of ncRNAs in establishing/mediating the 3D genome organization and in regulating gene expression. This work was supported by the Russian Science Foundation (grant 18-14-00011).

Genome dynamics and epigenetics

P.03-001-Mon
Epigenetic reprogramming by naïve conditions establishes an irreversible state of X chromosome reactivation in human ESCs

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Female human pluripotent stem cells (PSCs) have variable X-chromosome inactivation (XCI) status. One of the X chromosomes may either be inactive (Xi) or display some active state markers. Long-term cultivation of PSCs may lead to an erosion of XCI and partial X reactivation. Such heterogeneity and instability of XCI status might hamper the application of human female PSCs for therapy or disease modeling. We attempted to address XCI heterogeneity by reprogramming human embryonic stem cells (hESCs) to the naïve state. We propagated five hESC lines under naïve culture conditions. PSCs acquired naïve cells characteristics although these changes were not uniform for all of the hESC lines. Transition to the naïve state was accompanied by a loss of XIST expression, loss of Xi H3K27me3 enrichment and a switch in Xi replication synchronously with active X, except for two regions. This pattern of Xi reactivation was observed in all cells in two hESC lines. However, these cell lines were unable to undergo classical XCI upon spontaneous differentiation. We conclude that naïve culture conditions do not resolve the variability in XCI status in female human ESC lines and establish an irreversible heterogeneous pattern of X reactivation.

P.03-002-Tue
The role of SCD1 in thyroid hormone dependent control of epigenetic modification in cardiomyocytes

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The main factors of epigenetic regulation are enzymes involved in alteration of chromatin structure, i.e. DNA methyltransferases (DNMTs), histone methyltransferases (e.g. lysine-specific demethylase 1 [LSD1]), and histone deacetylases (HDACs). Moreover, epigenetic modifications which might be induced by thyroid hormones (TH) action are involved in pathogenesis of several cardiovascular diseases. TH through nuclear TH receptors can modulate the expression of various genes involved in epigenetic control (e.g. DNMT1). Moreover, TH through regulation of protein kinase A (PKA) can rapidly change chromatin methylation level and structure. Furthermore, one of the main enzyme that regulates lipid metabolism, stearoyl-CoA desaturase 1 (SCD1), which gene expression is under TH control, affects the level of DNA methylation in 3T3 adipocytes. Therefore, the aim of the presented study was to investigate the role of SCD1 and TH in regulation of epigenetic modifications in the heart. To induce hyperthyroidism SCD1+/+ and SCD1-/- mice were injected with triiodothyronine (T3). Performed analyses showed, that SCD1 deficiency increased LSD1 and HDAC4 protein content as well as PKA phosphorylation level in cardiomyocytes. Moreover, we observed decreased global DNA methylation level and DNMT1 protein level in SCD1-/- heart. Interestingly, TH caused increase in global DNA methylation level and LSD1 protein level in SCD1-/- heart when compared to WT controls. Furthermore, in hyperthyroidic SCD1-/- cardiomyocytes we observed drop in phosphorylation of PKA, what was correlated with elevated HDAC4 protein level. Therefore, obtained results emphasize the important role of SCD1 expression in epigenetic modifications in the heart and suggest that SCD1 is an important element of response to TH action in the heart. National Science Center (Poland) grant UMO-2014/13/B/NZ4/00199.

P.03-003-Wed
Clarifying the role of DNA methylation in tree phenotypic plasticity

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In a context of global climate change, trees as sessile and long lifespan organisms need to develop mechanisms enabling them to adapt and to survive. These last years, epigenetic mechanisms such as DNA methylation have been proposed as a valuable resource since they can be triggered by the environmental conditions in a reversible-way. However, evidences for their role in tree phenotypic plasticity are still lacking (Bräutigam *et al*., 2013; Plomion *et al*., 2016). In this context, we develop different complementary approaches:

1 A correlative approach with simultaneous analysis of methylome and transcriptome dynamics in the shoot apical meristem (center of shoot morphogenesis) of poplar in various environments (Gourcilleau *et al*., 2010; Lafon-Placette *et al*., 2013; Bastien *et al*., 2015; Le Gac, 2017; Lafon-Placette *et al*., 2017).
2 A reverse genetic approach, using RNAi clones of *Populus tremula x alba* (Zhu *et al*., 2013; Condé *et al*., 2017; Le Gac *et al*., in prep) hypo or hypermethylated and grown under environmental constraints.
3 A population approach, using natural populations from diverse geographic origins to explore microevolutive adaptation to local environment and phenotypic plasticity (Project 'EPITREE' ANR 2018-2021, S. Maury). Our previous data highlight a relationship between DNA methylation in the shoot apical meristem biomass productivity and a possible connection with phytohormone signaling in response to abiotic stress. New data will be also presented concerning the stability of the epigenetic modifications and their genetic diversity in populations. Altogether, our data provide new insights into how trees modulate their epigenomes to ensure developmental plasticity and adaptation in a changing environment.

P.03-005-Tue
Role of stearoyl-CoA desaturase 1 in epigenetic control of pancreatic islet cells identity

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Type 2 diabetes (T2D) is multifactorial disorder characterized by chronic hyperglycemia due to peripheral tissue insulin resistance and progressive loss of pancreatic islets function. Recent studies have shown that the loss of endocrine cells identity may be one of the main reasons for development of pancreatic islets dysfunction in T2D. The maintenance of identity of insulin- and glucagon-secreting cells in pancreatic islets depends on dynamic control of transcription factors (TFs) expression. The gene expression patterns in pancreatic islets have been influenced by fatty acids (FAs) through epigenetic mechanisms. In the present study we tested the hypothesis that stearoyl-CoA desaturase 1 (SCD1), a significant control point in FAs metabolism, affects expression patterns of TFs involved in maintenance of pancreatic islet cells identity via epigenetic modifications. The experiments were carried out in vivo on SCD1 knock-out (SCD1 KO) mice and in vitro on INS-1E β-pancreatic cell line, where lipotoxicity was induced by palmitic acid treatment. Our data show that pancreatic islets of SCD1 KO mice are characterized by different microarchitecture and decreased protein level of TFs which are crucial for maintenance of β-cells identity (Pdx1, FoxO1, Isl1) and increased of those characteristic exclusively for α-cells (glucagon, Arx) comparing with wild type mice. Furthermore, we noticed that inhibition of SCD1 activity as well as silencing of SCD1 gene expression, lead to global DNA hypomethylation and decrease in methyltransferase 1 (Dnmt1) protein level in INS-1E cells. In addition, we also observed significant changes in methylation pattern within promoter regions of identity TFs (Pdx1). Obtained results suggest that SCD1 activity/expression and DNA methylation pattern alterations may lie beneath maintenance of pancreatic islets functional identity. This study was supported by NCN UMO2013/10/E/NZ3/00670 and National Centre NCBR grant STRATEGMED 3/305813/2/NCBR/2017

P.03-006-Wed
Transforming growth factor beta 1 (TGF-b1) treatment as a new in vitro model of osteoarthritis

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Osteoarthritis (OA) is a rheumatic disease characterized by cartilage degradation, inflammation and osteophyte formations. Osteophytes are initiated by the process of chondrocytes hypertrophy. Recent studies showed that blockage of TGF-β1 signaling in cartilage of OA mouse models prevents osteophyte formations. Furthermore, it has been demonstrated *in vivo*, that inhibition of Enhancer of zeste homolog 2 (EZH2), Histone 3 lysine 27 methylase, attenuates hypertrophy. Our study aimed to evaluate the hypertrophic effect of TGF-β1 in human articular chondrocytes and to investigate whether EZH2 inhibition would attenuate TGF-β1-induced hypertrophy *in vitro*. Human primary

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chondrocytes were incubated with TGF-β1 up to 72 h. Hypertrophy was evaluated by Runt-related transcription factor 2 (Runx2), collagen type X (COL10A1), and Vascular endothelial growth factor (VEGF) expression as well as Matrix Metalloproteinases (MMP) 13 expression and release. Cartilage anabolism was investigated by Sox9, aggrecan and type 2 collagen (COL2A1) expression. Glycosaminoglycans (GAGs) synthesis was measured by Blyscan assay. Protein and mRNA expression were analyzed by western blot and RT-PCR, respectively. Histone methylase activity of EZH2 was inhibited using EPZ6438. We found that TGF-β1 decreased dramatically cartilage anabolism as evidenced by the reduction of GAG synthesis as well as COL2A1, aggrecan and Sox9 protein and mRNA expression. At contrary, the expression of hypertrophic genes was increased by TGF-β1. Moreover, as expected, EZH2 inhibition attenuated TGFβ-induced reduction of Sox9, as well as the upregulation of COL10A1 and MMP13. In conclusion, we confirm the involvement of TGF-β1 in the initiation of hypertrophy in chondrocytes, and establish a new OA model. Moreover, we confirm further that EZH2 inhibition counteracts hypertrophy and therefore represents an interesting issue to assess in clinical trials.

P.03-007-Mon
Role of MSL2 domains in specific recruiting of the dosage compensation complex on the X chromosome in *Drosophila* melanogaster

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The Male-Specific Lethal (MSL) complex regulates dosage compensation of the male X chromosome in *Drosophila*. The MSL2 protein is expressed only in males and is a key component of the MSL complex that includes also MSL1, MSL3, MOF and MLE proteins and two lnRNAs named roX1 and roX2. The MSL complex specifically recognizes approximately 200 sites on the X chromosome, named High Affinity Sites (HAS), but mechanism of its recognition remains unknown. Previously it was found that the CLAMP protein binds to HAS, but also many other sites at all chromosomes. It was identified that the CXC domain of the MSL2 protein specifically recognizes HAS. Here we showed direct interaction between CLAMP and MSL2. To study role of different MSL2 domains in recruiting of the MSL complex to the X chromosome, we used previously established model system based on the fact that expression of MSL2 in females results in recruitment of MSL complex to the X chromosome. As a result, we found that the proline-rich domain of MSL2 is required for stimulation of the roX2 promoter. Deletion of proline-rich region in MSL2 prevents roX transcription that results in MSL recruiting only to HAS in females. In males deletion of the proline-rich region in MSL2 does not significantly affect amount of roX RNAs and spreading of the MSL complex along the X chromosome. We also found that inactivation of either CXC-domain or CLAMP interacting domain in MSL2 precludes activation of roX2 and binding of the MSL complex to the X chromosome in females. At the same time, inactivation of the same domains in males only partially affects recruiting of MSL to the X chromosome. The work was supported by the Russian Scientific Foundation grant No. 17-74-20155.

P.03-008-Tue
Interaction of PARP1 and its regulatory protein, YB-1, is modulated by PAR

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Y-box-binding protein 1 (YB-1) is a multifunctional protein involved in a lot of cellular processes. YB-1 is a DNA- and RNA-binding protein. By binding to nucleic acids, YB-1 is engaged in many DNA- and mRNA-dependent processes such as DNA replication and repair, transcription and mRNA translation. Currently, YB-1 is considered as one of non-canonical proteins of base excision repair (BER). YB-1 has an increased affinity to damaged DNA and interacts in vivo and in vitro with several BER proteins, regulating their activities. Recently, it was shown that YB-1 also interacts with poly(ADP-ribose)polymerase 1 (PARP1), the key regulator of BER. PARP1 binding to damaged DNA results in its activation followed by synthesis of nucleic acid-like polymer of ADP-ribose (PAR) originating from NAD^+. As a protein post-translational modification, PAR performs numerous functions in the regulation of BER. The main target of PARylation is PARP1 itself. It was found that PARP1 modify YB-1 with PAR, and YB-1 is able to stimulate the activity of PARP1. Interestingly, YB-1 also interacts non-covalently with PAR. Therefore, it can be proposed that PAR generated during poly(ADP-ribosyl)ation process may regulate YB-1 interaction with PARP1. In the present work we explored the influence of purified PAR on YB-1-PARP1 interplay during PARylation. Our data demonstrate that PAR in low concentration may promote interactions of YB-1 and PARP1, resulting in increased modification of both proteins. Conversely, YB-1 modification level and PARP1 activity are decreased by excess PAR due to disconnection of functional coupling of YB-1 and PARP1. To conclude, we found that the interaction of PARP1 and regulatory protein YB-1 may be modulated by PAR during PARylation process. This work was supported by grant from RSF (14-24-00038).

P.03-009-Wed
Not only the active site: importance of remote interactions in the recognition of oxidative DNA lesions

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DNA glycosylases of the helix–two-turn–helix superfamily are crucial for repair of oxidative DNA damage in bacterial and human cells. A prototypical member of the superfamily, formamidopyrimidine–DNA glycosylase (Fpg) excises 8-oxoguanine (oxoG), an abundant pro-mutagenic lesion, together with many other redox-damaged purines and pyrimidines. Although several X-ray structures of Fpg are known, they hardly explain the range of Fpg substrate specificity revealed in biochemical experiments. Static protein structures may be supplemented with molecular modeling and evolutionary analysis to provide useful information on the substrate recognition and conversion. Such combined approaches are especially useful to reveal important elements outside the immediately evident active site. We have analyzed coevolution of amino acid residues in the Fpg family, and performed molecular dynamics of Fpg bound to various DNA molecules to

infer possible roles of the residues and their interactions. Interestingly, a cluster of residues that showed the largest differences in motion between the natural substrate pair of Fpg, oxoG:C, and its least preferred substrate, oxoG:A, was found outside the apparent active site and DNA-binding groove, near the linker that connects the two domains of the protein. Additionally, we have identified a conserved cross-groove bridge, a structure involving several residues that connect the domains non-covalently. We have tested the importance of these structural elements by making site-directed mutants in *E. coli* and *L. lactis* Fpg. The scanning alanine mutagenesis of the near-linker region surprisingly resulted in the enzyme variants that had better specificity towards oxoG:C. On the contrary, elimination of the cross-groove bridge inactivated Fpg, making it unable to bind DNA, and also increased the cooperativity of protein thermal unfolding, indicative of stabilizing interactions mediated by the bridge. The work was supported by RSF (grant 17-14-01190).

P.03-010-Mon
The effect of PTENP1 pseudogene methylation on PTENP1 and PTEN transcription in human cells

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Genetic mutations in the tumor suppressor gene *PTEN* are often found in human malignant cells, and such genome changes are especially characteristic of endometrial cancer. At the same time the three transcripts of *PTENP1* pseudogene – sense RNA, and antisense RNAs α and β – are involved in regulation of *PTEN* gene expression. In our previous research, we found that 5'-region of *PTENP1* was methylated in normal endometrium, as well as in endometrial hyperplasias and carcinomas of middle-aged and elderly women. The aim of this study was to investigate the impact of *PTENP1* methylation on *PTENP1* pseudogene and *PTEN* gene expression in human cells. Genomic DNA was isolated from 8 cell cultures (HEK 293, MRC-5V2, MCF-7, Hep G2, SCOV-3, SKBR-3, COLO 375 and HeLa) using standard methods. The methylation status of *PTENP1* 5'-terminal region was studied using the methylation-sensitive PCR. *PTENP1* antisense-α and β–transcripts were detected by RT-PCR. Relative levels of *PTENP1*-sense and *PTEN* RNAs were determined by RT-qPCR (Litvak method). It was shown that *PTENP1* was methylated in 5 of 8 cell lines (MRC-5 V2, MCF-7, HepG2, SCOV-3 and HeLa). We found that *PTENP1* antisense-α transcription was completely suppressed in all cell lines with *PTENP1* methylation. Nevertheless, *PTENP1* antisense-β transcription remained unchanged in these cells (except of HepG2). It was shown that *PTENP1* methylation correlated with a decreased *PTENP1* sense RNA expression. We also detected an elevated *PTEN* gene expression in 4 out of 5 cell lines with the *PTENP1* methylation (MRC-5V2, HepG2, SCOV-3 and HeLa). Thus, we assume that *PTENP1* methylation may act as a positive regulator of *PTEN* gene expression through a suppression of the *PTEN* transcription negative regulator (*PTENP1* antisense-α RNA).

P.03-011-Tue
Different performance of PTEN in the lung tissues and in the blood of pneumoconiosis

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Silicosis is a respiratory disease due to long-term silica dust exposure. Our previous study has demonstrated that silica dust mediates the activation of PI3K/PTEN/AKT/MAPK/AP-1 pathway in human embryo lung fibroblasts (HELFs). The purpose of this study is to identify genome-wide aberrant DNA methylation profiling in lung tissue from silicosis patients. We all know that the key to epigenetic study is the selection of target organs. In the study of pathogenesis of many diseases, due to unable to get target organs, we can only choose relatively easily acquired organs and tissues to do our studies. Thus the partial information of epigenetic studies are lost. This time, we were very lucky to collect lung tissue samples from patients with pneumoconiosis and to extract DNA. This provides a gold standard for clarifying DNA methylation changes in the lung tissue of patients with pneumoconiosis. We performed genome-scale DNA methylation profile of lung tissues from silicosis patients to identify DNA methylation patterns in silicosis through Illumina Human Methylation 450K Bead chip. We found 86770 CpG sites and 79660 CpG sites significantly differed in methylation status in early-stage and advanced-stage compared with normal lung methylation data from GEO, respectively. PTEN promoter hypermethylation might be associated with the decrease of PTEN protein. In turn, lung tissue samples are difficult to obtain in daily physical examination. We will test the results of the lung tissue sample studies in the blood of patients with pneumoconiosis. We found that there was an significant hypomethylation of PTEN in the blood of patients with silicosis, and at the same time, there was an significant increasing of the mRNA of PTEN. So it is a very interesting results that there was an total opposite performance of PTEN in the lung tissues and in the blood of silicosis.

P.03-012-Wed
In vivo visualization of chromatin interactions in an ecdysone-dependent locus of *D. melanogaster*

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The in vivo dynamics of chromatin interactions in polytene chromosomes of *D. melanogaster* under the ecdysone stimulation still remains an unstudied question. We have designed the genetic constructions, that will allow to replace three different regulatory elements in the eiP74EF gene by means of specific recombination-mediated cassette exchange (RMCE). To visualize the dynamics of chromatin in this locus in vivo we are applying the technique, previously developed by Thomas Gregor and Kerstin Bystricky. Two specific bacterial centromere-like sequences (termed ParS) and their specific fluorescently-labeled protein ligands (ParB) were used for labeling the locus of interest on the chromosome. There are also two labels available for in vivo visualization of the transcription events: the MS2 and PP7 phage-originating stem-loop forming sequences and their protein ligands (fluorescently labeled capsid proteins of these phages). According to the concept of our experiment, we are planning to make a series of fly lines by the RMCE. In all fly lines the two ParS sequences will be inserted in the boundary regions, while the internal regulatory element will be labeled

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with MS2-stem-loop repeats. Each of the three named regulatory elements will be deleted in a separate fly line, so that the comparison of the average distances between these point on the genome could be evaluated by the LSCM. This relatively simple evaluation will give valuable information on: (1) in vivo dynamics of spatial interactions inside the 74EF locus in its silent and ecdysone-induced states; (2) possible differences in the mode of chromatin interactions between the polytene and the corresponding normal chromosome; (3) the role of the three main regulatory elements of the eiP74EF gene in the transcriptional response to ecdysone. This work was supported by Russian Scientific Foundation grant 16-14-10346.

P.03-013-Mon
UHRF1 depletion induced cellular senescence by decreasing DNMT1 transcription

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Global loss of DNA methylation has been implicated in chronological aging and cellular senescence. In our previous study, we revealed that Ubiquitin-like with PHD and Ring Finger Domains 1 (UHRF1)/DNA Methyltransferase 1 (DNMT1) axis is critical at initiation of senescent reprogramming in human diploid fibroblast (HDF) model. However, the underlying mechanism has not been investigated in detail. In this study, we found that UHRF1 increased DNMT at transcription level. UHRF1 increased ubiquitination of p53 as E3 ligase and subsequently inhibited the transcription activity of p53, a transcriptional repressor of DNMT1. Consistently, DNMT1 promoter regions possess p53 binding sequence and p53 knockdown increased DNMT1 promoter activity and its. Moreover, UHRF1 knockdown increased p21 by increasing transcriptional activity of p53 and induced HDF senescence. Our results suggest that UHRF1 depletion could downregulate DNMT1 by enhancing p53 transcriptional activity, leading to a cellular senescence. Further detailed study on the ubiquitination of p53 by UHRF1 is currently under investigation.

P.03-014-Tue
Bioinformatic ChIP-Seq-based approach to identify PARP1-dependent genes in cancer cells

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Chromatin immunoprecipitation followed by new generation sequencing (ChIP-Seq) allows tracing histone modifications and association of particular proteins with chromatin. Using ChIP-Seq data and Galaxy (version 18.01.rc1) we searched for regions in the genome, which were occupied by PARP1 as well as characterized by histone marks typical for active gene promoters (H3K4me3, H3K27ac), active (H3K4me1, H3K27ac) and inactive enhancers (H3K4me3, H3K27me3). Reads were mapped to the human genome (b37: hg19) with Map with Bowtie for Illumina, peaks were called in MACS and overlapping intervals were analyzed by using BEDTools. Only about 1% of total PARP1 was found in gene promoters. A similar number of peaks was also observed in active and inactive enhancers. Furthermore, the abundance of PARP1-positive inter- and intragenic enhancer was comparable. Surprisingly high number of PARP1 peaks occurred in areas characterized by single histone marks in the following descending order: H3K4me1>H3K4me4>H3K27me3>H3K27ac. Further analysis revealed that some H3K4me1/H3K27ac/PARP1 enhancers were associated with close and actively transcribed genes and PARP1 contribution to activation of their transcription was confirmed by PARP1 silencing. Concluding, the identification of PARP1-bound genomic regions, which are featured by gene regulatory histone marks may predict PARP1-dependent genes. This study has been supported by Polish National Science Centre grant UMO-2013/11/D/NZ2/00033.

P.03-015-Wed
Experimental evolution of *S. cerevisiae* - evolutionary stability of functional modules in yeast genome

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Experimental evolution involves long-term maintenance of a large, reproducing population and is considered as essential tool for investigating clonal dynamics, competition, fitness and genetic basis of complex traits. The purpose of our research was to conduct experimental evolution of selected *S. cerevisiae* strains, coupled with whole genome sequencing which allow us to perform detailed analysis of changes in so-called "functional modules". Functional modules are groups of genes (between which genetic interactions occur) participating in the same biological process (e.g. transport). It might determine whether these modules have a common evolutionary path and what is the scale of genetic interactions in genome. Previous studies suggest that the loss of function of one of the genes from given module often leads to the inactivation of another gene from that module, which compensates the previous loss. Presumably, this is due to the fact that the deletion of a given gene causes module damage—so that the entire mechanism is malfunctioning and therefore it is preferable to remove other modular genes. For research we chose *cog7Δ* and *nup133Δ* genes mutants. Cog7 is a part of vesicular transport module whereas Nup133 is one of nuclear pore complex protein. Both of them have many putative compensatory interactions and show growth rate defects compared to the wild-type strain. After about 300 generations mutant strains (and wild-type strain as a control) were subjected to genome re-sequencing for identification of compensatory mutations resulting from each primal mutation. We found that majority of mutations identified in this study occurred in genes involved in cell aggregation and stress response. Moreover compensatory evolution did not promoted substantial divergence across populations. The work is supported by the grant from the National Science Centre of Poland, number 2014/13/B/NZ8/0471.

P.03-016-Mon
Investigation of TIMP3, FBN-EDA and FBN-EDB gene expression levels in patients with thoracic aortic aneurysm

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Thoracic aortic aneurysms are characterized by extracellular matrix breakdown associated with progressive smooth muscle cell rarefaction. Hypertension and presence of congenital bicuspid

aortic valve (BAV) are risk factors for the disease, but a genetic predisposition also plays a prominent role in etiology. For many years, it has been known that patients with Marfan syndrome (MFS), an autosomal dominant syndrome, are predisposed to thoracic aortic aneurysm. MFS results from mutations in Fibrillin1 gene, which encodes fibrillin-1, a component of elastin-associated microfibrils. The study was completed with 16 cases. Tissue samples obtained during surgery were freezed immediately in liquid nitrogen and stored at -80°C until RNA isolation. Beta actin gene used as control for RNA quality and quantity normalization. Gene expression analysis was performed by qRT–PCR. Analysis of the obtained data was done with SPSS 17.0 program. ANOVA, Fischer and Chi square tests were used for characteristic demographic and other comparative data representations. Scatter Plot used in the representations of the expression evaluations. Expression of FBN-1, FN-1 and TIMP-3 gene did not show any significant difference between TAA patients and healthy subjects. Figure: TIMP-3, FBN-1, FN-1 Genes Fold Change Graphs. Uncontrolled increasing of MMP activity plays an important role pathogenesis of acute and chronic diseases by degradation of ECM. Difference between MMP and TIMPs expression cause ascending aorta dissection. According to the results of this study these genes were expressed at a higher level but it was not gained statistical significant results. The present work was supported by the Research Fund of Istanbul University (Project No. 28248.).

P.03-017-Tue
Identification of a new insulator-associated protein in *Drosophila*

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The *Drosophila* GAGA factor (GAF) binds to GA-repeats and involved in multiple nuclear processes incl. activation and silencing of gene expression, nucleosome organization and remodeling, higher order chromosome architecture and mitosis. These functions are provided by multiple interaction partners of GAF protein. In previous study using affinity purification coupled with high throughput mass spectrometry we have identified GAF associated protein complexes, including proteins from LBC and CP190 complexes, including insulators. Moreover, we identified a number of factors with DNA-binding potential that together with GAF could be involved in recruitment and functional activity of insulator complexes to chromatin. One of them is the Sry-delta, a protein containing a cluster of six C2H2-type Zinc finger motifs that are predicted to bind DNA. In current study using yeast two-hybrid (Y2H) assay we performed a search of Sry-delta direct partners. As a result, we have found that Sry-delta directly interacts with the BEAF-32, a known insulator DNA binding protein. Using deletion analysis, we precisely mapped the domains required for interactions between Sry-delta and BEAF-32. This finding suggests the existence of cooperative interactions between different DNA-binding factors that could bind together to provide a greater selectivity and affinity for protein complexes recruitment to different set of loci. The reported study was funded by RFBR according to the research project No. 17-54-45098 and by No. GAP0494.

P.03-019-Mon
Epigenetic regulation of inflammatory macrophage polarization by histone deacetylase-dependent mechanisms in experimental atherosclerosis

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Monocyte (Mon)-derived macrophages (Mac) are versatile cells adapting their phenotype in response to the microenvironment in the process of atheroma formation. Mac actively orchestrate important inflammatory and oxidative reactions. Two major Mac populations with different major phenotypes have been described: the pro-inflammatory (M1) and anti-inflammatory (M2). Histone acetylation enzymes control epigenetic mechanisms implicated in the regulation of cell phenotype. The aim of this study was to find approaches to mitigate atherogenic Mac functions by modulating histone deacetylases (HDAC)-dependent pathways in experimental atherosclerosis. Human THP-1 Mac and Mac derived from freshly isolated mouse Mon were polarized into M1 and M2-Mac. The cells were further exposed (24 h) to vehicle or structurally distinct HDAC inhibitors (SAHA, tubacin or CI-994). Human non-atherosclerotic and atherosclerotic samples and ApoE-/- mice were used. The mice, maintained on normal (ND) or high-fat cholesterol-rich diet (HD), were randomized to receive vehicle/suberoylanilide hydroxamic acid (SAHA). HDAC1 and 2 proteins were induced in M1-Mac as compared to resting Mac (M0) and M2-Mac. Pharmacological inhibition of HDAC reduced the gene/protein expression of inflammatory molecules (MCP-1, TNFα, TLR2/4, NOS2, CD80/86) and the activation of STAT1 in M1-Mac. Immunohistochemical staining of human atherosclerotic lesions revealed that HDAC proteins are up-regulated within media and Mac-rich areas ($CD68^+/CD45^+$). The augmented expression of HDAC proteins and M1-Mac markers correlated with the severity of atherosclerotic lesions, were found in the aortas of ApoE-/- mice. Treatment of ApoE-/- (HD) mice with SAHA down-regulated the markers of M1-Mac and reduced the extent of atherosclerotic lesions. The data suggest the pharmacological targeting of HDAC-dependent pathways may be an effective therapeutic strategy in atherosclerosis. Work supported by UEFISCDI (PN-III-P4-ID-PCE-2016-0665).

P.03-020-Tue
Differences in methylation of iPSCs, obtained by integration and integration -free methods, are determined only by transgene silencing

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Induced pluripotent stem cells (iPSCs) generating from somatic cells have the ability to self-renew, to differentiate into any type of somatic cells, and are promising tool for basic research and regenerative medicine. The reprogramming factors (oct 4, sox 2, klf 4, c-myc) can be delivered to the cell either by vectors integrating into the genome (lentiviruses, retroviruses) or by non-integrative methods (e.g., plasmids, Sendai virus, synthetic mRNAs). To evaluate the contribution of the reprogramming method to epigenetic characteristics of iPSCs, isogenic system should be utilized. Here we report analyses of methylation

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profiles for iPSC lines derived from fibroblasts of healthy donors via integrating (retro- and lentiviral transfection) and non-integrating (Sendai virus infection) reprogramming using an Illumina 450K Methylation BeadChip platform. The data of the GEO and the ArrayExpress portals, as well as our home data set of isogenic iPSCs, were used. Overall, iPS obtained by different methods, are indistinguishable in the analysis of DNA methylation. There were differences only in 8 CpG sites that were located in the exons of the MYC, KLF4 and SOX2, and OCT 4 genes and hypermethylated in integrative viruses-derived iPSCs. Differences are reproduced both in isogenic lines, and in the general set of samples. We assumed that these CpGs reflect the methylation of the exogenic reprogramming genes. We validated our data by bisulfite sequencing of exogenic and endogenic KLF and C-myc. It turned out that exogenic inserts are fully methylated in IPSCs of good quality, while in imperfect clones hypomethylation occurs. Thus, we can determine the quality of IPSCs using methylation data of just several CpGs. At the same time, negligible differences in the methylation of iPSCs established by different methods make it possible to combine data on IPSCs obtained by various methods into large datasets for further analyses. The work was supported by RSF 14-15-00930.

P.03-021-Wed
Phenotype does not necessarily follow genotype: Identification of an incompletely penetrant novel POLR1D variant as a likely cause of Treacher Collins syndrome

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Inherited in an autosomal-dominant manner, Treacher Collins syndrome (TCS; MIM 154500) is a disorder of craniofacial development that occurs with an estimated incidence of 1:50,000 live births. TCS is characterized by a combination of hypoplasia of the facial bones, bilateral downward slanting of the palpebral fissures, colobomas of the lower eyelids, cleft palate, malformation of the external ears, atresia of the external auditory canals, and bilateral conductive hearing loss. 8% of individuals with TCS have a pathogenic variant within two genes (*POLR1C* and *POLR1D*), each of which codes for a subunit of RNA polymerase I. The current study presents the case of a two-year-old male patient with clinical manifestations suggestive of TCS, such as malformed external ear, underdeveloped chin and serious respiratory problems. Sequence analysis of genomic DNA from the patient and his parents revealed that the patient inherited the novel *POLR1D* c.299Tin silico modeling, we predict that the substitution of the evolutionarily conserved leucine with a proline in the *POLR1D* product (AC19) destabilizes the larger of the two α-helices normally involved in extensive intersubunit contacts. Overall, we identified a pathogenic new variant within *POLR1D* that leads to TCS in the patient but displays incomplete penetrance in the parent carrying the same variation.

P.03-022-Mon
The evolution of homeologous gene expression in a recent allotetraploid *Capsella bursa-pastoris*

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Polyploidization and subsequent sub-, neofunctionalization or loss of duplicated genes is one of the major mechanisms of plant genome evolution. *Capsella bursa-pastoris* (shepherd's purse) is a recently emerged allotetraploid and a promising model object for the studies of the early consequences of polyploidization. Its sequenced genome and close relativeness to classic model object *Arabidopsis thaliana* make *C. bursa-pastoris* especially convenient. We have identified 16,798 pairs of homeologous genes in two subgenomes of *C. bursa-pastoris*. We have studied differential expression of homeologs in ten samples covering the main parts of plant. 44% of homeologous pairs were differential expressed in at least one sample. Generally, one gene from pair was downregulated in one or more samples or upregulated in the same manner, but in rare cases homeologs showed discordant expression. In such pairs homeolog from one genome was downregulated in some samples gene and upregulated in other. This pattern of differential expression is a possible reflection of ongoing processes of subfunctionalization. Time-series gene expression data are a powerful tool for the analysis of the dynamic biological processes and can provide a detailed view to the differences in homeolog's behavior. Transition to flowering is a well-studied on *A. thaliana* process and can serve as a model system for gene subfunctionalization analysis. Time series of gene expression in Capsella meristems during floral transition were used for homeolog expression analysis. Differentially expressed pairs of genes were studied for the evidences of post-polyploidization changes.

P.03-023-Tue
Characterization and in silico modelling of bi-allelic POLR3A mutations as a cause of Wiedemann–Rautenstrauch syndrome

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Wiedemann–Rautenstrauch syndrome (WRS; OMIM: 264090), neonatal progeroid syndrome, is a rare autosomal recessive

disorder with fewer than fifty-five reported cases to date. Clinical characteristics include prenatal and severe postnatal growth retardation, unusual facial features, dental anomalies, lipoatrophy and sparse scalp hair. There is no specific disease-causing gene for WRS, although the possible association of WRS with bi-allelic *POLR3A* variants (c.1909+18G>A, c.2617C>T) has recently been shown in a single patient. The current study presents the case of a four-year-old female with clinical manifestations suggestive of WRS, such as premature aging phenotype, bilateral ponto-mesencephaly, osteopenia, lipoatrophy in the caudal areas, and alopecia areata. Two novel compound heterozygous *POLR3A* variants (c.3337-11T>C, c.3568C>T [p.Q1190*]), which have been inherited from both parents, were found to be associated with WRS through sequencing. Based on *in silico* analyses, the c.3337-11T>C variant was predicted to cause abnormal gene splicing, while the truncating p.Q1190* variant was predicted to reduce the catalytic activity of RNA polymerase III and/or impair the interactions between the *POL3RA* product (C160) and its interacting partners, such as several other components of the enzyme and assembly factors/class III transcription factors. Absence of the POLR3A gene may contribute to disrupted interactions of POLR3A with DNA and have downstream effects on transcription factors important for neurologic and cellular function. To conclude, the proband's genotype suggests alternative phenotypic roles for *POLR3A* variants and may expand the clinical spectrum. Further studies on the functional significance of both *POLR3A* variants as well as possible more patients diagnosed with WRS are required to confirm the increasingly recognized association between *POLR3A* and WRS.

P.03-024-Wed
Dynamic changes in the replication complex during normal and perturbed replication

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The mechanisms of DNA synthesis are closely inspected throughout almost the entire cell cycle. The licensing of chromatin, the initiation, elongation and termination of replication are subjected to specific control by different mechanisms. During unperturbed replication they monitor for balanced distribution of exact DNA copy number in prodigy cells. When replication is compromised, the S-phase checkpoint is activated in order to stabilize the replication complex and to provoke the repair mechanisms for damage processing. In order to obtain a complex picture of the entire process of DNA replication, the specific characteristics of the dynamics of interactions in-between the replisome proteins must be obtained and evaluated. That is why, by means of highly sensitive and precisely aligned microscopy system, we study the changes in the dynamics of different replication-associated proteins throughout replication. Our live-cell imaging approach permits precise detection of process fluctuations that are in a range of microseconds. It aims to obtain quick and user-friendly model for genome instability diagnostics and drug-testing.

P.03-025-Mon
Targeting single-strand DNA breaks for DNA repair

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Single-strand DNA breaks (SSBs) is the prevalent form of endogenous DNA damage in the living cells. They are frequently caused by reactive oxygen species, which directly disintegrate backbone sugars and often produce damaged 3' and 5' termini surrounding the break. SSB repair (SSBR) often engages proteins dedicated to base excision repair (BER) and is considered as its sub-pathway. In higher eukaryotes one of the major proteins detecting such breaks and orchestrating SSBR is poly(ADP-ribose) polymerase 1 (PARP-1). Elongating RNA polymerase II (RNAP II) also participates in detection of these lesions. SSBs in template strand (TS-SSBs) of a transcribed DNA cause arrest of some RNAPs with efficiencies varying significantly depending on the 5' and 3' backbone end chemistries at the break and the presence or absence of adjacent abasic sites. In contrast, SSBs in non-transcribed strand (NT-SSBs) are shown to inhibit RNAPs only during transcription through a nucleosome. The effect of TS-SSBs on transcript elongation by *E. coli* RNAP and RNAP II was not yet investigated in details. In the present work we established an *in vitro* experimental system for investigation of transcription of histone-free and nucleosomal DNA in presence of randomly or uniquely introduced TS-SSBs. The effect of TS-SSBs on elongation by *E. coli* RNAP was studied using the system. Damaged templates were transcribed *in vitro* by *E. coli* RNAP, and efficiency of elongation through the lesion and enzyme stalling were evaluated. The system can be adapted for transcription by RNAP II and will be used to study the role of DNA repair machinery during ongoing transcription and to reveal how PARP-1 affects elongation on the damaged templates. This work was supported by RFBR grant No. 17-54-33045 and The Russian Federation Presidential Grant for Young Scientists No MK-2377.2017.4 (Agreement of the Ministry of Education of the Russian Federation No 14.W01.17.2377-MK).

P.03-026-Tue
Simultaneous but not separated overexpression of the XPC and HR23B genes increases resistance to genotoxic stress

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The massive amount of data accumulated at this point on the genes functions and the mechanisms in which they are involved made possible selecting targets for the regulation of the properties of cells and organisms. In particular, it enabled us to control cell resistance to ionizing radiation and other genotoxic agents. Approaches utilizing CRISPR/dCas9 system allowed us to regulate several genes simultaneously in their natural chromosomal context. In present work we overexpressed *XPC* and *HR23B* genes, products of which are functionally interdependent and act as DNA-damage recognition complex, using VP64-p65-Rta (VPR) activator fused to nuclease-null Cas9. We showed for the first time that HEK293T cells with the overexpression of both

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genes were more than 20% more resistant to radiation in doses of 1, 2, 3, 4 and 6 Gy than control cells, transfected with sgRNAs plasmids without the activator. Same results were obtained when cells were treated with 100 μM paraquat. At the same time, the overexpression of *HR23B* alone did not lead to changes in the stress-resistance of the cells. Moreover, the resistance to ionizing radiation was reduced by the overexpression of the *XPC* gene alone which is also known from studies that used various *in vitro* and *in vivo* models. Survival analysis was conducted using the fluorometric microculture cytotoxicity assay in 24 biological replicates for each data point. We assumed that the simultaneous overexpression of some functionally interdependent stress-response genes (for example, coding subunits of a single complex or units of a single cascade) that did not disrupt the control of the cell cycle, or apoptosis, or other malignancy protection systems, could present a promising approach for increasing stress resistance of cells, tissues and organisms. The study was supported by a Grant of the President of the Russian Federation (MK-2929.2017.4).

P.03-027-Wed
Yeast protein Nhp6A binds to short GC-rich genes

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Nhp6A is a small non-histone chromosomal yeast protein that nonspecifically binds and bends DNA. This protein is present at many promoters and transcribed regions of genome and is involved in regulation of transcription. Recently it was shown that Nhp6A participates in destabilization of the nucleosomal structure *in vitro* that may explain its preferential localization at gene promoters and other regulatory loci. The protein is also found along the entire length of 176 genes, including the open reading frame. Its function in the coding regions remains unknown. Aiming to discover the mechanism of action of Nhp6A, we have further studied this group of genes. We calculated length distribution and GC-content of the genes including nearby areas and compared with an average across the yeast genome. We demonstrated that Nhp6A predominantly binds to the coding regions of short GC-rich genes. The observed interaction is not associated directly with the high content of GC-pairs in these DNA loci; therefore a specific, possibly regulatory mechanism involving Nhp6A for this group could be proposed. Since a part of Nhp6A-associated genes retain some features of the ancestral bacterial genome, we suggest that this genomic distribution of Nhp6 is related to the mechanisms of regulation or maintenance of gene transcription that appeared early in the course of evolution. This work was supported by the Russian Science Foundation (project no. 14-24-00031).

P.03-028-Mon
Dynamic control of the replication protein And1

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One of the most important tasks of the living cell is to duplicate its genome and propagate. When there is an obstacle for correct replication, the genome stability is altered and genetic disorders or cancer can appear. That is why many proteins from the Replication complex are under tight control in order to precisely execute the dynamic program of the replication machinery. In our work we focused on the study of the control over a key protein from the human Replication complex – And1. It is necessary for the stable association of Pol α to chromatin. It also links GINS to Pol α and thus harmonizes both DNA helicase and polymerase. As And1 has a fundamental role in replication, it is of major importance to determine its behavioural alterations that follow the cell cycle progression. All significant changes of its kinetics can be interrelated to the dynamic program of the replication process. That is why, by means cutting-edge live-cell imaging approaches, we studied the pattern of And1 protein levels throughout the cell cycle and in the context of the dynamics of DNA replication.

P.03-029-Tue
Monitoring of castration-resistant prostate cancer patients using liquid biopsy-based DNA methylation biomarkers

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Prostate cancer (PCa) is the most prevalent non-cutaneous oncologic disease in elderly men and has high mortality rates due to the development of castration-resistant PCa (CRPC). Despite significant improvements in CRPC management, reliable molecular biomarkers are needed for well-timed personalized treatment decisions and for monitoring disease progression. Utilizing epigenome-wide technologies, our group identified several novel PCa-specific epigenetic biomarkers, *PRKCB*, *CCDC181*, and *ADAMTS12*, whose frequent methylation in tumors (N = 151) correlated with their downregulated expression ($P < 0.001$). We further analyzed the suitability of this biomarker panel for liquid biopsy-based CRPC monitoring. Methylation of the 3 genes was detectable in urine of patients diagnosed with CRPC (N = 133) as well as with localized PCa (N = 54). In urine collected from CRPC patients before the first-line therapy with abiraterone acetate (AA), higher methylation levels of *ADAMTS12* and *GSTP1* ($P < 0.050$) were observed in cases that experienced short-term disease progression. Pairwise analysis revealed decreased methylation levels of the genes when positive response to AA was observed for >6 months. In summary, our study led to identification of a set of DNA methylation-based biomarkers that could be utilized for noninvasive monitoring of CRPC progression and prediction of treatment response.

P.03-030-Wed
The effect of the risk allele A of rs200395694 associated with SLE in Swedish patients on MEF2D gene regulation and alternative splicing

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Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease with diverse clinical manifestations and complex etiology. The common associated genetic variants do not fully explain the disease heritability, which may suggest the contribution from rare and low frequency variants, undetectable in GWAS. We identified a novel rare variant rs200395694 located in the *MEF2D* gene encoding for the myocyte-specific enhancer factor 2D transcription factor as being associated with SLE in Swedish patients (504 SLE patients and 839 healthy controls, $P = 0.014$, CI = 1.1–10), and studied the potential effect of the risk allele A on gene regulation and splicing. The SNP shows an effect on protein-DNA binding and luciferase expression when analyzed by EMSA and luciferase reporter assay thus supporting the presence of an active cell-specific enhancer. The total expression of the *MEF2D* isoforms was studied by quantitative RT-PCR using RNA isolated from cell lines. Both endogenous α1 and α2 transcripts were detected in human peripheral blood mononuclear cells (PBMC), monocytic cells THP-1, myelogenous leukemia cells K562 and B cell line Daudi, but only the major α1 transcript was detected in Jurkat T cell line, HeLa cervical cancer cells and HEK293 human embryonic kidney cells. The effect of the SNP on alternative splicing was studied using minigene constructs with different genotypes transfected into 4 cell lines. No allelic difference was detected for the α1 isoform transcribed from the minigene. The alternative isoform α2 generated from the minigene with the rare A allele was significantly repressed in all tested cells. In summary, we identified a novel rare regulatory variant associated with SLE in Swedish patients. The risk allele exerts an effect on a putative enhancer and also inhibits the splicing of the alternative MEF2D isoform.

P.03-031-Mon
A novel role of spliceosomal proteins in intercellular communication of glioblastoma cells

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Alternative pre-mRNA splicing dramatically increases protein diversity within the cell. Besides being a critical mechanism for development and regulation of cell-type-specific functions, splicing is also involved in multiple pathologies, including cancer. Dysregulations of splicing were shown to play a major role both in malignant transformation and cancer progression. It is currently thought that spliceosomal proteins perform their functions only within the cell. However, our study provides the first evidence that various spliceosomal components may play an important role in intercellular communication. By analyzing blood and ascites samples from glioblastoma and ovarian cancer patients we found that cancer cells can specifically secrete RNA and protein components of spliceosomes after the courses of chemo- and radiotherapy. Further experiments revealed that spliceosomal proteins are exported by apoptotic cancer cells within exosome-like extracellular vesicles (EVs). These EVs induce pre-mRNA splicing changes in recipient glioblastoma cells and promote proliferation, migration, therapy resistance and aggressive mesenchymal phenotype of glioblastoma patient-derived neurospheres both in vitro and in vivo. Mechanistically, we identified RBM11 as a representative splicing factor that is upregulated in tumors after therapy and shed in EVs upon induction of apoptosis. Once internalized in recipient cells, exogenous RBM11 switch splicing of MDM4 and CyclinD1 towards the expression of more oncogenic isoforms. Clinically, the elevated expression of these isoforms in glioblastoma is associated with worse patient prognosis. Taken together, these findings reveal a novel role of spliceosomal proteins in intercellular communication between apoptotic and surviving tumor cells and suggest that targeting of EV-mediated splicing changes may be exploited to decrease tumor aggressiveness that would eventually improve cancer patient outcome.

The structural organization of the cell

P.04-001-Mon
Defects in ER-Golgi morphology and disturbed locations of selected actin nucleators in developing spermatids of MYO6-deficient mice

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Myosin VI (MYO6) is an actin-based motor protein that has been implicated in a wide range of processes including endocytosis, maintenance of Golgi morphology and regulation of actin dynamics. In *Drosophila,* MYO6 plays a key role in the late phase of spermatogenesis and mutant male flies lacking MYO6 are sterile. The fundamental mechanisms that control spermiogenesis are conserved between evolutionally distinct animal species, thus we hypothesised that MYO6 may also have a potential role during this developmental process in mammals. To test this hypothesis, we examined MYO6 expression and localisation and

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found that two splice variants – with a small insert and no insert – are expressed in developing spermatids in mice. We further found that these MYO6 isoforms are associated with key actin structures involved in sperm maturation. We next examined developing spermatids in MYO6 KO mice (*Snell's waltzer*; *sv/sv* mice), whose fertility is also reduced. Our ultrastructural analysis has revealed morphological disruptions, especially of the Golgi complex, the endoplasmic reticulum (ER) as well as the acrosome. These results suggest that MYO6 may play a role in actin organisation and dynamics crucial for sperm development not only in *Drosophila* but also in mice. We further compared the localisation of marker proteins for the Golgi complex and the ER and of a number of actin-nucleating proteins in developing spermatids of wild type and MYO6 KO mice. Our results show significant changes in the distribution of all the marker proteins tested in the absence of MYO6. In summary, our findings suggest for the first time that MYO6 may play a role in actin organisation and maintenance of Golgi and ER morphology during spermiogenesis in mammals.

P.04-002-Tue
In cellulo nanocrystallization of the nucleoid in bacteria and spores

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Survival of living organisms in constantly changing environmental conditions is possible due to universal hereditary strategies of adaptation to various types of stress, based on structural, biochemical and genetic rearrangements. One of the strategies implemented in bacterial cells is related to the protection of the nucleoid from unfavourable environmental conditions by binding of DNA to specific histone-like proteins, the main one being the protein Dps (DNA binding protein from starved cells), and condensation of DNA with DPS in nanocrystalline complex which was recently discovered in gram-negative bacteria. A series of diffraction experiments to study structural response to stress or stress-induced biocrystallization in bacteria E. coli, spore -forming bacteria Bacillus cereus, in cells and spores of mycelial fungus Umbelopsis ramanniana was performed for the first time. Zones of increased intensity at resolution 90 A and 44 A indicate an ordered organization (most likely nanocrystalline) of bacterial nucleoid in E. coli cells. For the starving bacteria Bacillus cereus peak at a resolution of 45 A, apparently, also associated with the presence of nanocrystalline complexes of DNA with histone-like proteins. The spores of the fungus Umbelopsis ramanniana BKM F-582, as well as the spores of Bacillus cereus create ordered sets of DNA molecules with DNA condensing acid - soluble proteins SASPs. Starving, dehydrated mycelial cells of the fungus Umbelopsis ramanniana, form ordered structures at resolutions of 27 A to 55A. The series of peaks reflects the formation of a number of orderly protein formations with continuously changing characteristic size of the interplanar spacings. This work was supported within frameworks of the state task for ICP RAS 0082-2014-0001. State registration # AAAA-A17-117040610310-6.

P.04-003-Wed
Regulation of expression efficiency of genes encoding cytoskeletal proteins in sperm of mice after long-term modeling microgravity

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Weightlessness conditions or its modeling on the Earth lead to a change of the genes expression level in the different cells, but how this regulation is carried out is still unclear. Therefore, the aim of this work was to determine the transcription levels of genes encoding cytoskeletal proteins (by qPCR), the total methylation level of the genome (by EpiJET DNA Methylation Kit, Thermo Scientific), the 5hmC content (by dot blotting with specific antibodies, Abcam), the content of DNA-methylase/demethylase, acetylase/deacetylase of histones (by Western-blotting) in mice sperm. Modeling of microgravity effects was carried out by 30-day antiorthostatic suspension (group 30HS, n = 7), simulated an early period of readaptation by 12-hour recovery after suspension (group 30HS+12 h, n = 7) and formed the corresponding control group (group C, n = 7). The relative mRNA content of the genes encoding the actin cytoskeleton proteins did not change or increased after 30-day suspension, but after 12-hour recovery it increased significantly compared to the control group. At the same time, the total methylation level of the sperm genome in the 30HS group did not differ from the control one, but in the 30HS+12 h group it decreased by 23% ($P < 0.05$), while the content of 5hmC did not change. Also, the DNA methylases content (Dnmt1 and Dnmt3a), DNA demethylases (TET1, TET2, TET3) did not change. However, the HAT1 acetyltransferase content in the 30HS+12 h group significantly increased by 10% relative to the control level ($P < 0.05$) in the absence of changes in the HDAC1 deacetylase content. One can propose a hypothesis that an increase of the transcription efficiency of the cytoskeletal genes under recovery after modeling of weightlessness is associated with a decrease genome methylation level, which in turn is due to an increase of the histone acetylation. The study was supported by program of the fundamental research SSC RF – IBMP RAS and program of RAS presidium "Molecular and cell biology".

P.04-004-Mon
Mitochondrial cristae changes as reflected by 3D super-resolution dSTORM microscopy

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3D super-resolution microscopy is a powerful method for accessing changes of objects that could be normally resolved only by electron microscopy. Despite the still impossible cristae resolution, we have previously demonstrated that mitochondrial cristae became widened in HepG2 cells after 72 hour hypoxic adaptation at 5% oxygen, with simultaneous breakdown of dimers of ATP-synthase leading to less dimer population within the widened cristae [1]. In this work, we applied the direct stochastic optical reconstruction microscopy (dSTORM) with primary Alexa-Fluor-647-conjugated antibodies and thus monitored certain morphological changes, such as in cristae width by dSTORM of ATP-synthase subunit F1α. Obtained 3D images were analyzed with the help of Ripley's K-function modeling spatial patterns or transferring them into distance distribution function. Resulting histograms of distances frequency distribution provide most frequent distances (MFD) between the localized single antibody molecules. We studied the above mentioned hypoxic adaptation

of HepG2 cells, and confirmed the previously reported [1] cristae widening. In contrast, in fasting state of model pancreatic β-cells, INS-1E, MFD between F1α were ~80 nm at 0 and 3 mM glucose, whereas decreased to 61 nm and 57 nm upon glucose-stimulated insulin secretion (GSIS) at 11 mM and 20 mM glucose, respectively. Shorter F1α interdistances reflected cristae width decrease upon GSIS, since such repositioning of F1α correlated to average 20 nm and 15 nm cristae width at 0 and 3 mM glucose, and 9 nm or 8 nm after GSIS (11, 20 mM glucose, respectively). *Supported by GACR 17-08565S*.

Reference

[1] Plecitá-Hlavatá L, et al., Ježek P. Hypoxic HepG2 cell adaptation decreases ATP synthase dimers and ATP production in inflated cristae by mitofilin down-regulation concomitant to MICOS clustering. FASEB J., 2016 30:1941–1957.

Stem cells

P.05-001-Mon
The role of deuterium in the biological properties of human adipose derived mesenchymal stem cells in vitro

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Depending on the ratio of deuterium (D) and protium (H) in water, its physicochemical properties change. Water, with the changed isotope composition D/H, has a number of unexpected biological properties, including antitumor, antidote and metabolic effects, but studies in this direction are insufficient. The aim of work was to study the effect of deuterium on the biological properties of human adipose derived mesenchymal stem cells (ADSC) *in vitro*. ADSC was prepared from lipoaspirate by enzymatic treatment. The resulting cell suspension was cultured on MEM-α, prepared from a dry powder by dilution with water (MilliQ) (D/H = 150 ppm), deuterated water (D/H = 99 abs. at.%) and deuterium depleted water (ddw) (D/H = 10 ppm). In addition, 10% FBS, 2 mM glutamine, and 1 μg / ml FGF-2 were added to the culture medium. Cultivation was carried out in multi-gas incubators with automatic maintenance: t 37°C, absolute humidity, 5% of CO_2 and O_2. The parameters were determined: population doubling time (PDT), viability (using PI and FDA dyes after 24/72 h) and metabolic activity by Alamar Blue after 24/72 h. PDT showed the lowest values in the control (MilliQ) and ddw, the largest - in deuterated media, which indicates a decrease in the proliferation of the culture. The acute and chronic toxicity of deuterated water was shown (of 2 times in comparison with the control), as well as the chronic toxicity of ddw. Metabolic activity decreased in the deuterated culture medium of 2 times in comparison with the control. At the same time, in ddw there was an increase in metabolic activity of 1.5–2 times in comparison with the control. The results show that deuterium plays an important role in intracellular processes that are responsible for the metabolism and viability of ADSC *in vitro*, which can be used in further research in the field of biology and medicine. The publication was prepared with the support of the "RUDN University Program 5-100".

P.05-002-Tue
Somatic cells reprogramming and genome editing for Stargardt disease modeling

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Degeneration of the retina occurs both in relation to age, and as a consequence of hereditary pathologies. A clinically similar pattern is often associated with different molecular pathways and gene mutations. The arsenal of therapeutic approaches for these patients is very limited. Modern advances in cellular reprogramming and genome editing make it possible to establish a model for the disease investigation and treatment. In this study we established induced pluripotent stem cells (iPSCs) from patients with a clinical diagnosis of Stargardt's disease. Mutation in the *peripherin* 2 gene was found and it was shown that the mutation does not affect the efficiency of differentiation in the pigment epithelium of the retina. Using the CRISPR / Cas9 system the mutation was corrected in the patient's iPSCs. As a result, isogeneic iPSC lines with a corrected mutation have been generated for establishing of an *in vitro* model of the disease and potentially suitable for personalized therapy of Stargardt disease.

P.05-003-Wed
Immunosuppression mediated by mesenchymal stem cells involves cell-cell contact mechanism

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Human adipose-derived mesenchymal stem cells (hASCs) are candidate for regenerative medicine treatments primarily due to their immunosuppressive properties. Various molecular mechanisms can be involved in hASC-mediated immune suppression. These mechanisms underlying hASCs-mediated immunosuppression include soluble factors and cell-cell contacts, which possibly depend on expression of cell adhesion molecules. To dissect cell contact-dependent mechanism of hASC-dependent immune suppression in vitro co-culture system consisting of hASCs and phytohemagglutinin-activated peripheral blood lymphocytes (PBL) was established. hASCs inhibited proliferation of activated PBL both in trans-well and cell-cell contact culture system as assessed by CyQuant cell proliferation assay. Activation of lymphocytes with hASCs has resulted in an increased attachment of immune cells to hASCs. Flow cytometry analysis revealed that activated CD4+ T cells were capable to bind hASCs more effectively in comparison to non-activated. We showed that hASCs-dependent suppression of activated T-cell proliferation is not associated with the reduction of IL-2R (CD25) expression or apoptosis. The increased level of IDO mRNA was observed in hASCs cocultured with activated PBMC in cell-cell contact system in comparison to separate cultures. Taken together our data support the substantial role of cell contact-dependent mechanism in hASC-mediated immune suppression.

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P.05-004-Mon
Aryl hydrocarbon receptor promotes differentiation in early mouse embryogenesis

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Early mammalian embryogenesis is a complex process controlled by pluripotency and differentiation, at the beginning of embryogenesis dedifferentiation is necessary to generate totipotent cells, that will later differentiate to specify cell lineages. Recent studies had reported relevant roles of AhR in pluripotency and stemness. Here, we have analyzed if AhR could help modulate the balance between differentiation and pluripotency in development of mouse preimplantation embryos. We have used AhR-deficient (AhR-/-) and wild-type (AhR +/+) embryos obtained from 0.5 to 3.5 d.p.c. that were analyzed for protein expression. The results showed a basal up-regulation of OCT4, NANOG and SOX2 in AhR-/- embryos, indicating that AhR could participate in maintaining differentiation at the beginning of embryo development. Immunofluorescence analysis showed a different intracellular location of these factors in both genotypes. Remarkably, OCT4 was excluded from the cell nucleus from 16-cell to early blastocyst, while in AhR-null embryos had nuclear location through all development. Further analyses, revealed that AhR showing an opposite pattern with respect to OCT4 distribution, thus suggesting a negative regulatory mechanism of OCT4 by AhR. It is known that Hippo signaling needs to be repressed at the in the outermost cells to specify trophectoderm lineage. In order to address how AhR-null affects this process, we analyzed YAP protein expression. AhR-null embryos showed reduced levels of nuclear YAP, indicating that AhR deficiency could be upregulated Hippo. This is also supported by the up-regulation of β-catenin and Last1/2 mRNAs in AhR-/- embryos. Further, these results are consistent with the overexpression of OCT4 in AhR -/- embryos. In summary, we show that absence of AhR promotes an undifferentiated phenotype in early preimplantation mouse embryos. AhR could, therefore, regulate differentiation and pluripotency through the modulation of pluripotency genes and Hippo signaling.

P.05-005-Tue
The role of the components of ubiquitin-proteasome system in the process of reprogramming of mammalian cells

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It is well known that ubiquitin-proteasome system plays a significant role in a protein homeostasis, however, the mechanisms of maintenance of this balance in embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have been studied rather poorly. ESCs and iPSCs are not only interesting objects of fundamental research, but also extremely promising in various fields of applied medicine, primarily regenerative medicine. Proteasome – the main proteolytic complex – consists of a core 20S particle and 19S regulatory particles. Under certain conditions, the constitutive catalytic subunits of the 20S particle beta1, beta2, and beta5 can be replaced by special subunits – beta1i, beta2i and beta5i to form immunoproteasomes (IPs) and it can also be regulated by a special regulator PA28. It is known that IPs plays role in differentiation of murine ESCs, but it has not been established whether IPs can take part in the process of reprogramming. To address this issue, we induced reprogramming of mouse embryonic fibroblasts into iPSCs using lentiviruses encoding Oct4, Klf4, Sox2, and c-Myc. During the process the cells were treated with selective inhibitor of beta5i, PR-957, and the inhibitor of beta5 and beta5i subunits, MG-132. Alkaline phosphatase staining, a marker of pluripotent cells, showed a significant decrease in the formation of iPSC clones, which emphasizes the important functions of both proteasomes and immunoproteasomes in the process of cellular reprogramming. Analysis of reprogramming of murine embryonic fibroblasts derived from beta2i- and PA28-deficient embryos also showed a strong decrease in the ability to give rise to iPSC clones, again, implying important functions of proteasomes, immunoproteasomes, and regulatory particle PA28 in the process of reprogramming. This study was by supported by the RSF 16-04-10343

P.05-006-Wed
3D bioprinting of human stem cells to construct blood vessel structures

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Three dimensional (3D) bioprinting combines cells, growth factors, and biomaterials to reconstruct living tissue and organs preferably using the patient's own cells. The aim of this study was to create blood vessels-like structures using human amniotic fluid stem cells (AFSC) and BioInk® hydrogel photopolymerized in a 3D network vascular tissue. Differentiation into muscular and endothelial cells was done using specific media supplemented with growth factors. Cells characterization was performed by flow cytometry, followed by FACS sorting of stem cell subpopulation. The differentiated AFSC were printed in 2 concentric layers (muscular layer at exterior and endothelial layer at interior). Printing was done using a 150 μm diameter needle, under 1 bar pressure, 150 mm/min speed rate, diameter of vascular tubes 6 mm, thickness and height of each layer 0.3 mm, followed by 5 s polymerization of each layer. The viability and distribution of cells in blood vessel structures were evaluated after 21 days using biochemical and histological tests. Our results showed that AFSC remain viable after 21 days of cultured in BioInk® hydrogel by MTT assay and fluorescent microscopy. Flow cytometry assay revealed that AFSC expressed endothelial markers such as CD31, CD105, CD133, CD144 and VEGFR2. Furthermore, histological (H&E, Trichromic Masson) and immunohistochemical (CD31, vimentin, a-SMA, F-actin, tubulin, vinculin) section of the constructs showed that cells are interconnected in each layer, with a relatively uniform distribution and stain positive for specific markers. In conclusion BioInk enables growth and differentiation of AFSC being a good printable biomaterial for vascular tissue engineering. For more information about blood vessel development, functional analyzes of these constructs (contractibility and elasticity by myograph techniques) and experiments using animal models will be performed. Project PN-III-P1-1.1-PD-2016-1660.

P.05-007-Mon
Toll-like receptor 2 tracks the emergence of hematopoietic progenitors and stem cells in pre-circulation embryo

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Toll-like receptors (TLRs) play a central role in host cell recognition and defense responses to pathogens. Direct pathogen sensing of adult bone marrow hematopoietic stem and their progenitors via TLRs has been shown to direct the cell fate towards enhanced myelopoiesis, enabling rapid generation of immune effector cells. However, despite the critical role of TLRs in adult hematopoietic cells, the functional expression of TLRs in embryonic hematopoietic progenitors has not been addressed. We show that TLRs are initially detected on short-lived maternal myeloid cells, which are gradually replaced by myeloid cells of embryonic origin. Interestingly, embryonic erythro-myeloid progenitors, which appear at day 7.5–8.5 of mouse embryogenesis (E7.5-E8.5), also express TLRs, and respond to TLR2 triggering by an enhanced proliferation and myeloid differentiation rate in a MyD88 adaptor protein dependent manner. Using *in vivo* fate mapping, we show that the *Tlr2* locus is already active in E7.75 yolk sac-derived hematopoietic precursors and at E8.5 in progenitors which contribute to adult long-term HSCs. Our results thus demonstrate that (i) *Tlr2* locus is active in yolk sac derived erythro-myeloid progenitors as well as precursors of adult hematopoietic stem cells at as early as E8.5; and (ii) TLR2 triggering endows these cells with the ability to boost the production of myeloid cells, revealing the so far unrecognized activation of pattern recognition transcription program in developing embryonic hematopoietic cells.

P.05-008-Tue
Extracellular vesicles from dental pulp stem cells suppress LPS-induced activation of NFκB signalling pathway in human microglial cells

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Increasing evidence suggests that chronic neuroinflammation has a causal role in pathogenesis of neurodegenerative diseases. We and others have demonstrated, that extracellular vesicles (EVs) derived from human stem cells display neuroprotective, immunosuppressive and anti-inflammatory properties. In the present study we investigated the effects of EVs derived from human dental pulp stem cells (DPSCs) on NFκB signalling pathway in human microglial cells. For this purpose immortalized human microglial cells (purchased from ABM) were transiently transfected with NFκB reporter plasmid pNiFty2-SEAP (Invivogen). Nucleofection was performed with 4D-Nucleofector instrument (Lonza). After 30 h microglial cells were subjected to one of the following treatments: 5 μg/mL of LPS (Sigma); 0.5 μg/mL of anti-TLR4 blocking antibody (Ab) (Abcam); 3.65 x 10^8 of EVs (as determined by NTA measurements using NanoSight LM10, Malvern); LPS+Ab; LPS+EVs; Ab+EVs and LPS+Ab+EVs. SEAP activity in supernatants was determined after 12, 24, 48, 60 and 72 h using colorimetric assay (OZ Biosciences). Our results demonstrate that EVs downregulated basal activity of NFκB after 36, 60 and 72 h by 10%, 13% and 20%, respectively. EVs also suppressed activity of NFκB signalling pathway in LPS-treated human microglial cells after 12, 24, 48, 60 and 72 h by 10%, 13%, 10%, 8%, 20%, 10% and 24%, respectively. Importantly, treatment with anti-TLR4 blocking antibody in LPS-treated cells resulted in a very similar downregulation of NFκB activity. Interestingly, combined treatment with EVs and anti-TLR4 blocking antibody resulted in an attenuation of the inhibitory effect on the NFκB activity suggesting a possible interference through a competition for TLR4 signalling pathway. Our findings demonstrate that EVs derived from human DPSCs suppress LPS-induced activation of NFκB signalling pathway in human microglial cells.

P.05-009-Wed
CRISPR-based secretome engineering of human endometrial stem cells

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Keeping with the latter observations, the paracrine activity of mesenchymal stem cells (MSCs) is assumed to underlie the positive effects of stem cell–based therapy. Thereby, the possibility to augment beneficial and diminish deleterious effects of MSCs paracrine activity seems to be an urgent issue in the regenerative medicine. Among the existing approaches of MSCs secretome modification genetic manipulations are on the cutting edge. Here we provide an optimized approach based on CRISPR/Cas9 genome editing technology for human endometrial stem cells (hMESCs) secretome manipulation. In order to obtain long-term transgene expression we selected lentiviral delivery method. Initially, we focused on the optimization of hMESCs transduction parameters that would be effective and would not affect stem cell properties. In order to increase transduction efficiency we applied either polybrene (Pb) or protamine sulfate (Ps). Though we were able to select effective transduction parameters for each compound, hMESCs transduced in presence of Pb demonstrated reduced proliferation and migration rates, impaired adipogenic and osteogenic differentiation capabilities, at least in part due to senescence induction indicated by enhanced SA-β-Gal staining, increased cell size and activation of p53/p21/Rb pathway. Fortunately, hMESCs transduction supplied with Ps had no significant impact on the main stem cell properties. Having chosen the optimal conditions for hMESCs transduction, we applied two CRISPR/Cas9 systems – GeCKO and SAM for gene knockout and overexpression, respectively. By using CRISPR/Cas9 systems we were able to obtain plasminogen activator-1 (PAI-1)-knockout and PAI-1-overexpressing hMESCs. Finally, we managed to acquire PAI-1-enriched and -depleted conditioned media reflecting the effectiveness and specificity of the CRISPR/Cas9 based approach for MSCs secretome managing. This work was supported by the Russian Science Foundation grant 14-50-00068.

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P.05-010-Mon
Stem cells for metabolic disease modeling and treatment

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Diabetes, one of the most common metabolic disorders for which a cure remains elusive. Mutations in the insulin (INS) gene occupy the second most frequent place among the causes of diabetes in children in the first 6 months of life. Most of the mutations described in the INS gene are missense mutations, in some cases deletion of the gene or the formation of a stop codon are observed. The clinical manifestations of diabetes associated with mutations in the INS gene largely depend on the location of the mutation. They violate the biosynthesis of insulin and are associated with various forms of diabetes. Most homozygous and compound heterozygous mutations in this gene are located in the promoter zone and the signal peptide. In comparison with heterozygous mutations in the INS gene, manifesting on the average at the 9th week of life, patients with homozygous and compound heterozygous mutations are characterized by an early onset of the disease (few days after birth) and low mass-growth at birth, evidence of intrauterine insulin deficiency. Variable manifestation time even for the same mutations is supposed to be due to the individual rate of β-cell apoptosis and the ability of pancreatic β-cells to regenerate. Human induced pluripotent stem cells (hiPSCs) have become a major focus of research during the last decade. Numerous hiPSCs have been generated from patients with a wide variety of diseases and a number of clinically relevant protocols of directed differentiation to specific cell type have been developed. In our study we decided to investigate regenerative potential of beta cells differentiated in vitro from patient's iPSCs carrying the INS gene mutations. We established hiPSC lines from patients with heterozygous mutations in the INS gene and generated control cell lines where point mutations were reversed using CRISPR/Cas9 genome editing tools to investigate precise mechanisms of β-cell apoptosis.

P.05-011-Tue
In vitro studies of repair efficiency of mesenchymal stem cells by co-culture with fibrosis hepatocytes

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Liver fibrosis presented at repeated inflammation, generated extracellular matrixes (ECM) between liver cells, caused organ fibrosis, hepatocyte death, and liver function loss, finally becomes cancer or liver cirrhosis. Stem cell therapy has great potential in disease treatment. Stem cells are undifferentiated cells, through self-differentiation or self-renewal capabilities, give the hope to cure diseases. In the studies, human liver cancer cell HepG2 treated with thioacetamide (TAA) to simulate liver fibrosis *in vitro*. Mouse bone marrow mesenchymal stem cells (BMSCs) were investigated the feasibility of BMSCs repairment on damaged HepG2 by trans-well co-culture (TWCC) system. After treating 0.02 g/mL TAA in culture medium at 30, 60, 90 and 120 min, DNA breakage of HepG2 didn't show by TUNEL result but fragile appeared at the cell membrane. Reactive oxygen species (ROS) content ascended by DCFDA staining. Liver fibrosis-related genes analyzed by qPCR, the expression of inflammation gene *Tnf* and anti-apoptotic gene *Bcl2* raised; functional gene *Alb* declined and ECM related gene *Col 1α1*, *Mmp9* decreased; antioxidant gene *Sod1*, *Gpx1*, and *Cat* expression were inhibited. TAA caused STAT3 protein and its phosphorylation decreased. After co-culture with BMSCs by TWCC, cell activity of TAA treated HepG2 was improved; expression of *Tnf* was decreased; *Col 1α1*, *Mmp9*, *Alb*, *Sod1*, *Gpx1*, *Cat*, and *Bcl2* were increased. STAT3 protein and its phosphorylation expression of HepG2 were improved under co-cultured with BMSCs. In conclusion, TAA caused HepG2 cell activity decreased, generated ROS damage, and inhibit ECM related gene expression. But from co-culture results, the paracrine effect of BMSCs significantly help HepG2 promoted cell activity, restored genes expression and increased antioxidant, anti-apoptotic genes expression. With TWCC system, BMSCs helped damaged HepG2 activated STAT3 protein expression.

P.05-012-Wed
Effects of age on biological features of MMSC: limitation for treatment with autologous MMSC in aged patients

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Recent experimental studies showed that the functional properties of stem cells could change with age of organism due to the development of mitochondrial dysfunction and cell senescence. Appropriate alterations in therapeutic effects also could take place. The present study addresses estimation of the key parameters of MMSC metabolism at various stages of ontogenesis and analyzing their therapeutic efficiency in a traumatic brain injury model (TBI). We have analyzed proliferation, ROS production, mitochondrial functioning and aging markers in MMSC from perinatal and adult human tissues. Adult MMSC had a lower proliferation rate, compared to perinatal MMSC. In contrast, ROS production in perinatal MMSC was significantly lower, which indicates a high probability of oxidative stress in "aged" MMSC. Moreover, analysis with MitoSOX specific probe has shown that ROS production generally occurs in mitochondria of such MMSC, which indicates the possible age-related damage of these organelles. We observed the accumulation of cell aging marker lipofuscin in MMSC from aged donors, which indicates the senescence of these cells. Interestingly, the maximal accumulation of lipofuscin was observed in high-passages MMSC cultivation. This fact should be taken into account when attempting to obtain a large amount of cell biomass by long-time cultivation for therapeutic use. The therapeutic activity of MMSC was assessed on cells from rat perinatal and adult (18 m-old) bone marrow. Cells were administered to rats after a TBI. Cells from adult animals MMSC demonstrated a significantly lower neuroprotective efficiency than perinatal MMSC. Thus, we have shown that despite the general preservation of stem cell phenotype, MMSC from adult donors accumulate aging markers, demonstrate decreased proliferation and oxidative stress development. These factors may significantly limit the use of autologous MMSC in aged patients. The study was supported by President's research grant MD-2065.2018.4.

P.05-013-Mon
Effect of IGF-1 and HGF induced bone marrow mesenchymal stem cells on focal segmental glomerulosclerosis in Sprague-Dawley rats: a pilot study

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Addition of growth factors to cell culture media can change many properties such as cell survival, proliferation, adhesion, and migration. Mesenchymal stem/stromal cells (MSCs) are able to gain proinflammatory or antiinflammatory properties due to their induction by certain growth factors as IGF-1 expressing MSCs were shown to enhance repair in kidney, heart, liver and pancreas. Migration of MSCs increases upon HGF treatment. The aim of this study was to investigate the effect of HGF and IGF-1 induced bone marrow MSCs (iMSC) on the Focal Segmental Glomerulosclerosis (FSGS) rat model. Rat (Sprague-Dawley) FSGS model was technically induced by injection of intraperitoneal single-dose Puromycin Aminonucleoside (PAN) (150 mg/kg). The occurrence of FSGS were assessed by biochemistry (increased urinary protein/creatinine (Up/Uc) ratio), immunohistochemistry (decrease in glomerular SYNPO protein level) and electron microscopy (podocyte effacement). After PAN administration, we injected $2x10^5$ naive MSCs or iMSCs (induced by 10 ng/mL IGF-1 and 20 ng/mL HGF) intravenously to the rats on days 3 (early-stage) and 5 (late-stage). The effect of treatment was assessed by Up/Uc ratios in twenty-four hour urine samples collected on the days 3, 9 and 20. Based on Up/Uc ratios, proteinuria completely disappeared on day 9 (Up/Uc:1.22 mg/mg) by early-stage iMSC treatment whereas proteinuria persisted by naive MSC treatment on day 9 (Up/Uc:68.13 mg/mg),which eventually decreased on day 20 (Up/Uc:8.02 mg/mg). In late-stage treated rats after the injury, proteinuria disappeared completely on day 9 by naive MSCs as well as iMSCs. Our results indicate that IGF-1 and HGF induced MSCs are capable to repair glomerular injury both at early stage and late stage of acute injury. Treatment by naive MSCs may be insufficient at early stage, but can be as effective as iMSCs at late stages.

P.05-014-Tue
The difference in stress response of quiescent and proliferating human endometrial mesenchymal stem cells exposed to heat shock

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Stem cell transplantation has emerged as a promising therapy for various diseases. Most stem cells applied for transplantation are in the proliferative state. During the transplantation stem cells undergo stress and of prime concern is a massive death of transplanted cells. The aim of the present study is to compare the stress response of proliferating and quiescent (synchronized in G0/G1 phase) cells. Human endometrial mesenchymal cells (eMSC) were employed as adult stem cells. Sublethal heat shock (HS) was used as a stress factor. eMSC were accumulated in G0/G1 phase after serum withdrawal. After 30 h of serum starvation the cell proliferation was initiated by addition of standard serum-containing medium. Proliferating cultures were maintained under standard culture conditions. 2 h after proliferation induction when the cells were mainly still in the G0/G1 phase they as well as proliferating cells were heated (45°C, 30 min). Both cell types after HS were returned under standard culture conditions for 72 h. HS response was monitored by cell proliferation, DNA damage response, ROS level and premature cellular senescence (SIPS). It was founded that quiescent cells more rapidly repair DNA and resume proliferation and are less prone to SIPS than growing cells. Unlike quiescent cells HS caused drastically increased ROS level in proliferating cells. Enhanced ROS level in the proliferating culture was accompanied by augmented expression of genes involved in antioxidant defense: superoxide dismutase (*sod*), catalase (*cat*), peroxiredoxin (*prdx*) and thioredoxin (*txn*). Collectively, our study has shown that quiescent cells are more resistant to stress than proliferating cells. Higher stress resistance of quiescent eMSC makes them more feasible for cell transplantation. *The work was supported by the Russian Science Foundation (project 14-50-00068).*

P.05-015-Wed
Derivation and characterization of induced pluripotent stem cells lines with inactivation of the beta-2-microglobulin gene by CRISPR/Cas9 genome editing

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The main cause of tissue rejection during transplantation is the mismatch of HLA haplotypes between donor and recipient. The discovery of induced pluripotent stem cells (iPSC) likewise the development of targeted differentiation protocols opens up broad prospects for the progress in regenerative medicine. Reprogramming technology allows establishing autologous iPSC that resolve the issue of immune rejection. However, obtaining patient-specific iPSC is very expensive and time-consuming, and requires the characterization and the quality control of each reprogrammed cell line. One possible solution is a creation of universally compatible characterized iPS cell lines that will be suitable for transplantation to any patient. HLA I proteins form heterodimers that consist of a polymorphic heavy α chain and a light β-2-microglobulin (*b2 m*) chain. The inactivation of *b2 m* in iPSC leads to shortage of HLA I expression on cell surface, thus, these cells should have reduced immunogenicity to allogeneic CD8+ T cells. It should be noted that cells that do not carry the HLA class I molecules on their surface may become targets for NK cells. In the present study, we derived *b2 m* knockout iPS cell lines by CRISP/Cas9-mediated genome editing using transfection of pSpCas9(BB)-2A-GFP plasmid containing Cas9 and guide RNA followed by GFP-based cell sorting. Selected clones were analysed by PCR analysis and sequencing. Flow cytometry analyses revealed that both surface b2 m and HLA I were not expressed on KO iPSC and their derivatives. The genetic manipulation and the disruption of HLA I expression did not affect pluripotency characteristics. Immunogenicity of KO cell lines was tested according to standard immunological protocols.

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Differentiated iPSC derivatives were resistant to allogenic CD8+ T cell-mediated killing in vitro. Similar cytotoxic tests will be conducted in co-cultivation with NK cells. This work was supported by the Russian Science Foundation grant #17-75-10206.

P.05-016-Mon
Generation of folliculogenic human dermal papilla cells from induced pluripotent stem cells

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Hair follicle morphogenesis is a complex process that occurs during the development of skin and as a part of the hair cycle. An important role in regulation of this process is played by hair follicle dermal papilla cells (DPCs). DPCs represent mesenchymal cell subpopulation with stem properties which provide an opportunity to apply them in the treatment of hair loss (alopecia). However the isolation and propagation of human DPCs for tissue engineering purposes remains a challenge because they quickly lose intrinsic biological characteristics, especially hair inductive capacity, with passaging, not allowing one to increase cell population sufficient for treatment. One of the promising and reasonable ways to develop cell products for hair loss treatment is to obtain trichogenic DPCs by differentiating them from induced pluripotent stem cells (iPSCs). iPSCs can differentiate into cells of all three germ layers in vitro and in vivo. Here we develop a strategy to differentiate human iPSCs into DPCs that can reconstitute the mesenchymal component of the hair follicle. The iPSC-derived DPCs show a similar marker expression signature as human DPCs directly isolated from human hair follicles. Human iPSC-derived DPCs are capable of generating hair follicles including the hair shaft, when transplanted into the skin of Nude mice. These results suggest an approach for generating a large amount of human DPCs for tissue engineering and new treatments for hair loss. The work was funded by the Russian Science Foundation (Project No. 16-14-00204).

P.05-017-Tue
Applying of immortalized myoblasts for the study of the cellular mechanisms of the muscle tissue replacement by connective tissue in the in vitro model of facioscapulohumeral muscular dystrophy (FSHD)

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The goal of this work was to describe immortalized myoblasts (IM) and to investigate the cellular mechanisms of fibrosis in FSHD. FSHD is a common autosomal dominant disorder that presents progressive weakness of the skeletal muscles. The main form - FSHD1 is associated with a reduction in the number of repeat units of the D4Z4 array and two polymorphisms (4qA161 and 4qA). As a result, the expression of a number of proteins is changed, in particular DUX4, which turns out to be toxic to the muscles. On the other hand, the analysis of the transcriptome of FSHD muscle biopsies revealed a high level of expression of proteins associated with a nonspecific response to inflammation. Perhaps such a response is an attraction factor for mesenchymal stromal cells (MSCs), which can differentiate into some types of cells, leading to fibrosis. The limited proliferative capacity and inaccessibility of primary myoblasts (PM) hampers their use in *in vitro* studies. Immortalization of cells allows obtaining an endless standardized resource for pathology study. IM cultures (Institute of Myology, Paris) from healthy and FSHD donors; PM cultures and MB transfected with DUX4, as control, were used. The MSCs migration to myoblasts was analyzed in the Transwell system. To study the migration mechanism we used neutralizing antibodies to SDF1α, inhibitor CXCR4 AMD3100, RT PCR, immunocytochemical method. MB from FSHD donors have morphological defects in *in vitro* differentiation. It was found that FSHD IM can stimulate MSC migration due to the secretion of SDF1, and the soluble factors of MSCs increase the expression of SDF1 and VEGF in IM. In addition, increased expression of a number of pro-inflammatory proteins was detected in FSHD MB. The results demonstrate that IM are applicable as a model for researching the cellular mechanisms fibrosis in FSHD and developing new targets for therapy. The work is supported by the state program of fundamental scientific research IDB RAS 0108-2018-0008.

P.05-018-Wed
Optimizing the preparation of MS1 extracellular matrix for the amplification of mesenchymal stem cells

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Bone marrow-derived mesenchymal stem cells (BM-MSCs) have great potential for cell therapies. However, the scarcity and loss of stemness during amplification has been a challenge for their application. Our previous study indicated that de-cellularized extracellular matrix (ECM) derived from microvessel endothelial cell line Mile Sven 1 (MS1) can effectively preserve the BM-MSCs stemness including proliferation and differentiation capacities. To optimize the preparation and application of this novel material, we tested various methods to collect, homogenize and coat MS1-ECM on the surface of culture dish, and then evaluated the re-gelification of MS1-ECM by scanning electronic microscopy. To test whether the optimized preparation can also preserve the stemness compared to the conventional culture condition and/or the hypoxic culture condition, we evaluated the properties of MSCs including morphology, self-renewal, tri-linage differentiation capacities and immunomodulatory potential after two passages. We found that our optimized preparation of MS1-ECM can provide BM-MSCs a better niche to preserve the stemness. Moreover, the major content of MS1-ECM and the responses of BM-MSCs cultured on various microenvironments will be assessed by proteomic and transcriptomic analysis to begin our journey to dissect the potential factors that are critical for maintaining the stemness of BM-MSCs. In conclusion, we have developed a novel and effective method to prepare and apply the MS1-ECM for expanding the primary BM-MSCs.

P.05-019-Mon
Creation of a sensor system for independent detection of expression of the pluripotency factor Oct4

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Molecular events inside living cells and tissues are characterized with connections and causation; they are hidden in time and in most cases we only can see them as an imprint of specific states under specific, mostly created artificially, conditions. To explore such hidden events, we need more independent methods, indicating processes that take place not now but ever inside objects of interest. Our gene of interest – transcriptional factor Oct4, a master regulator of pluripotency. A big number of works has been created to clarify its functionality and regulation. But this works mostly concentrated on stem cells and induced stem cells. We think that boundary stages are also important, maybe even more important, because they contain reasons that lead to finite states. For our purposes, we have created a system based on standard methods for tracing or inducing gene expression *in vitro* and *in vivo*. They have similar properties: as a presence of site-specific recombinases and stop cassettes which can be deleted under the influence of certain stimuli. We combined two types of such recombinases (Flp and Cre), selective and fluorescent markers, and genome editing methods in such a way that once induced (no matter gene is expressed or not when we are providing a trigger) it can trace activation of expression of a certain gene at any time when expression of the gene of interest occurs. Use of this system allows us to find a way to stages with rare or temporal expression dynamics of Oct4 and gives us an opportunity to explore them. The work was supported by the Russian Science Foundation (grant 17-14-01407).

P.05-020-Tue
Search for potential targets of LRRK2 kinase associated with Parkinson disease

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The phosphorylation of proteins is major mechanism of cellular regulation and signaling. Up to 90% of all proteins of eukaryotic cells are phosphorylated and dephosphorylated as a result of coordinated functioning of protein kinases and phosphatases. Violation of the consistency of these processes can lead to the development of various pathologies at the cellular, organ and organism level, which makes the study of the peculiarities of this regulatory system extremely topical. Mutations in Leucine-rich repeat kinase 2 (LRRK2) gene are associated with an autosomal dominant form of Parkinson's disease. The most common mutation G2019S leads to an increase in LRRK2 kinase activity, which potentially can contribute to the etiology of Parkinson's disease Since substrate specificity and regulatory pathways involving LRRK2 kinase are poorly understood, the removal of gaps in this knowledge can make a significant contribution to understanding the pathogenesis of Parkinson's disease. Proteomics allow identifying qualitative and quantitative changes in the protein composition of cells, tissues and organs (including various post-translational changes in proteins) associated with these pathological conditions. To identify potential targets of the mutant G2019S form of LRRK2 in dopaminergic neurons we used the iPSC-based model of Parkinson's disease, specific LRRK2 kinase inhibitors and label-free discovery phosphoproteomics for quantitative evaluation of phosphoproteins content. We identified a number of proteins in dopaminergic neurons in which the intensity of phosphorylation changes in the presence of LRRK2 kinase inhibitors. Possible regulatory pathways associated with Parkinsonism in which these proteins are involved are discussed. This work was supported by Russian Scientific Foundation grant #14-15-00930.

P.05-021-Wed
Mesenchymal stem cells in spheroids improve fertility in model animals with damaged endometrium

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One of the important reasons of female infertility is Asherman's syndrome (AS) caused by the destruction of the endometrium because of repeated or aggressive curettages and/or endometritis. The traditional AS therapy is hormonal medications or surgical resection of intrauterine adhesions, but in many cases, these methods are ineffective. Mesenchymal stem cell (MSC) transplantation may offer a potential cure for severe AS. To date, MSC have been established from different tissues including fragments of endometrium in the menstrual blood that is noninvasive and easily available material for isolation of endometrial MSC (eMSC). High proliferation activity during long-term cultivation, genetic stability, lack of tumorigenicity and low immunogenicity make the eMSC a promising source of stem cells for future clinical applications. A major impediment in the cell-based therapy is a high death rate and poor engraftment of cells in sites of damage. An approach to improve the efficacy of MSC-based therapy is MSC preparation as three-dimensional spheroids. In this study, we examined a therapeutic potential of human eMSC in the treatment of endometrium injury in the rat model of Asherman's syndrome and compared the therapeutic effect of these cells maintained in monolayer and spheroids. eMSC organized in spheroids retain all properties of eMSC in monolayer: growth characteristics, expression of CD markers and differentiation potential. Synthesis of angiogenic and anti-inflammatory factors drastically increased in eMSC assembled into spheroids. eMSC organized in spheroids were more therapeutically effective than the cells in monolayer. After transplantation of eMSC in spheroids the pregnancy outcome and litter size in rats with AS was higher than in rats received autologous rat bone marrow cells. It suggests the therapeutic plausibility of heterologous eMSC in case of failure to use autologous cells.

P.05-022-Mon
VEGF secretion in mesenchymal stem cells exposed to doxorubicin and small molecule anticancer drugs

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Growth and trophic factors secreted by mesenchymal stem cells (MSC) play an important role in the therapeutic effect they exhibit. In animal models transplanted MSC improve the repair of damaged myocardium after infarction due to the paracrine secretion of vascular endothelial growth factor (VEGF). There are conflicting data on the role of this factor in the tumor growth. The study of the mechanisms underlying MSC tumor support or suppression is necessary for successful application of cancer drugs in therapy. It is known that chemotherapy with doxorubicin (DX) often causes damage and fibrosis of myocardial tissue. In the present study, we investigated the viability and secretory functions of MSC after treatment with DX and small molecule

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anticancer drugs which signaling pathways are known. Two MSC cultures obtained from the desquamated endometrium from different donors were used. Both lines secrete VEGF. Treatment of MSC with DX stopped proliferation, caused stress-induced premature senescence, but did not reduce the secretion of VEGF in both MSC lines. Selective ALK inhibitor of TGFβ/Activin pathway (SB431542) inhibited secretion of VEGF and did not reduce proliferation. Specific inhibitor of c-Jun N-terminal kinase JNK (SP600125) suppressed secretion and arrested proliferation. p38 MAP kinase inhibitor (SB203580) also inhibited secretion, but stimulated proliferation. Like DX, allosteric phosphoinositide-dependent protein kinase-1 agonist (PS48) caused cellular senescence and maintained the secretion of VEGF in both MSC. PS48 is an activator of PDK1 and induces downstream pathways, primarily PI3K-Akt. Thus, PI3/Akt signaling in MSC can be the main regulator of survival and secretion of MSC after DX treatment. However, due to the ability of MSC to migrate towards tumor blood vessels, VEGF secreted by them can stimulate angiogenesis and growth of certain tumors. This work was supported by Russian Science Foundation project 14-50-00068.

P.05-023-Tue
Stimulation of peripheral nerve regeneration after trauma by hepatocyte growth factor gene therapy in mice

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Traumatic peripheral nerve injury is common and results to loss of function and/or neuropathic pain. One of the most promising non-surgical methods of peripheral nerve damage treatment is gene therapy with prolong local expression of neurotrophic and angiogenic factors. Hepatocyte growth factor (HGF) is one of the most promising pleiotropic factor for peripheral nerve gene therapy due to its angiogenic, neuroprotective, antiapoptotic, antifibrotic and anti-inflammatory effects. In our study, we examined the efficacy of HGF plasmid gene therapy (pC4W-hHGF) to restore the effects of traumatic nerve damage in mice using a model of unilateral traumatic crush injury of common peroneal nerve (*n. peroneus communis*). Effects were assessed using electrophysiological, histological and behavioral tests. Treatment of pC4W-hHGF resulted in the restoration of the nerve structure and functional recovery in comparison to similar parameters in control animals treated with empty vector. Compound action potentials (CAP) in the experimental group treated with pC4W-hHGF showed an increased amplitude and a decrease latency in comparison with the control group. Immunofluorescent staining analysis Immunofluorescence staining analysis against the axon marker protein NF-H showed axon marker protein NF-H showed a threefold increase in the number of axons in the nerve located distal to the crush region in HGF-treated mice compared to the control (29.16 ± 3.19 in HGF100 group vs. 9.17 ± 0.55 in control group, pcs/1000 μm^2; $P < 0.001$). Furthermore, significant functional recovery n. peroneus communis caused by pC4W-hHGF gene therapy was observed using a trace analysis and sciatic function index (SFI). Results of present study provide evidence for use of plasmid-based HGF gene therapy to treat traumatic of peripheral nerve injury in diabetic neuropathy often combined with diabetic peripheral vascular disease. This study was supported by Russian Science Foundation (RSF Grant no. 16-45-03007).

P.05-024-Wed
Differential effects of experimental hypoxia conditions on cancer cell behaviours in HCC

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Hypoxic microenvironments are important for invasion, metastasis and drug resistance in solid tumors. In literature, two different models have been using to create hypoxic conditions; $CoCl_2$ which is a chemical inducer of hypoxia and hypoxia chamber or hypoxia bags. In our experiments we performed these two methods on the HuH-7 parental, cancer stem cell subpopulation (EpCAM+/CD133+) as well as non-stem cell subpopulation (EpCAM-/CD133). After magnetic separation, we constituted hypoxic conditions with different hypoxic methods in these cells. We observed that there were no differences in motility of HuH-7 presence of $CoCl_2$, whereas when we incubated HuH-7 cells in hypoxic bag motility of HuH-7 was significantly increased. Since hypoxia is very important for regulation of cancer stem cells, we then examined motility of EpCAM+/CD133+ and EpCAM-/CD133- subpopulations under two different hypoxic conditions. In the presence of $CoCl_2$ motility of both EpCAM+/CD133+ and EpCAM-/CD133- were decreased, contrary motility of these cells were increased when hypoxic conditions were created with hypoxia bag. We next examined spheroid formation of these cells under two different hypoxic conditions and we determined that spheroid numbers of HuH-7 was not changed by hypoxia treatment. However, hypoxia created by $CoCl_2$ were decreased spheroid numbers in EpCAM+/CD133+ hepatic stem cell population, whereas there was no significant differences were observed in cells incubate in hypoxic chamber. In EpCAM-/CD133- subpopulation, spheroid numbers were decreased with $CoCl_2$, however spheroid numbers were increased in cells in hypoxia chamber. As a result, methods of hypoxia have differential effects on the behaviours of HCC cells in context dependent. The biological consequences of these differential effects needs further investigations.
[Correction added on 25 April 2019, after first online publication: The abstract title of P.05-024-Wed has been corrected to "Differential effects of experimental hypoxia conditions on cancer cell behaviours in HCC" in this version.]

P.05-025-Mon
Fusion and reprogramming of human cardiomyocytes with human mesenchymal stem cells

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According to the classical belief, heart cells cannot be regenerated and repair the damaged tissue following irreversible damage. Recently, cellular therapies have emerged as the most innovative treatment strategies. In cardiovascular diseases, a clear understanding of cardiac reprogramming and fusion mechanisms is necessary to achieve the goals of cellular therapy. The main

objective of this study is to identify the molecular targets of the changes in the methylome status of cardiomyocytes after fusion with MSCs underwent hypoxia to clarify the role of MSCs in reprogramming and to understand the most effective mechanisms in MSC-cardiomyocyte fusion. Fusion with MSCs was induced in untreated AC16 cells and AC16 cells which was treated with hypoxia. Reprogramming to cardiomyocyte was determined with immunohistological assays in normal-hybrid and hypoxic-hybrid cells after fusion. Methylome profiles and reprogrammed genes of MSC, AC16, hypoxic AC16, normal-hybrid and hypoxic-hybrid cells were identified to define the key pathways responsible for cardiomyocyte differentiation. Bioinformatic analyses showed that MSC and cardiomyocyte hybrid cells maintained cardiomyocyte properties rather than stem cell properties and MSCs did not have reprogramming features and guided differentiation of hybrid cells towards characteristics of somatic cells rather than stem cells. According to the results of the methylome analyses, changes targeting 'chemokine signalling pathway, cytokine-cytokine receptor interaction pathway, TGF-beta signalling pathway were identified. Results of the methylome analyses were compared to the transcriptome analyses and the genes of which promotor methylation were identified. This study is the first study which investigated the fusion between MSCs and human cardiomyocytes under cell culture conditions. We strongly believe that, the results of this study will contribute to the development of new strategies for therapies of cardiovascular diseases.

P.05-026-Tue
Possibility of usage of endometrium functional layer autologous cells at pregravid preparation of women with chronic endometritis

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Cell therapy based on the regenerative potential of stem cells of patient's own endometrium can be one of the new possible approaches in the treatment of infertility. To study the possibility of autologous stem cells using during the pregravid therapy of patients with inflammatory diseases of the uterus, the cells from the diagnostic pipelle biopsy samples of endometrium functional layer of the women with a diagnosis of chronic endometritis were isolated by enzymatic dissociation method and cultivated. According to the study by flow cytometry, light microscopy and cytological methods, the cultured cells were mesenchymal stem cells according to morphological, phenotypic characteristics and functional properties. The obtained cell cultures also contained pericytes, the precursors of endothelial and epithelial cells. Fourteen autologous mesenchymal stem cells cultures were obtained from 14 tissue samples of the endometrial functional layer. The cells were passaged once a week. The number of 20–30 $\times$ 10^6 cells, the estimated dose for injection, could be grown as a result of cultivation for three passages. Thus, the viable mesenchymal stem cells of early passages can be obtained in the amount necessary for autologous transplantation from the pipelle biopsy samples of the functional endometrial layer of women with chronic inflammatory diseases and fibrotic changes in the endometrium, that can be used for treatment of chronic inflammatory processes of the endometrium, restoration of its morphofunctional state and implementation of reproductive function. The study was supported by project No. VI.62.2.1 of the Basic budgetary financing (2017–2020).

P.05-027-Wed
The effect of adipose tissue derived mesenchymal stem cells on PTEN/AKT/FOXO3A signaling pathway cyclophosphamide induced ovarian toxicides in rats

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It is also known that cyclophosphamide leads to the activation of primordial follicles via PTEN / Akt/ Foxo3a pathway, thus destroying the ovarium reserve. In this study, we aimed to investigate the restorative effect of mesenchymal stem cells against the toxic effect of cyclophosphamide on the ovary and its effect on the PTEN / Akt / Foxo3a pathway. In the study, a total of 18, 8 weekly, Wistar Albino female rats weighing 150 $\pm$ 15 grams were studied. Three groups were formed: Group 1: Control group, Group 2: Cyclophosphamide group, Group 3: Cyclophosphamide + Mesenchymal stem cell, Group 2 and Group 3 were injected intraperitoneally with 50 mg / kg cyclophosphamide on the first day and 8 mg / kg cyclophosphamide was injected daily for the following 13 days in the same groups. 15 days after the first day of cyclophosphamide injection, 50,000 mesenchymal stem cells were injected into both ovaries of the rats. Follicular count were investigated and PTEN, pPTEN, AKT, PAKT, FOXO3a and pFOXO3a expression were evaluated by immunohistochemistry and western blot methods. Immunohistochemical staining were similar, PTEN, pPTEN, AKT, PAKT, FOXO3a and pFOXO3a in GROUP1,2,3. The mean primordial follicle count was lowest in Group 1 and group 2 and the mean primordial follicle counts were higher in Groups 3 than in Group 1,2. But secondary and tertiary follicle higher in group 2 and 3 than group 1. Histological evaluation of the tissues also demonstrated a decrease in granulosa cell damage, and stromal degeneration, after Stem cell administration. Cyclophosphamide was found to cause remarkable degenerative effects in normal ovarian tissue,. Although stem cell therapy did not effect on PTEN, pPTEN, AKT, PAKT, FOXO3a pathway. It can protect ovarian tissue from the toxic effects of cyclophosphamide. Mesenchymal stem cell, PTEN, premature ovarian failure, cyclophosphamide.

P.05-028-Mon
Urokinase expression is induced in healing myocardial infarcts and may regulate cardiac stem cell proliferation

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Urokinase-type plasminogen activator (uPA) is a major mediator in cardiac repair processes, which lead to ECM degradation, either directly by proteolytic cleavage of extracellular matrix components, or indirectly through the activation of matrix metallo-proteinases, regulation of growth factor activities and cell functions. Here, we hypothesize that urokinase generation in the heart in response to injury may determine the accumulation and proliferation properties of c-kit+ cardiac progenitor cells (CPC). Myocardial infarction (MI) was induced by anterial descendant coronary artery ligation in mice. Urokinase expression level were analyzed by Western blot and RT-PCR methods. C-kit+ CPC were isolated from hearts (WT and uPA-/- mice) by retrograde perfusion according to Langendorff method followed by immunomagnetic selection. Histological and pathological analyses were

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performed. The expression pattern of uPA during the post-infarction period showed significant increase during the early (3 days) and remote periods (7 and 14 days after MI) at both transcriptional and protein levels. An increase level of urokinase correlated with activation of the c-kit+ CPC pool. Already on day 3, massive infiltration of CPC occured in the peri-infarction area (289 +/- 154 cells / mm2) and the damage zone (252 +/- 118 cells / mm2). Similar dynamics persists on day 7 after acute damage (264 +/- 143 cells / mm2) and gradually decreased by the 14th day after injury. In addition, an elevated level of urokinase caused an increase of CPC cell proliferation in the area of damage. In vitro studies have shown that CPC obtained from uPA-/- mice possessed reduced proliferative capacity in comparison with CPC from wild-type mice. The present study demonstrates that urokinase expression in the heart in response to injury may induce activation of CPC and increase their proliferation properties, which may be important for starting a successful regeneration. This work was supported by RSF grant 17-15-01368.

P.05-029-Tue
Gene expression is modulated in human umbilical cord Wharton's jelly-derived mesenchymal stem cells by TSH-TSHR interactions

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We reported that TSH-TSHR interactions modulated gene expression in human bone marrow-derived mesenchymal stem cells. In this study, we demonstrated that human umbilical cord Wharton's Jelly-derived mesenchymal stem cells expressed a functional thyroid stimulating hormone (TSH) receptor (TSHR). To understand whether TSH-TSHR interactions has any effects in gene expression of human umbilical cord Wharton's Jelly-derived mesenchymal stem cells, we performed gene expression studies at mRNA levels by using a gene array assay and RT-PCR gene profiling arrays. Human umbilical cord Wharton's Jelly-derived mesenchymal stem cells were isolated from human umbilical cords. Alternatively, we purchased human umbilical cord Wharton's Jelly-derived mesenchymal stem from American Type Culture Collection (ATCC) as well. TSH treatment modified several gene expression of mesenchymal stem cells in all samples. We also found that in terms of gene expression, response of human umbilical cord Wharton's Jelly-derived mesenchymal stem cells to the TSH treatment was different from those of human bone marrow-derived mesenchymal stem cells. Based on these findings, we concluded that TSH-TSHR interactions might contribute proliferation, migration, and differentiation of human mesenchymal stem cells.

Photosynthesis and plant biochemistry

P.06-001-Mon
Influence of *Chlorella vulgaris* on the photosynthetic apparatus of rice plants under cadmium stress

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Cadmium (Cd) is one of the most toxic heavy metals due to its high solubility in water, its easy absorption through the roots and its accumulation in plant tissues. The aim of this study was to investigate the effect of the green algae *Chlorella vulgaris* on the growth parameters and the functions of the photosynthetic apparatus in rice plants grown under Cd stress. PAM chlorophyll fluorescence, oxygen evolution (measured with Clark-type and Joliot-type electrodes), P_{700} photo-oxidation measurements and pigment content were used to assess the changes in the functions of the photosynthetic apparatus under Cd stress (150 μM $CdCl_2$) of rice seedlings grown hydroponically in the presence or the absence of *Chlorella vulgaris* (OD_{760} = 1.2). Several important markers associated with oxidative stress (lipid peroxidation, H_2O_2 and proline production) were also evaluated. Cadmium stress led to inhibition of the plant growth, the functions of the photosynthetic apparatus and increased levels of the oxidative stress markers. Our data revealed that the presence of green algae *Chlorella vulgaris,* during Cd exposure of the rice seedlings, improved notably the growth, the photochemical activities of both photosystems, the electron flow from Q_A to plastoquinone, the kinetic parameters of the oxygen-evolving reactions, pigment content and decreased lipid peroxidation, H_2O_2 and proline production. This study suggests that the green algae *Chlorella vulgaris* applied in the rooting medium has a protective effect against Cd toxicity in rice plants. The possible molecular mechanisms involved in the defensive effect of the green algae on the function of photosynthetic apparatus are shown. The authors thank to Ivan Iliev (IPPG-BAS) for providing the *Chlorella vulgaris*.

P.06-002-Tue
Distinct patterns of activation of papain-like cysteine protease Triticain-α in vivo

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Papain-like cysteine proteases (PLCPs) in plants and animals participate in numerous physiological processes. They are synthesized as preproenzymes consisting of N-terminal signal peptide, inhibitory prodomain and proteolytic domain. The proenzyme activation occurs through autocatalytic mechanism implying intra- or intermolecular hydrolysis of prodomain or by other proteases acting upstream in the proteolytic cascade. PLCPs commonly localize in lysosomes or vacuoles being activated and stable at low pH levels. Despite recent progress in studying of PLCPs, there is a lack of knowledge concerning *in vivo* properties of these proteases, for most of which the enzymatic cascades, subcellular localization, and specific substrates remain unknown.

Triticain-α is a PLCP from *Triticum aestivum L*, which was detected in wheat seedlings. Previously, we have cloned Triticain-α, identified domain structure of the enzyme necessary for its maturation and demonstrated its glutenase and collagenase activities. We also developed a cheap and efficient protocol for producing the active recombinant enzyme. In this study, using non-aqueous fractionation method we have demonstrated for the first time that the mature endogenous Triticain-a is specifically accumulated in vacuoles of *T. aestivum L* leaves, whereas the full-length protein and its low MW variants are localized to non-vacuolar fractions. MS analysis of Triticain-α fragments allowed us to identify sites of the enzyme proteolysis specific to vacuolar and non-vacuolar fractions. Based on this data, we suggest that autocatalytic maturation of Triticain-α is characteristic of vacuoles, whereas outside these compartments the maturation is held by other intracellular proteases. Since homolog of Triticain-α was shown to be a substrate of metacaspases involved in cell death of xylem elements, we suggest a possible association of Triticain-α with this process as well. This work was funded by the Russian Science Foundation (grant #16-15-10410).

P.06-003-Wed
Study of plant glycated proteome

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Protein glycation is referred to as a non-enzymatic modification of proteins with reducing sugars or carbonyl products of their degradation. Resulting advanced glycation end products (AGEs) are pro-inflammatory in mammals, whereas these products were only recently reported in plant protein hydrolyzates. However, no information about the mechanisms, underlying glycation in plants, was available. Therefore, here we address the mechanisms and patterns of protein glycation in plants. For this, formation of AGEs was characterized under standard growing conditions and in presence of environmental stress. Age- and stress-related changes in plant status were characterized by a panel of reliable physiological and biochemical tests. Additionally, reactivity and glycation potential of individual carbohydrates were characterized *in vitro* in reactions with model synthetic peptides. For characterization of glycation reactions an integrated omics approach was applied. Analysis of protein glycation relied on LC-MS-based bottom-up proteomic approach, whereas for metabolomic studies GC-MS was employed. The proteomic analysis revealed characteristic stress-specific glycation patterns, dominated with polypeptides involved in protein metabolism and transcriptional regulation. The plants treated with Cd(II) demonstrated the most abundant patterns of glycation products, mostly represented by arginine-derived modifications. Interestingly, this kind of stress promoted AGE formation from methylglyoxal while drought – from glyoxal. Both ageing and environmental stress resulted in dramatic increase in the contents of specific carbohydrates and glycation rates of individual sites in multiple proteins. Importantly, these up-regulated sugars demonstrated a high reactivity and glycation potential *in vitro*. Protein homology modeling revealed possible effects of protein structure on glycation rates at specific residues. The work is supported by the Russian Scientific Foundation (No. 17-16-01042).

P.06-004-Mon
A link between BABA-priming effect of enhanced defence and amino acid homeostasis

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Plants are constantly exposed to a wide range of adverse effects from the environment when among the most important factors affecting plant growth and development belong pathogenic microorganisms. Unfortunately, host resistance against pathogens is not available for many plant species, and a significant proportion of global crops is annually destroyed. Non-biogenic amino acid β-aminobutyric acid (BABA) has been for the long time associated with plant-priming phenomenon associated with a state of enhanced defence against an exceptionally broad spectrum of stresses. BABA-priming effect has been extensively studied on various plant systems when recently, in *Arabidopsis* IBI1 gene encoding an aspartyl-tRNA synthetase (AspRS) binding specifically only (R)-BABA, was identified. In this study, for the first time we demonstrate that BABA is able to prime not only plant systems but also suspension cells and this primed state is transmitted to the next progeny. Further, to clear-up relation between BABA priming phenomena and amino acid homeostasis, in different plant species we measured amino acids levels after various treatments of BABA and its analogues with higher cell permeability. Noticeably, competition experiments between BABA and aspartate significantly reduced BABA priming effects and proved competition about binding site of enzyme AspRS as a crucial factor in BABA biological activity. Moreover, previous results determined on insects and our preliminary results of BABA effect measured on human immune cells show for general BABA mechanism among diverse organisms.

P.06-005-Tue
Challenges in the identification and quantification of K-acetylation sites on proteins from *Arabidopsis* leaves

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Cellular signaling pathways are regulated in a highly dynamic fashion in order to quickly adapt to distinct environmental conditions. The acetylation of lysine residues within proteins is a highly conserved and reversible post-translational modification which occurs in all living organisms. Upon lysine acetylation, the function of proteins can be modulated due to the loss of the positive charge of the ε-amino group of a lysine residue. The acetylation process is catalysed by lysine acetyltransferases (KATs) and the reverse reaction by lysine deacetylases (KDAC). Recent advances in mass spectrometry-based proteomics have considerably increased the number of identifiable acetylation sites per experiment in mammalians. However, in plants a more complex approach has to be carried out. Here, we describe the reproducibility of antibody-based lysine acetylation enrichment, the influence of dimethyl-labeling hereon and how to increase numbers of acetylation per experiment derived from plant material. Moreover, there is a variety of putative acetyltransferases in *Arabidopsis* possibly showing either NAT- or KAT activity. Determination of the activities of yet unknown acetyltransferases and classifying their preferences via HPLC and fluorophore-based enzyme assays combined with mass spectrometry and immunoblots should be achieved.

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P.06-006-Wed
Phytaspase activity levels in edible plants: champions and outsiders

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Phytaspases are plant subtilisin-like proteases possessing unusual, 'caspase-like' cleavage specificity. Phytaspase precursor proteins are constitutively processed in plants to give rise to active enzymes targeted to the apoplast. Apart from plant targets, phytaspases were shown to hydrolyze and thus inactivate mammalian gastrointestinal peptide hormones, gastrin and cholecystokinin, *in vitro*. A possibility to modulate levels of these hormones by ingestion of plant food was thus envisaged. As a first step to address this possibility, here we characterized phytaspase activity levels in extracts of edible plants using fluorogenic peptide substrates of phytaspases. This analysis revealed that phytaspase activity in different plant species strikingly varies. Exceptionally high levels were observed in coriander, estragon, and celery among herbs, in pepper among vegetables, and in avocado among fruits. To characterize an enzyme from phytaspase-rich source, we purified pepper *Capsicum annuum* phytaspase (*Ca*Phyt) to homogeneity and identified the enzyme through mass spectrometry analysis. After *Ca*Phyt gene cloning and production of the recombinant protein, properties of the purified *Ca*Phyt were determined. Substrate specificity analysis revealed strict Asp specificity of hydrolysis, with the VNLD-based peptide substrate being far the most efficiently cleaved among commonly employed phytaspase substrates. Also, *Ca*Phyt was found to efficiently hydrolyze synthetic human gastrin and cholecystokinin. *Ca*Phyt displayed proteolytic activity in a broad pH interval (pH 3.0–8.0), with pH optimum at pH 5.5–6.0. Furthermore, *Ca*Phyt specific activity was approximately ten-fold higher than that of a closely related tomato phytaspase. Availability of highly active *Ca*Phyt provides now an opportunity to explore its possible effects on gastrin and cholecystokinin levels using animal models. This work was supported by the Russian Science Foundation grant # 16-14-10043.

P.06-007-Mon
Engineered monomeric and dimeric fluorescence recovery proteins refine understanding of the regulatory cyanobacterial photoprotection mechanism

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Photosynthetic organisms balance between photosynthesis and photoprotection. Cyanobacterial antenna complexes, phycobilisomes (PBs), gather light and ensure the energy flow to the photosystems. Under high light, the photoactive orange carotenoid protein (OCP) binds to PBs to dissipate the excessive absorbed energy into heat, whereby preventing the photodamage. The fluorescence recovery protein (FRP) completes the regulatory cycle by causing detachment of OCP from PBs and restoration of their fluorescence. However, the exact molecular mechanism of this process is largely unknown. Despite the sequence identity below 50%, FRP homologues from *Synechocystis*, *Anabaena* and *Arthrospira* form complexes with *Synechocystis* OCP at 1:1 or 2:1 stoichiometries, suggesting that OCP binding may involve monomerization of the otherwise dimeric FRP, but for an unclear reason. To address this question, we designed tentative monomerizing mutations in the FRP dimeric interface or introduced pairs of Cys residues to have an opportunity of fixing the dimeric protein conformation upon oxidation. The mutants were produced in *Escherichia coli*, purified to homogeneity and then comprehensively analyzed. The oligomeric status of both types of engineered FRPs (either monomeric or dimeric) was confirmed by analytical size-exclusion chromatography and chemical crosslinking; differences in the tertiary structure and stability associated with the oligomeric status, as well as the functional consequences of their interaction with OCP, were analyzed by spectroscopy techniques. The monomeric FRP is relatively unstable and, adopting an altered alpha-helical conformation, is poorly efficient in OCP binding and inactivation. On contrary, the fixed dimeric FRP was able to tightly interact with OCP, regulate its photocycle and the ability to quench PBs fluorescence. We conclude that FRP monomerization is not obligatory for the FRP function and the subunit interface is not directly involved in contacting OCP.

P.06-008-Tue
Detail description of the heat induced stress response of the photosynthetic machinery of bean plants monitored by analyses of chlorophyll fluorescence signals and 820 nm light reflection

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The photosynthetic machinery is very sensitive to environmental changes and plant stress thus it is a very useful indicator for evaluation of the stress response and tolerance of important agricultural plant species. A very perspective method for studying the changes in the photosynthetic apparatus (PSA) is measuring the chlorophyll *a* fluorescence emitted from Photosystem 2 (PS 2) antennae complexes. This approach has proven itself superior to all other techniques due to its fast, non invasive nature and the huge amounts of data it provides in a single measurement. Illumination of a dark adapted plant sample induces a rise in the chlorophyll fluorescence signal drawing an induction curve which gives significant information about every step of the photosynthetic process. The Multifunctional Plant Efficiency Analyzer (MPEA) is constructed to measure three types of signals simultaneously - Prompt chlorophyll fluorescence (PF), Delayed chlorophyll fluorescence (DF) and modulated light reflection at 820 nm (MR820). Our group developed a new approach called Dark Drops of PF which gives additional data about the reduction state of the PS 2 electron acceptors and can contribute to the overall analysis. In our study we followed the changes in the photosynthetic electron transport chain induced by post effects of moderate heat stress in bean plants (*Phaseolus vulgaris*) by analyzing the changes in PF, DF, MR820, Dark Drops of PF and selected photosynthetic parameters. We found that light heat stress can accelerate the electron flow through PS 1 while inhibiting the functions of the PS 2 protein complexes which changes the balance between the two photosystems. Temperatures of 40 degrees and above lead to inactivation of the reaction centers of PS 2 and ungrouping of the light harvesting complexes. Higher temperatures can influence the membrane stability thus affecting the ability to form proton gradient across the thylakoid membrane.

P.06-009-Wed
Light control of lipid metabolism in the brown alga *Undaria pinnatifida*: consequences for chloroplast ultrastructure and photosynthetic competence

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Light produces a variety of effects on algal lipid metabolism and therefore lipid composition. Glycolipids determine the physicochemical properties of the thylakoid membrane that are essential in supporting the photosynthetic capacity, and, hence, they should be included into the concept of light acclimation of photosynthesis. Long-term exposure to varying light intensity (100, 290 and 400 μmol photons/m^2/s) influenced the lipid and fatty acid composition of *U. pinnatifida*, as well as on growth rate and photosynthetic activity. The changes in total lipid content were accompanied by variations in lipid classes. Each lipid class exhibited a specific fatty acid composition and changed considerably at a different light intensity. Under adaptation to low light, besides the increased synthesis of glycolipids, monogalactosyldiacylglycerol MGDG, sulfoquinovosyldiacylglycerol SQDG, and phosphatidylglycerol PG we observed also active n-3 desaturation with the formation of 18:3n-3, 18:4n-3 and 20:5n-3 acids. These changes were accompanied by the active synthesis of Chl a+c and fucoxanthin pigments as well as a significant increase in the PSII maximum quantum efficiency and the electron transport rate. In contrast, the high–light samples displayed a significant decline in n-3 polyunsaturated fatty acid content, increase in the amounts of storage lipids, triacylglycerols, rich in 16:0 and 18:0 and a decline in photosynthetic activity accompanied with increase in malondialdehyde content. The consequent accumulation of n-3 fatty acids in glycolipids could facilitate the thylakoid membrane fluidity, and, therefore, the velocity of electron flow involved in photosynthesis during light acclimation. Thus, a prolonged exposure to low and high light considerably affects lipid synthesis, suggesting a correlation between the activity of photosystems and the process of synthesis and desaturation of fatty acids. The work was supported by the Russian Science Foundation (14-50-00034).

P.06-010-Mon
What happens with tobacco metabolism if protein casein is the only source of nitrogen?

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The well-known paradigm that plants must rely on soil fauna for the breakdown of organic matter has been challenged by many scientists now. We took a closer look on what happened with tobacco metabolism if grown *in vitro* in sterile conditions with casein as a sole nitrogen (N) source. Casein promoted the root growth, providing the plant with a robust root system. Also, the protein and total free amino acid content together with the C/N ratio were not significantly reduced in comparison with the other two experimental groups grown with inorganic forms of N. We determined an increased concentration of free branched-chain (Ile, Leu) and basic (Arg, His, Lys) amino acids in the plants supplemented with casein. Their proteomic analysis of the root exudates revealed three classes of proteases as being secreted to the medium, and that some apoplastic proteases in the roots were downregulated, except for aleurain-like protease that was significantly upregulated. However, most of proteins involved in N metabolism remained unchanged. We detected that the plants supplemented with casein showed inducible proteolytic activity at lower pH than neutral. Their high activity of endo-1,3-β-glucanase in the roots was related to the cell wall adjustment resulting in the loosening of the transport pathways for metabolites, including peptides and proteins. Taken together, our findings suggest that tobacco plants accumulate mainly branched-chain and basic amino acids from casein, secrete proteases into the medium and break the peptides in the apoplast. This project was supported by Charles University (UNCE 204025/2012).

P.06-011-Tue
Defining a metabolic core module of stress response in *Arabidopsis thaliana*

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Abiotic stress exposure of plants induces metabolic reprogramming which is a tightly regulated and complex process affecting signalling cascades, transcriptional, translational and metabolic regulation. The complexity of resulting interconnected stress-responsive networks impedes the functional understanding of molecular plant stress response compromising the design of breeding strategies and biotechnological processes. Consequently, defining a molecular network enabling the prediction of a plant's stress mode promises to significantly promote the understanding of stress-induced biochemical reprogramming and its technological application. In the presented study, *Arabidopsis* wild type plants and three mutant lines with enzymatic deficiencies in the central carbohydrate metabolism were grown under ambient conditions as well as under low temperature/high light stress conditions. Stress-induced dynamics of metabolome and proteome were quantified in a mass spectrometry-based high-throughput experiment. Together with chlorophyll fluorescence parameters, which indicated a significant stress impact on photosystem reactions, this multidimensional molecular data set was used to train a machine learning algorithm to predict stress behaviour of all genotypes. Based on multivariate statistics and machine learning approaches on wild type data, a core module consisting of 20 proteins was identified enabling the prediction of the stress mode of all mutant lines. Further, among these protein candidates a protein-protein interaction network was identified connecting transcriptional regulation with regulation of primary and secondary plant metabolism. In summary, the identified stress-responsive core module defines a tightly regulated molecular network being preliminary for reprogramming of metabolism during stress and for the predictability of complex biochemical regulation during environmental fluctuation.

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P.06-012-Wed
Possible correlation of MADS-RIN expression with carbohydrate content in fruits of both wild and cultivated tomatoes

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Fleshy fruit of tomato Solanum lycopersicum is a model for fruit development studies. In tomato cultivars, fruit ripening is accompanied by the hexoses accumulation, and green-to-red (yellow/orange) color changes due to lycopene and beta-carotene synthesis. In certain wild tomato species (Solanum sect. Lycopersicon), the lack of color changes during ripening was shown. Thus, these fruits may differ in biochemical content not only in carotenoids, but also in sugars. The present work is focused on profiling of carbohydrates and carotenoids during fruit development in wild and cultivated tomatoes, as well as key genes expression. Fruit carbohydrates and carotenoids were measured at four developmental stages in red-fruited (RF) (S. cheesmaniae, and S. lycopersicum cv. Silvestre recordo and var. humboldtii) and green-fruited (GF) (S. arcanum, S. chmielewskii, S. peruvianum, S. habrochaites) species. We observed that during ripening, compare to RF fruits accumulating glucose and fructose, GF fruits of evolutionary more ancient wild species accumulate sucrose, low amounts of beta-carotene, and do not contain lycopene. In cultivated tomato, it was previously shown that both the carotenoids biosynthesis and the sucrose to hexose conversion are believed to be under the control of the MADS transcriptional factor RIPENING INHIBITOR (RIN). This allowed us to propose that in wild GF fruits the lack of sucrose degradation and carotenoid biosynthesis is due to the loss/reduced activity of RIN. On the base of fruit biochemical analysis and the revealed co-expression pattern of RIN, carbohydrate-related genes TAI (vacuolar invertase) and InvInh (invertase inhibitor), and carotenoid-related PSY gene (phytoene synthase) in fruit at different developmental stages, the hypothetical scenario of RIN-mediated regulation of the gene network for carbohydrate and carotenoid metabolism in RF and GF fruits was proposed. The study was supported by the Russian Science Foundation grant 16-16-10022.

P.06-013-Mon
Effects of salt stress on the functions of the photosynthetic apparatus and the antioxidant activity of Paulownia

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Salinization is one of the most important global environmental problems, which limits plant growth and development and thus decreases the crop yields. The aim of this study was to investigate the impact of salinity on the antioxidant capacity and the functions of the photosynthetic apparatus of *Paulownia tomentosa x fortunei* and *Paulownia elongata x elongata*, grown in Hoagland solutions with different concentration of NaCl (0 – 200 mM) for different periods of time. The effects of NaCl on *Paulownia* plants were assessed by measuring of PAM chlorophyll fluorescence, P_{700} photo-oxidation, pigment content, antioxidant activity, antiradical activity and levels of flavonoids and proline. Data revealed that treatment with high NaCl concentration (≥150 mM) for 10 days led to decrease of the amount of chlorophylls and carotenoids, the maximum (Fv/Fm) and the effective (Fv'/Fm') quantum yields of PSII, the effective quantum yield of the photochemical energy conversion (Φ_{PS2}), the photochemical quenching (q_P) and the linear electron transport rate (ETR) as well as the restriction of the electron flow from Q_A to plastoquinone. The analysis of P_{700} photo-oxidation by far-red light suggested that the NaCl induced changes in the both populations of PSI as the effect was stronger on the PSI in the grana margins. Furthermore, data revealed that high NaCl concentration (≥150 mM) for 10 days led to increase of antioxidant activity, antiradical activity, flavonoids and proline amount. In addition, data suggest that prolonged influence (15 days and 25 days) of NaCl leads to adaptation of the studied *Paulownia* lines to high salt concentrations. The possible adaptation mechanisms and the differences in the sensitivity to high salt concentrations of the studied *Paulownia* lines are shown. This study is supported by the project № ДФНП-17-135/01.08.2017 of Program for career development of young scientists, BAS.

P.06-014-Tue
DnaJ homologues from *Synechocystis* sp. PCC6803, and their role in Rubisco biosynthesis

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Carboxylase / oxygenase ribulose-1,5-bisphosphate (Rubisco) is the enzyme responsible for attaching atmospheric CO_2 to pentose and therefore for initial step of biomass generation. Rubisco is the only enzyme in nature that allows for incorporation of inorganic into organic matter for a large scale. Because it is an enzyme that is very slow and inaccurate, scientists have been looking for ways to improve its kinetic parameters. The major obstacle to this venture is the complex Rubisco biosynthesis process that requires a multiple chaperone proteins, which makes it difficult to test potential kinetic mutant enzymes. The prokaryotic Rubisco from *Synechocystis* sp. PCC6803 unlike other cyanobacteria, does not fold in *E. coli*, suggesting a lack of specialized folding factor or insufficient homology of existing factors in this bacteria. However, it has been showed recently, that plant Rubisco assembly in *E. coli*, cells with five chloroplast chaperones including BSD2 which has a zinc-finger domain and is considered to be a Hsp40 family member (*Hayer-Hartl at all, Science 2017*). According to this information it is possible that Rubisco from *Synechocystis* sp. PCC6803 also requires additional chaperone to fold properly in bacterial system. It is suggested that this missing factor is one of DnaJ homologues (which also like BSD2 protein has zinc-finger domain), as this cyanobacteria genome encode seven homologues of the DnaJ protein the Hsp40 family members. Here we present the results after testing which out of all seven DnaJ homologues from *Synechocystis* sp. PCC6803 binds Rubisco large subunit from this cyanobacteria.

P.06-015-Wed
The pigment binding behavior of water-soluble chlorophyll proteins (WSCPs)

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Water-soluble chlorophyll proteins (WSCPs) from Brassicaceae are, by contrast to membrane bound chlorophyll (Chl) proteins

not involved in photosynthesis. Their biological function remains unclear, but has been proposed to act as a carrier for Chl or Chl precursors/derivates in the Chl biosynthesis or metabolism pathways. WSCPs are remarkably stable towards high temperature, harsh pH and protease treatments. Moreover, recombinant WSCP apoprotein can extract Chl from thylakoid membranes, liposomes, detergent micelles and organic solvents. Upon Chl binding, the WSCP apoprotein forms a tetrameric complex with one Chl per apoprotein and no other pigments bound. In this study, we investigate the scope of pigments bound and the mechanism of the binding reaction *in vitro* employing recombinant WSCP. We developed an approach to monitor the pigment binding to WSCP by using its visible circular dichroism (Vis-CD). Vis-CD measurements allowed rapid screening of various tetrapyrroles as potential binding partners of WSCP. The protein was found to bind a broad range of tetrapyrroles including porphyrins, chlorines and bacteriochlorins. Furthermore, the kinetics of the pigment-protein complex formation was measured by time-resolved Vis-CD. Interestingly, measurements with liposomes revealed changes in the binding kinetics depending on the lipid composition, suggesting that WSCP apoprotein interacts strongly with membranes containing negatively charged lipids. These results may help to understand the mechanism by which a water-soluble protein is able to extract Chls from membranes.

P.06-016-Mon
pH-induced conformational switching in LHCII-SR chimeric protein as a model for regulation of light harvesting in photosystem II of green algae

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Stress-related pigment-protein complex LHCSR3 expressed in high-light conditions is responsible for regulation of the non-photochemical quenching in green algae. It uses pH-sensitive C-terminus as a switch button upon acidification of the lumen. In the recent experiment, the recombinant protein LHCII-SR built from the LHCII main antenna by replacing the C-terminus with the LHCSR3 sequence exhibited pH-dependence of the fluorescence decay (FD) rate. We studied pH-induced conformational changes in this protein and their impact on the structure of the exciton states and their properties. The 3D structure of the complex was reconstructed using the primary sequence for the LHCII-SR and the tertiary structure for the LHCII. Constant-pH molecular dynamics was used to reveal stable conformations in the pH interval from 4.4 to 7.5 responsible for variations of the FD rate. Exciton energies of the complex for stable conformations were modeled using exciton Hamiltonian method. Diagonal elements of the Hamiltonian were calculated as excitation energies of individual pigments; exciton couplings were evaluated from their transition densities. Those properties were calculated using QM/MM approach with TDDFT for QM part, and polarizable force field AMBER02 for MM part. Absorption, linear, and circular dichroism spectra were modeled based on eigenvectors of the Hamiltonian. Homogeneous spectral broadening was accounted for by using the Redfield theory, the inhomogeneous one – by the random shift method. The spectra were compared with the experimental ones in order to check the validity of the computations. Chlorophyll-xanthophyll exciton couplings responsible for FD were compared for conformations obtained earlier demonstrating significant increase. These results can be combined in future with theories for quantum dissipative dynamics and lead to time-dependent description of chlorophyll-xanthophyll energy transfer. The study was funded by RFBR (grant no. 18-34-00700).

P.06-017-Tue
Structural studies reveal protein dynamics crucial for protein carotenoid uptake and delivery

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A family of soluble carotenoid proteins having the ability to transfer its carotenoid to other proteins was recently described. These proteins are homologs of the C-terminal domain (CTDH) of the Orange Carotenoid Protein (OCP) that is involved in cyanobacterial photoprotection. CTDHs were suggested to have a mediating role in a carotenoid transfer mechanism from the thylakoid membrane to the Helical carotenoid proteins (HCPs), which are paralogs of the N-terminal domain of the OCP. In this work, the three-dimensional structure of a carotenoid-free CTDH variant from Anabaena (Nostoc) PCC 7120 is presented. Obtaining this structure required the urea-induced disruption of a large, crystallizable oligomeric form of the CTDH. This CTDH belongs to clade 2, which contains a cysteine amino acid at position 103. Two dimer-forming interfaces were observed – one between the two monomer's disulfide-bond regions (head-to-head) and the second between each monomer's β-sheets domain (back-to-back). SAXS measurements confirmed that this class of proteins has two different kinds of dimeric interactions in solution that are consistent with the oligomeric forms seen in the crystal structure. In addition, the X-ray structure revealed that in apo-CTDH, the C-terminal tail (CTT) undergoes a dramatic change in its position. Mutational analysis revealed that the CTT facilitates both carotenoid uptake and carotenoid delivery, thus playing an important role in carotenoid transfer mechanism. Finally, the CTT movement seems to occur also in OCP upon photoactivation, suggesting that it could also have a similar role in carotenoid transfer as in CTDH.

P.06-018-Wed
Plastidial lysine acetyltransferases of *Arabidopsis* and their relevance for photosynthesis

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Post-translational modifications, such as lysine acetylation, allow cells to rapidly respond to varying environmental conditions. Lysine acetylation has the potential of altering localization, interactions or enzymatic activities of proteins. Specific

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acetyltransferases catalyze the acetylation of the ε-amino groups of internal lysine residues by transferring acetyl-groups donated by the metabolite acetyl-CoA. Whereas lysine acetylation of histone proteins has been extensively studied, the role of non-nuclear lysine acetylation is so far only poorly understood. With the help of modern high-resolution mass spectrometry it has been found that lysine acetylation is particular abundant on photosynthesis-related proteins. The aim of this project is the identification of putative plastidial lysine acetyltransferases (KATs) by localization assays and *Arabidopsis* knockout mutant analysis as well as by testing recombinant proteins concerning their KAT activity.

P.06-019-Mon
What are the effects of protein nutrition on photosynthetic parameters and stress markers in tobacco plants?

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Despite of plant mineral nutrition being a well-studied topic, organic-bound nitrogen has only recently attracted more attention as a part of plant N nutrition for sustainable agriculture. The aim of our study was to find out how protein casein as a sole N source affected photosynthetic performance and stress level of tobacco grown *in vitro*. Casein, similarly as inorganic N limitation, reduced plant growth. Photosynthetic parameters evaluated by chlorophyll *a* fluorescence induction using the JIP test confirmed also decreasing photochemical efficiency during the primary light phase of photosynthesis. Casein-supplied plants showed a significantly lower performance index (PI_{ABS}), which characterizes the function of the whole electron transport. In casein-grown plants, specific parameters showed also an increasing portion of energy dissipated by various mechanisms, not used for photochemistry in PSII. The N-limited plants exhibited the same trend, but their parameters were less declining. In order to determine the level of stress caused by casein as a N source, we measured the content of phenolic compounds, reactive forms of oxygen and the activity of superoxide dismutase. In the plants supplemented with casein, the content of hydrogen peroxide was comparable and the superoxide radical was slightly lower than in the inorganic N-abundant plants. Moreover, the plants grown with casein possessed beside Cu/Zn isoform of superoxide dismutase also Mn-isoform. The content of phenolic compounds was correlated with the antioxidant capacity. This project was supported by Charles University (UNCE 204025/2012).

P.06-020-Tue
The interplay of light, cytokinins and cytokinin receptors during induced senescence of *Arabidopsis* leaves

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Plant hormones cytokinins have been studied for many decades and their ability to slow down or postpone the process of leaf "ageing" (senescence) belongs to their well-known effects. Senescence is essentially connected with a decrease in chlorophyll content and photosynthetic activity and with an increase in lipid peroxidation. Cytokinins are known to retard all these changes. However, we show that cytokinins can have the opposite effect – they can accelerate senescence if their concentration exceeds a certain threshold. Our studies further revealed that not only cytokinin concentration, but also the light intensity radically affects the result of cytokinin activity. In order to understand the interplay between cytokinin and light more deeply, we applied cytokinins exogenously (0, 10^{-7}, 10^{-6} or 10^{-5} M 6-benzylaminopurine, BAP) on detached leaves of *Arabidopsis*. For these experiments we have used three *Arabidopsis* mutants which have functional only one of the three known cytokinin receptors (AHK2, AHK3 or AHK4). These leaves were kept under various light conditions and changes in photosynthetic performance, lipid peroxidation and levels of endogenous cytokinins were analysed after six days. While AHK3 was the main receptor mediating the effect of cytokinins on chlorophyll content and photosynthetic function, AHK4 primarily mediated the cytokinin effect on lipid peroxidation. AHK2 was able to mediate both of these effects, but only partially. We found that the threshold concentration of BAP that leads to slowdown/acceleration of senescence-induced changes was different for different parameters and was also affected by light.

P.06-021-Wed
The mutation disrupting the wax layer formation impact on soluble sugars and total phenolics content of rye near-isogenic lines under soil drought

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Rye has a number of positive attributes, excellent drought tolerance would be the most interesting one considering the increased summer temperatures and limited rainfall periods. This trait may be connected with the intense waxy bloom on the stem and leaf sheath. In our study the connection between wax covering green parts of rye and biochemical responses to soil drought stress was investigated. The aim of the study was to determine the differentiation of rye near-isogenic lines (NILs) in terms of soluble sugars content (SSC) and total phenolics content (TPC) as a result of the mutation disrupting the wax layer formation impact. The research material consisted of two pairs of rye inbred lines

(811,811bw;L35, L35bw). Each pair comprised of "waxy" and "waxless" line ("bw" suffix) bearing recessive mutation leading to disorder in formation of wax coating. All lines were exposed to a 3-week drought stress, then the flag leaves were cut off, frozen and lyophilized. The analysis of SSC and TPC content were conducted. The experiment was carried out in 2 years. During both years of experiment a decrease of both parameters was induced by water shortage. Among plants grown in water deficit during the first and the second year, the maximum TPC level was observed for waxless line L35bw. The results obtained during both years revealed significant differences among tested pairs. Each of the differences (12–78%) indicated higher amount of TPC in waxless lines over both treatments and each year of the trial. The SSC content differed between the years. After the first year of experiment under both treatments waxless lines showed higher amount of SSC than their waxy equivalents, line 811bw had almost 3 times more soluble sugars than line 811. The second year showed some changes in comparison to second year, under drought stress line 811bw accumulated higher SSC amount than the waxy 811, whereas L35 exhibited opposite results by lower accumulation of SSC than the L35. The research was funded by the National Science Center, Poland, under project no. 2015/17 / B / NZ9 / 01694.

P.06-022-Mon
The mutation disrupting the wax layer formation impact on photosynthetic pigments content of rye near-isogenic lines under soil drought

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Drought caused by soil water deficit is one of the most important constraint for crop improvement all over the world. Rye has a number of interesting features, such as outstanding cold hardiness, strong disease resistance and valued excellent drought tolerance. The aim of the study was to analyze the differentiation of near-isogenic lines (NILs) of rye in terms of selected biochemical parameters as a result of the mutation disrupting the wax layer formation impact. Two pairs of NILs were tested (811,811bw; L35,L35bw), each one consisted of the typical line ("waxy") and the one with recessive mutation causing disorder in forming proper wax coating ("waxless", denoted as "bw" suffix). Tested lines were exposed to a 3-week drought stress, then the wax layer was collected, later on weighted (mg per g of leaf Drought Weight) and analyzed by GC-FID. The flag leaves were cut off, frozen and lyophilized for photosynthetic pigments content (PPC) analysis. All lines subjected to drought stress reacted by lowering the PPC level: the concentration of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll *a*+*b* (TChl) and carotenoids (Car). The differences between waxless and waxy lines under water deficit was observed within all parameters and lines, biggest variety occurred between the concentrations of Chl *a*. Waxless line 811bw demonstrated significantly higher values of Chl *a,* Chl *b* and TChl than the waxy 811, whereas Chl *a* and Car content was higher for the waxy line of pair L35,L35bw. The wax content varied among lines, from 13 to 15 mg/g DW and from 12 to 22 mg/g DW under control and reduced soil moisture conditions, respectively. Almost all lines increased wax content under drought conditions. The wax composition stated as hydrocarbon chain length showed higher accumulation of odd chain hydrocarbons by lines bearing mutation under both conditions, at the same time even chains were lowered. Differences between the lines were noticed. The research was funded by the National Science Center, Poland, under project no. 2015/17 / B / NZ9 / 01694.

P.06-023-Tue
Actin depolymerization could result in increased plant resistance to pathogens

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The integrity of actin cytoskeleton plays a key role in plant immunity, both by providing a physical barrier and by its involvement in the transport of callose, antimicrobial compounds and cell wall components to the site of infection. Consequently, it is generally assumed that actin disruption reduces plant resistance to pathogen attack. However, in a previous study, we reported that actin depolymerisation triggers the salicylic acid (SA) signalling pathway (Matoušková et al. 2014). Increased SA level in plants is associated with enhanced plant resistance to pathogen attack. Here we present the attempt to solve this apparent inconsistency by showing that the relationship between actin depolymerization and plant resistance is more complex than previously thought. We investigated the nature of this relationship using two completely different plant pathosystems: i) a model plant (*Arabidopsis thaliana*) and a bacterial pathogen (*Pseudomonas syringae*), and ii) an important crop (*Brassica napus*) and its fungal pathogen (*Leptosphaeria maculans*). We demonstrated that actin depolymerization induces a dramatic increase in SA levels and that in this case SA is biosynthesized by the isochorismate synthase pathway. In both pathosystems, disruption of actin filaments led to significantly increased resistance toward the infection.

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P.06-024-Wed
Arsenic accumulation and speciation in Pteris cretica and Pteris straminea

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Arsenic (As) contaminated soils are becoming major global environmental and human health risks due to ubiquity of As and its carcinogenicity. The identification of natural hyperaccumulators of As opens the door for phytoremediation of the arsenic contaminant and sparked the research interest in the biology of As in (hyper)accumulating ferns. Here we report on As accumulation and speciation analysis in two cultivars of *Pteris cretica* and in *Pteris straminea*. The concentrations of As accumulated in ferns grown in greenhouse in soils amended with 20, 100 and 250 mg

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As per kg dry weight (dwt) followed the order *P. cretica* cv. Albo-lineata >> *P. straminea* ≥ *P. cretica* cv. Parkerii; As in roots and fronds of cv. Albo-lineata was more than 10 times greater than in *P. straminea*. The highest As concentrations were observed in cv. Albo-lineata grown for 90 days in the presence of 250 mg As per kg. The extracts obtained from disrupted cells of roots and fronds of investigated ferns were fractionated by using the size exclusion chromatography, followed by ICP-OES and RP-HPLC analyses of eluted As species. These analyses identified all As eluting in late fractions corresponding to unbound As. Our analyses excluded the possibility of As binding with peptidaceous ligands, such as phytochelatins, and indicate that investigated ferns deposited As in cells as inorganic or methylated species, irrespective of their accumulation status. This study was supported by Czech Science Foundation, Grant No. 17-10591S.

P.06-025-Mon
Ubiquitination activities of SPL1 and SPL2, two E3 ligases of chloroplast outer membrane

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Ubiquitination plays a vital role in defining a faith of a protein, thus affecting growth and development of a plant. Ubiquitination depends on the activity of three enzymes: E1 activator, E2 conjugating enzyme and E3 ubiquitin ligase. The presence of different E3 ligases have been confirmed in many eukaryotic organelles, but only recently SP1, a RING-type ubiquitin E3 ligase, has been discovered in the chloroplast outer membrane of a model plant *Arabidopsis thaliana (Ling & Jarvis, 2012)*. The protein regulates the TOC (translocon on the outer chloroplast membrane) machinery in response to abiotic stresses by UPS (ubiquitin-proteasome system) *(Ling & Jarvis, 2015)*. In addition, two homologs of SP1, SPL1 and SPL2, have also been identified. To confirm their putative enzymatic activities we heterologously expressed both proteins and conducted *in vitro* ubiquitination assays. We also present our approach to identify their substrates and thus their physiological role in the plant cell.

P.06-026-Tue
Effect of cadmium and its combination with low and hight temperature on activity of antioxidant enzymes in wheat

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Wide range stresses directly or indirectly lead to development of oxidative stress in plants. Plants possess a number of protective mechanisms, including activation of enzymatic and non-enzymatic compounds of antioxidant system that serve to neutralize reactive oxygen species (ROS) and prevent oxidative stress. The comparative study of antioxidant enzymes activity in wheat plants in response to cadmium (Cd) influence in combination with normal (22°C), low (4°C) and high (37°C) temperatures was performed. 7-days-old wheat seedlings (cv. Moskovskaya 39) subjected to cadmium (100 μM) treatment or its combination with low (4°C) of high (37°C) temperature for 1 week. According to the results of electrolyte leakage, MDA content, Cd accumulation in wheat leaves, it was found that in combination of cadmium with low or high temperature, the temperatures have dominant effect, and reaction of plants was similar with the response to separate effect of cold or heat. The activity of superoxide dismutase (SOD) increased in wheat leaves under all stress conditions. Whereas the catalase (CAT) activity increased on 7th day of exposure to cadmium at normal temperature and in combination with heat and dropped under combination of cadmium with low temperature. Moreover guaiacol - dependent peroxidase (G-POD) activity did not changed under combination cadmium with heat and increased at cadmium and its combination with cold. Under all stress conditions on 7th day of experiment the decrease in hydrogen peroxide content was observed that can be a result of effective antioxidant enzymes activation. It was shown that combine effect of cadmium and unfavorable temperatures did not have additive negative effect on some parameters. Due to involvement of the general mechanisms of tolerance, in particular the activation of antioxidant enzymes, the plant realizes a protective cascade reactions directed at the survival of plants under these conditions.

Biotechnology

P.07-001-Mon
Surface functionalization dependent subcellular localization of superparamagnetic nanoparticle in plasma membrane and endosome

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In our research, we elaborate the application of thermal decomposition based Fe3O4 superparamagnetic nanoparticle (SPMNP) for subcellular fractionation in cell biology context. Here, we used thermal decomposition based seed mediated growth for the synthesis of 8 nm Fe3O4 core SPMNP. We performed surface functionalization of SPMNP with phospholipids (Pl) and dimercaptosuccinic acid (DMSA). Surprisingly, we observed surface functionalization dependent SPMNP localization in subcellular compartments such as plasma membrane, endosomes and lysosomes. By using SPMNP based subcellular localization with pulse-chase methodology, we could use SPMNP for high pure-high. Yield organelle (plasma membrane, endosomes and lysosome) fractionation. Previously, several groups have used SPMNP based subcellular fractionation to isolate endosomes and lysosomes with high purity-yield for subcellular omics. For example using Dextran coated SPMNPs, several research groups were able to decipher endosomal trafficking in lysosomal storage disorders. Due to generic nature of SPMNP, our methodology has additional advantage in isolating subcellular compartments such as plasma membrane, endosome and lysosome from any given adherent cells. With additional optimization step, our methodology could be applied for suspension cells. In addition, our methodology does not include any acidic treatment or antibody based pulldown and subcellular compartments are isolated under native physiological conditions. Hence, this methodology would facilitate enzymatic studies, isolating intact membrane protein complexes and structural studies. Thus, SPMNP that are distinctly localized in subcellular compartments can be used as technology for subcellular fractionation that can complement existing tools for cell biology research. As a future perspective, our methodology can be used to isolate subcellular compartments in primary cells and can be extended to in vivo analysis for biochemical and structural biology studies.

P.07-002-Tue
Engineering of cellulase N-glycosylation for boosting the enzymatic hydrolysis of cellulosic materials using combinations of the purified enzymes

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Cellobiohydrolases (CBH) and endo-1,4-β-glucanases (EG) are the major components of extracellular multienzyme systems of cellulolytic filamentous fungi that recently found applications in the growing biotechnology of lignocellulosic biomass conversion to second-generation biofuels and other useful chemicals. Protein engineering of cellulases for enhancing their activity against cellulosic materials remains a hot topic among researchers working in this field. One of the novel approaches is based on site-directed mutagenesis of cellulase N-glycosylation sites in order to remove the respective N-linked glycans from a certain region of a protein molecule restraining the enzyme activity. We used this approach to obtain the recombinant mutant forms of EG II (rPvCel5A), CBH I (rPvCel7A) and CBH II (rPvCel6A) from *Penicillium verruculosum* fungus with enhanced cellulase activities. The engineered forms of the mentioned enzymes having the highest specific activities against cellulosic substrates were used as components of the binary and ternary cellulase mixes in hydrolysis of microcrystalline cellulose (Avicel) and milled aspen wood. Using the mutant forms of rPvCel5A (N194A), rPvCel6A (N219A) and rPvCel7A (N45A) in the mixes together with extra added purified β-glucosidase resulted in significant boosting of the glucose formation from cellulose relative to the combinations of the wild-type enzymes of the same composition. The increase in glucose concentration after 72-h hydrolysis of Avicel and milled aspen wood was 31–36% and 27–40%, respectively, depending on the mix composition. These data will be useful in development of novel fungal strains secreting cellulolytic cocktails with enhanced hydrolytic performance in the processes of bioconversion of lignocellulosic feedstocks. This work was supported by the Russian Science Foundation (grant number 16-14-00163).

P.07-003-Wed
Luminescent properties and application of luciferase isoforms from copepod *Metridia longa*

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Secreted bioluminescent proteins are now widely used as the reporters in bioimaging technologies. The main advantage of using high-active secreted luciferases is a no-lysis protocol that allows the conduction of live cell assays and multiple assays on the same cells. In addition, the luciferases of the kind are also well suited for development of high-throughput screening technologies. Several isoforms of the secreted coelenterazine-dependent luciferase which are responsible for a blue bioluminescence (L_{max} = 480 nm) of the marine copepod *Metridia longa* have been cloned. The amino acid sequences of these proteins (MLucs) are very homologous and comprise a variable N-terminus with a signal peptide and a highly conservative C-terminal part which includes 10 cysteine residues that form up to 5 disulfide bonds. Here we report an effective production of Metridia luciferase isoforms using the baculovirus expression system that provides the obtaining of substantial amounts of pure correctly folded proteins with a high bioluminescent activity, and their biochemical and bioluminescent properties. The isoforms significantly differed in kinetics, temperature optimum of bioluminescent reaction, but all demonstrated a high thermostability and a high bioluminescent activity. The applicability of MLuc7 as reporter for *in vivo* and *in vitro* assays has been verified as well. The fusion proteins based on MLuc7 and a variable fragment of antibody specific to encephalitis virus have been constructed and successfully applied in model immunoassay. We have also shown this isoform to function well as a secreted reporter when expressed in mammalian cells (HEK293). This work offers Metridia luciferase isoforms as bioluminescent reporters with unique properties. Since the properties are different it allows application of these isoforms for various experimental tasks. These studies were funded by RFBR and Government of Krasnoyarsk Territory according to the research project No. 16-44-242099.

P.07-004-Mon
Generation and characterization of a human neutralizing monoclonal antibody against rabies virus using in vitro immunization method

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Rabies is one of the deadliest infectious diseases with fatality reaching up to 100%. Humanized or fully human monoclonal antibodies (mAb) that neutralize rabies virus (RABV) can represent a basis for powerful post-exposure prophylaxis of rabies in humans, possessing several significant benefits in comparison with human or equine rabies polyclonal immunoglobulin. In this study, the aim was to isolate a new human mAb capable of neutralizing RABV. As an alternative to the phage display for the isolation of human mAbs, an *in vitro* method of immunization was used, consisting in the production of human B-lymphocytes from the peripheral blood that produce human antibodies to the G protein of the rabies virus (RABVG). At first, as a result of the incubation of monocytes from human peripheral blood with RABVG in the presence of recombinant cytokines antigen-presenting dendritic cells were obtained. Heterohybridomas of human B-lymphocytes and murine myeloma cells were generated by somatic hybridization with PEG. Using the enzyme-linked immunosorbent assay, clones of heterohybridomas producing high affinity human antibodies against RABVG of IgG isotype were selected. Propagation of the selected hybridoma cells was made for the isolation of the immunoglobulin genes and carrying out immunochemical characterization of the produced antibody. Total RNA from the cells was extracted using TRIZOL reagent and variable genes of light and heavy chain of a new antibody to the RABVG were amplified using cDNA templates and designed specific primers. The antibody was transiently expressed by using a biplasmid expression system based on pcDNA3.4 vector in CHO-cells. The recombinant antibody was purified from cell culture supernatant. Its affinity and specificity assays were analyzed for various vaccine strains of rabies, and the neutralizing activity was determined. This work was supported by a subsidy of the Ministry of Education and Science of the Russian Federation (RFMEFI60716X0154).

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P.07-005-Tue
Mycoplasma HU proteins restore growth deficit of *E. coli* with double genetic knockout of hupA and hupB genes

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Omnipresent in bacteria, DNA-binding histone-like HU proteins (HUs) are nucleoid-associated proteins (NAPs) involved in DNA supercoiling, nucleoid compaction, modulation of DNA-dependent transaction and bacterial adaptation to stress conditions. The first small molecule inhibitor of *M. tuberculosis* HU developed by crystal structure-based drug design demonstrated antibacterial activity. The antimycoplasmic activity of HU inhibitors is supposed to be more profound, as the absence of HUs is lethal for organisms where they are the only NAPs available, e.g. Mollicutes. High genome plasticity of Mollicutes resulting in high diversity of their protein sequences, requires conducting additional in vitro and in vivo assessments of the abilities of newly designed inhibitors to affect target-proteins independently of their sequence and structural variations. In this study, mycoplasma HU proteins: HUSpm from *S. melliferum* - an insect parasite infecting honeybees and HUMgal from *M. gallisepticum* - a poultry pathogen causing severe respiratory disease in chicken and turkeys, were produced under control of arabinose promoter in *E. coli* cells with double knockout of the *hupA* and *hupB* genes coding heterodimeric *E. coli* HU. An electromobility shift assay performed with synthetic oligonucleotide duplexes detected that DNA-binding activities in cell-free extracts of *E. coli* expressing both mycoplasma HUs are similar to those of purified recombinant proteins. Arabinose induction of either HUSpm or HUMgal expression in a slow growing knockout $hupA^{-}/B^{-}$ *E. coli* cells restores the wild-type growth. These findings indicate that both HUs execute all essential functions of histone-like HU proteins. We suggest that *E. coli* cell-based complementation system represents a simple in vivo test for rapid HU inhibitor evaluation not requiring cultivation of the infectious bacteria. This work was supported by the Russian Science Foundation grant 15-14-00063P.

P.07-006-Wed
Efficient DNA-free nanoparticle mediated genome editing of potato using CRISPR-Cas9 RNP complex

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Due to its simplicity, practicality and efficiency CRISPR–Cas9 is rapidly developing as the emerging technology for genome editing on various eukaryotic systems including higher plants and animals. Here we describe new approach for delivery of pre-assembled Cas9–sgRNA ribonucleoproteins into dissected potato (Solanum tuberosum, cv Chicago) shoot apical meristems by chitosan nanoparticles using vacuum infiltration and regeneration of plants with edited alleles. As a model gene for genome editing, we have selected a gene encoding coilin, a major protein of Cajal bodies(CBs). In additional to its traditional role in assembly of CBs, coilin also participates in biotic (virus attack) and abiotic (high salinity) plant stress responses. Using this method of delivery, we have demonstrated DNA-free genome editing and recovery of potato plants with mutated (disrupted) coilin gene. The generated edited plants exhibit high level of resistance to potato virus Y and significant tolerance to osmotic and salt stress. This work opens up a new avenue for practical application of CRISPR-Cas9 technology to produce genome edited (transgene-free) potato plants and other crops. The present work was performed with the financial support from the Russian Science Foundation (grant No. 16-16-04019).

P.07-007-Mon
Development of potentiometric micro-sized urea biosensor based on urease-dextran complex

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Enzymatic sensors for the potentiometric determination of urea belong to the best known class of biosensors. Although potentiometric urea biosensors emphasising on better sensitivity or higher response range are reported, not much effort has been made in resolving the drawbacks of enzyme instability, difficulty in storage and handling, and fragility of the immobilization matrix. These drawbacks can be overcome by enhancing enzyme stability with neutral biopolymers for development of biosensor. Therefore in this study, development of micro-sized potentiometric urea biosensor has been purposed by using modified urease enzyme with dextran, immobilized on ammonium selective electrode surface via glutaraldehyde as a crosslinking agent. The potentiometric micro sized urea biosensor by enhanced stability against environmental conditions using urease-dextran complexes will not include inner reference electrode and inner reference solution. Potentiometric performance of urea biosensor based on urease-dextran complex (selectivity constants, linear working range, determination of limit, response time, pH working range, temperature affect, reproducibility, storage stability) is examined with a computer-controlled measurement system and results are compared with other biosensors based on unmodified urease. The urea biosensor prepared by using urease-dextran complex showed more effective performance than unmodified urease ones.

P.07-008-Tue
Identification of major bee venom allergen Api m 1 IgE epitopes and characterization of their corresponding mimotopes

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Immunotherapy with bee venom extracts is associated with high rate of side effects including severe systemic reactions. Short peptides that mimic IgE epitopes (mimotopes) offer an opportunity for development of safer immunotherapeutics. Due to their small size, they do not possess the ability to cross-link IgEs on effector cells. Moreover, in combination with a suitable immunogenic carrier, they are able to stimulate T cell immune response. Peptides were selected from phage-displayed random peptide libraries and used to computationally map IgE epitopes. Peptide mimotopes of identified epitopes were synthesized. Additionally, peptide mimotopes fused to bacteriophage coat protein pIII were isolated from *Escherichia coli* periplasm and tested for IgE binding with sera of bee venom-allergic patients in the immunodot assay. Basophil

activation test and cytokine production measurement were used to assess the allergenic activity and T cell response modulating potential, respectively. For the first time, two IgE epitopes of major bee venom allergen Api m 1 were identified. They are located in the polypeptide chains and do not include glycosylated moiety. Corresponding peptide mimotopes showed no allergenic activity. Moreover, in contrast to bee venom and Api m 1, which stimulated predominantly type 2 cytokine response, carrier-bound mimotopes induced T cell secretion of type 1 cytokines suggesting a shift from Th2 to Th1 immune response. Identification of Api m 1 IgE epitopes is a very important step for the better understanding of mechanisms involved in the pathogenesis of sting anaphylaxis and can further improve diagnosis and prognosis of bee venom allergy. Furthermore, their respective mimotopes represent novel candidates for the development of safer immunotherapy for bee venom allergy.

P.07-009-Wed
Biochemical activities of sweet-tasting protein brazzein in addition to its sweetness

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Low-calorie sugar substitutes are currently in high demand because the over-consumption of sugar and artificial sweeteners has a variety of side effects such as diabetes and obesity. To date, only eight sweet-tasting proteins are known to elicit sweetness, and these include brazzein, curculin/neoculin, egg white lysozyme, mabinlin, miraculin, monellin, pentadin, and thaumatin. Among these, brazzein is derived from the fruit of the West African *Pentadiplandra brazzeana* Baillon plant and has attracted much attention as a candidate sweetener, because of its potential sweetness, sugar-like taste, and good stability at high temperature and wide range of pH. In order to investigate the bioactivity of brazzein in addition to its sweetness, we utilized the Basic Local Alignment Search Tool (BLAST) program and Vector Alignment Search Tool (VAST) program from the U.S. National Center for Biotechnology Information (NCBI) to compare the primary and higher-order structure of different proteins or peptides. Moreover, in vitro assays were performed to verify the results obtained using BLAST and VAST. Furthermore, the antioxidant, anti-inflammatory, anti-allergic activities, melanogenesis inhibition assay, elastase inhibition assay, and procollagen synthesis assay of brazzein were investigated. Brazzein did not show antibacterial and antifungal activities, although the primary structure of brazzein showed approximately 42–45% similarity with defensin that has antimicrobial effect and drosomycin used as an antifungal agent. However, brazzein exhibited predominant antioxidant effect, showing ABTS radical scavenging activity and DPPH activity. Brazzein also showed an anti-inflammatory activity and an anti-allergic activity through β-hexosaminidase assay and cyclooxygenase-2 (COX-2) inhibition assay. Based on these results, brazzein can be used as a functional sweetener with various bioactivities.

P.07-010-Mon
Targeting aberrantly activated leukocytes in inflammatory bowel disease

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Inflammatory Bowel Disease (IBD) represents a group of chronic inflammatory conditions of the gastro-intestinal tract. Whereas healthy individuals acquired immunological tolerance to commensal bacteria in the gut, IBD patients lost this tolerance resulting in unwanted infiltration of leukocytes and uncontrolled intestinal inflammation. Current therapies rely on both traditional anti-inflammatory agents as well as novel biologics like antibodies against TNF-α. These therapies render the patients vulnerable to infections because they lack specificity. Therefore, we aim to target a specific subpopulation of leukocytes only and manipulate these cells using therapeutic nucleic acids. This population of leukocytes expresses the high-affinity conformation of $\alpha_4\beta_7$ integrin and these cells have been shown to home mainly to the gut. We designed and produced a recombinant protein that has the ability to target specifically these cells. Next, we generated lipid nanoparticles that allow efficient conjugation to this targeting moiety and we have shown that the resulting targeted lipid nanoparticles (tLNPs) bind exclusively to cells expressing the high-affinity $\alpha_4\beta_7$ integrin. The tLNPs can encapsulate high levels of nucleic acid like mRNA or siRNA and the results show that this payload efficiently internalizes into the target cells. Using *in vivo* models of colitis, we tested siRNA-loaded tLNPs to knock down specific genes. Currently we are investigating the therapeutic potential of these tLNPs by using colonoscopy and measuring the expression of pro-inflammatory markers. Furthermore, we are employing novel microPET/CT imaging techniques to improve IBD diagnostics using the targeting moiety conjugated to radioisotopes. To conclude, we designed tLNPs that exclusively target aberrantly activated leukocytes *in vivo* and results indicate that these tLNPs are able to knockdown pro-inflammatory genes making this a potential new IBD therapeutic.

P.07-011-Tue
Structural and functional features of *Lysobacter* sp. XL1 L1 and L5 proteins

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Bacteriolytic enzymes intended for hydrolysis peptidoglycans of competitive bacteria. That is why they are perspective agents as new antimicrobial remedy alternative to antibiotics. Genus *Lysobacter* produces the wide spectrum of different bacteriolytic agents. But their bacteriolytic enzymes are poorly studied. Gram-negative bacterium *Lysobacter* sp. XL1 secretes bacteriolytic enzymes L1-L5. Extracellular serine bacteriolytic proteases L1 and L5 are homologous. However they differ by type of secretion: L5 protein is secreted by means of outer membrane vesicle, and when the L1 protein is secreted by type II secretion system. The objective of the work was the study of structural and functional properties of them. It was obtained of the conditions for crystals appearance for these proteins were adjusted. Diffraction data were collected from L1 and L5 crystals with resolutions 1.35 A and 1.60 A, respectively. Protein L1 has high percent of structural identities to α-lytic protease from *Lysobacter enzymogenes*. The protein L5 has some structure features: compact crystal packing; two domains that different from identical loops of homologous

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enzymes. The protein L1 was co-crystallized with inhibitor AEBSF. It was found that AEBSF interacts with part of protein L1 molecules with atypical reversible inhibition. This type of interaction was not showed early. Also, the specificity of proteins L1 and L5 in respect to *Staphylococcus* peptidoglycan was compared. This revealed that L5 protein has both the amidase and endopeptidase activity, as well as L1 protein. However, protein L5 has much less intensity of hydrolysis of this substrate. For L5 protein was found the ability to amyloid formation. This fact explains the peculiarities of its topogenesis. Thus, obtained results can expand knowledge about structural and functional properties of bacteriolytic enzymes and have an obvious potential for practical usage in biomedicine for creation of a new antimicrobial preparations.

P.07-012-Wed
A novel trimeric design of an immunotoxin targeting colon cancer cells shows significant enhanced antitumor efficiency

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Immunotoxins are chimeric molecules, which combine the exquisite antibody specificity to recognize and bind with high affinity a tumor antigen (target domain), with the cytotoxic activity of a toxin (toxic domain). Thereby, these molecules cause specific cellular death according to the focused antigen presence over cell surface. Currently, the available designs present some limitations for *in vivo* tumor treatment, which drive us to develop new designs with optimized features. In this work we describe the production, purification, and structural and functional characterization of two new immunotoxins. Both are formed by the variable domains of an anti-CEA monoclonal antibody (scFvCEA) and α-sarcin, a potent fungal ribotoxin. The first construction is a single chain monomeric immunotoxin (IMTXCEAαS), while the other one consist in a novel trimeric format (IMTX-TRI-CEAαS). This trimeric immunotoxin is expressed as monomers that oligomerizes by the presence of the N-terminal trimerization region of collagen XVIII NC1. By their trimeric structure, these recombinant design owns enhanced characteristics in comparison to their monomeric analogues, regarding size, functional affinity, serum stability, biodistribution and lower blood clearance, awarding them with highly improved tumor targeting activity. Thereby, the application of this trimeric format to our previously monomeric designed immunotoxins could improve its known *in vivo* antitumoral activity. According to this hypothesis, our results show a significant improve by the trimeric immunotoxin, in binding to CEA-antigen and in specific cytotoxicity caused in in vitro assays, compared to the monomeric one. Furthermore, these findings correspond themselves with an increased activity inhibiting tumor growth in *in vivo* assays, using nude mice bearing colon cancer xenografts, for the trimeric immunotoxin. Therefore trimeric immunotoxins appear as improved immunotherapeutic candidates against cancer pathologies.

P.07-013-Mon
Antistaphylococcal beta-lytic protease from *Lysobacter capsici* VKM-2533T

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Currently, one of the key health problems is the growing antibiotic resistance of strains of *Staphylococcus aureus*, especially for clinical isolates. Thus, according to the European Center for Disease Prevention and Control, infections occurring in the field of surgical intervention caused by *S. aureus* are 20.3%, while according to the report on healthcare-associated infections in intensive care units, 23% of *S. aureus* isolates are resistant to oxacillin. In this regard, the search for new antibacterial agents, acting as an alternative to antibiotics and not causing adaptation of pathogenic strains, is particularly relevant. It was found that the strain *Lysobacter capsici* VKM-2533^T produces a number of extracellular bacteriolytic and antifungal agents. During our research a multistage scheme of proteins purification was developed. One of these proteins identified by MALDI-TOF as a beta-lytic protease (EC 3.4.24.32). The optimization of cultivation and purification procedures managed to get resulted in a high yield of this protein (1 mg/L). A partial characterization of the enzyme was carried out: the optimum of pH, the temperature optimum, the temperature of semi-activation, inhibitory analysis, the optimum concentration of the buffer. A study was carried out to optimize the scheme for obtaining beta-lytic protease crystals. Using the turbidimetric method, the bacteriolytic activity of the beta-lytic protease *L. capsici* VKM-2533^T on living cells of *S. aureus* was established with high efficacy. The study of physicochemical properties and the conduction of a structural and functional analysis of the beta-lytic protease from *L. capsici* will make it possible to create an alternative antibiotic unique antistaphylococcal preparation.

P.07-014-Tue
Withdrawn

P.07-015-Wed
Understanding principles of biological light harvesting through model antennas

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The mechanism that enables biological systems to efficiently capture and transduce light energy is still not fully understood, despite the intense research focus. Here is presented a highly flexible and customisable model light harvesting system for the study of the fundamental principles of biological light harvesting, emphasising some of the most distinct features of biological systems: establishment of energy gradients for energy transduction, precision of chromophore placement and system homogeneity. The model system is based on protein nanofibres comprised of idealised modular tetratricopeptide repeat (TPR) proteins; the TPR proteins provide a regular ordered surface suitable for the display of chromophores with desired spacing and density with atomic−resolution precision, thus allowing to control the extent of chromophore conjugation and to mimic biological arrangements. Individual TPR modules are functionalised with porphyrin chromophores and subsequently stepwise assembled into permanent supramolecular structures through unnatural amino acid-mediated cross-linking. The precise control over the assembly process results in homogenous assemblies, whereas the precise placement of the conjugated chromophores facilitates the establishment of energy gradients.

P.07-016-Mon
Unique image analysis technique for label-free cardiomyocytes stimulated through graphene-based biointerface

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Drug-induced pro-arrhythmic cardiotoxicity represents a major concern for drug discovery, as several blockbuster drugs were removed from the market due to their adverse effects on the cardiovascular system. Utilizing stem cell-derived human cardiomyocytes (CM) and high-content imaging provides an opportunity to overcome the limitations of current cardiotoxicity assays, leading to the increase in assay predictiveness and the decrease in drug development costs. Modern cardiotoxicity assays have to include both (a) dynamic cell stimulation technologies capable of generating different CM contraction rhythms, and (b) efficient monitoring/image of CM activity using optical methods. High-content imaging offers non-invasive monitoring of CM activity at the single-cell resolution. The majority of image analysis approaches requires a cell segmentation step, which makes the whole process very computer-time consuming and incompatible with demands of fast-paced drug screening assays. We use pixel intensity variance over time information from the high-speed transmitted-light greyscale movies to separate active and non-active areas of the image. We track control points by looking for a maximum correlation between points from neighboring frames. Contraction amplitude is calculated as an absolute value of pixel displacement over time and the phase is determined by both maximum amplitude and resting state information. As a result, we receive amplitude-phase space-time profile in an automatic way, allowing us to use the in-depth characterization of the contraction events. The optical setup and the software algorithm were specially designed for autonomous operation to provide reliable measurements from large-scale screens. In summary, our novel image-based analysis in combination with dynamic optical stimulation would allow to greatly improve in vitro assessment of drug-induced cardiotoxicity of new drugs.

P.07-017-Tue
Escherichia coli dual plasmid expression system capable of glycosylated alcohol dehydrogenase production

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As a recent trend in biotechnology, attention is paid to utilization of biocatalytic mechanisms for production of industrial compounds instead of using ineffective and environmentally noxious organic synthesis. We focused on alcohol dehydrogenase from bacteria *Rhodococcus ruber* (RrADH) because of its beneficial characteristics concerning thermostability and tolerance towards common organic co-solvents. Our work is aimed at its heterologous production in the host organism *Escherichia coli* that serves as a relatively simple and accessible means of recombinant proteins production. Current possibilities of protein expression in prokaryotic producers have lately been extended with recombinant protein glycosylation. We decided to apply this novel approach in *E. coli* expression system producing RrADH. The glycan conjugated to the enzyme molecule could serve as a reliable approach to achieve oriented covalent immobilization of RrADH that could bring advantageous reusing and potentially higher enzyme stability. We have successfully created an expression system based on *E. coli* containing the high-copy number vector pJexpress401-RrADH-CGH coding the enzyme conjugated with C-terminal sequences recognized by the oligosaccharyltransferase PglB, and the low-copy number plasmid pACYC-pgl2 containing genes coding enzymes participating in the mechanism of glycan assembly. Here we present the first promising results of our dual plasmid expression system indicating successful *in vivo* bacterial glycosylation process.

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P.07-018-Wed
Quantitation of alpha-ketoglutaric acid in athletic supplements by using D-phenylglycine aminotransferase

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Alpha-ketoglutaric acid (AKG) is a biologically important compound acting as an intermediate in Krebs cycle, a substrate for the synthesis of glutamic acid and glutamine, and a transporter of intracellular nitrogen to the urea cycle. Currently, supplements containing AKG are commercially available e.g. capsules and used for building muscle and maintaining the balance of nitrogen or ammonia levels in the human body. To determine AKG contents in these products, a novel method was developed based on the conversion of AKG to a product with high absorbance namely 4-hydroxybenzoylformic acid using D-4-hydroxyphenylglycine as a co-substrate and D-phenylglycine aminotransferase (D-PhgAT) as a catalyst. By this route, the initial rates of the reaction which were directly proportional to AKG concentrations were spectrophotometrically measured at 25°C by the increase of absorbance at 334 nm during the first 30 s and used for the construction of standard curve. The method showed good linearity (AKG of 20–160 μM, $r^2 = 0.9994$) with the limits of detection and quantitation of 4.09 and 13.62 μM, respectively. It was accurate, precise and repeatable and free from the interference by the excipients in capsules. Furthermore, it gave the results in agreement with HPLC method. Hence, the proposed assay is an efficient and facile alternative method for the quality control of these products.

P.07-019-Mon
Detection of chymase activity using the AuNPs-peptide probe, a specific peptide probe conjugated onto gold nanoparticles

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Gold nanoparticles (AuNPs) with the characteristics of fluorescence resonance energy transfer have the ability of quenching fluorescence with distant dependence. Based on this, we have established a sensitive and efficient biosensing method by modifying peptide-probe onto the AuNPs to detect enzyme activity. The method is designed for chymase activity detection and applied in renal disease diagnosis. In this study, 15-nm AuNPs were used to establish the AuNPs-based fluorescence peptide probe (AuNPs-peptide probe). The peptide sequence is FITC-Acp-DRVYIHPFHLDDDDDC which contains a fluorophore at the C-terminal end, enzyme (chymase) substrate (DRVYIHPFHL), a spacer (DDDDD) and a cysteine (C) used for conjugating to AuNPs. When the enzyme catalyzed the substrate sequence, fluorophore drifted away from AuNPs and the fluorescence emitting signal can be excited at 485 nm and detected at 515 nm. The results show, detection time of the AuNPs-peptide probe used for activity detection of chymase is only needed 15 min and a linear correlation from 10 to 120 ng/mL of chymase is acquired. Detection limit of the probe is about 5 ng/mL and detection sensitivity could be significantly increased using spacer-enhanced peptides, which designed with the concepts based on the length and charge of peptides. The chymase reaction would be obviously inhibited by adding a specific chymase inhibitor, chymostatin. The specificity of AuNPs-peptide probe was also identified by trypsin and chymotrypsin reactions. The AuNPs-peptide probe has been applied in detecting chymase activity as an indicator of nephropathy in a mouse model induced by aristolochic acid I (AAI) challenge. The probe was successfully used for the detection of renal chymase activity, and the results show that pathogenically increased chymase activity in the kidney tissue of nephropathic mice treated with AAI. This is the first study using an AuNPs-peptide probe for the detection of chymase activity.

P.07-020-Tue
Identification of novel genes coding for cold-active glycosidases with biotechnological potential

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Due to many unique properties of cold-active enzymes such as high catalytic efficiency at low temperatures associated with a high thermosensitivity, they have a significant potential to participate in various industrial processes. In this work, we were searching for genes coding for new cold-active amylases and cellulases from cold-adapted microbial strains. In the previous experiments, activities of the selected glycosidases at low temperatures were tested in different samples containing microorganisms originating from various cold areas. Due to the obtained temperature profiles, two yeast (*Mrakiella aquatica* and *Cryptococcus victoriae*) and one bacterial (*Arthrobacter* sp. C1-1) strains, showing the highest amylase or cellulase activities at low temperatures, were chosen for the identification of responsible genes in their genomes. First, gene-specific (GS) primers were designed based on the similarities found in available sequences of amylases or cellulases from the organisms most phylogenetically related to the chosen isolates. The microbial strains were then cultivated at 15 °C and used for the isolation of bacterial genomic DNA (gDNA) or yeast RNA. For the amplification of bacterial target genes, we performed PCR reactions using the GS primers and the gDNA as a template. In the case of the yeasts, a template DNA was obtained by reverse transcription of the isolated RNA. The combination of 3'RACE and 5'RACE methods was used to sequentially amplify the whole sequence of target genes using the set of GS primers and the prepared DNA. At the moment, we are still in the process of mapping the gene sequences. In future, the cold-active glycosidases coded by the identified genes will be produced in the form of recombinant proteins and deeply characterized.

P.07-021-Wed
Effective T2R and G alpha co-expression system using IRES bicistronic vector

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Human taste receptor type 2 (T2R) consists of 25 members belonging to the GPCR superfamily, which mediates bitter taste perception and protection against xenobiotic substances. The binding of ligands to T2R causes the increase in intracellular calcium level via activation of G protein α subunit (Gα) in taste cells. Thus, T2R and Gα co-expressing cells have been used to evaluate the function of T2R *in vitro*. To date, several

approaches for constructing T2R and Gα co-expressing cells have been reported, and the character of each T2R have been also revealed practically. However, constructing T2R and Gα co-expressing cells having enough sensitivity for ligands in a stable manner is not easy, which prevents the progress of the study. Here, we show the efficient method for constructing functional T2R and Gα co-expressing cells by using bicistronic vector containing internal ribosome entry site (IRES). In this study, we constructed two kinds of expression vector, T2R-IRES-Gα and Gα-IRES-T2R, that exchanged the arrangement of T2R and Gα each other across the IRES for evaluating the T2R sensitivity against the corresponding ligand. As a result, the cells transfected using Gα-IRES-T2R showed higher sensitivity for the corresponding ligand. It was reported that the efficiency of IRES-mediated translation was lower than that of cap-dependent translation in many cases. However, T2R expression level in Gα-IRES-T2R transfected cells was more than twice as high as in T2R-IRES-Gα transfected cells. Furthermore, IRES-mediated T2R expression level kept higher than that of cap-dependent expression, even though we changed the promoter, untranslated region and Gα sequences of the vector. The results obtained in this study indicate that Gα-IRES-T2R is a suitable design for constructing T2R and Gα co-expressing cells and can be useful for the progress of T2R study.

P.07-022-Mon
Development of marker-free pod borer resistant pigeonpea (*Cajanus cajan*)

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Pigeonpea (*Cajanus cajan* L.), a high-quality protein-rich grain legume of semi-arid tropics (SAT), is severely affected by lepidopteran insect pests. For sustainable resistance against this pests, chloroplast targeted synthetic version of the bioactive core component of a crystal protein (Syn cry1Ab) of *Bacillus thuringiensis* was expressed in pigeonpea under the control of green-tissue specific ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit (*rbcS*) gene promoter. *Agrobacterium*-mediated transformed plants generated with the expression cassette (*cry1*Ab-*lox-bar-lox*) showed high insect mortality rate (90%) *in-vitro* against *Helicoverpa armigera* in the T1 generation, indicating the insecticidal potency of Syn cry1Ab. Alongside, another vector with chimeric *cre recombinase* gene under the constitutive (2x35s) promoter was designed for the elimination of selectable marker *bar* (bialaphos resistance) gene. Crossing experiments were performed between T1 plants with single insertion site of *cry1*Ab-*lox-bar-lox* T-DNA and one T1 plant with moderate expression of cre recombinase with linked hygromycin resistance (*hpt*II) gene. Marker gene excision was achieved in hybrids with up to 35.71% recombination efficiency. Insect-resistant transgenic lines, devoid of the selectable marker (*syn bar* + *cre-hpt*II), were established in the subsequent generation through genetic segregation.

P.07-023-Tue
Obtaining of recombinant VP2 protein of chicken anemia virus

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Chicken anemia virus (CAV) is a small non-enveloped icosahedral ssDNA virus belonging to the genus Gyrovirus of the Anelloviridae family. Infection of young chickens with this virus leads to T cells and hemocytoblasts depletion resulting in anemia and severe immunosuppression. Weakened immunity, in turn, contributes to increased susceptibility of chicks to secondary viral and bacterial infections, which is a serious problem for poultry industry. CAV has circular genome of 2.3 kb encoding only 3 proteins. One of them – VP2 – is a dual-specificity protein phosphatase, which catalyses the removal of phosphate from both phosphotyrosine and phosphoserine/phosphothreonine substrates. It was shown also that VP2 possibly acts as a scaffold protein in virion assembly. We decided to obtain recombinant VP2 in a prokaryotic expression system for the possible use of it as an antigen in diagnostic test kits. To do this, a fragment corresponding to VP2 ORF was amplified by PCR from the genomic DNA of CAV strain circulating in Russia. Further, this fragment was cloned into plasmids pETmin and pET15mcs under the control of the inducible T7 promoter. The resulting vectors pCAV-VP2-H6 (based on pETmin) and pH6-CAV-VP2 (based on pET15mcs) encoded the target protein with a 6-histidine tag at the C- or N-end, respectively. *E. coli* Rosetta2 (DE3) cells were transformed with these plasmids, expression of the target genes was induced by the addition of IPTG (final concentration 1 mM). Interestingly, recombinant VP2 with N-terminal histidines accumulated in cytoplasmic fraction in soluble form, whilst VP2 with C-terminal histidines was detected predominantly in inclusion bodies. The yield of protein in both cases was approximately 30 mg/L. VP2 with N-terminal histidines was purified by metal-chelate chromatography on a Ni-Sepharose column. This work was supported by the Russian Science Foundation (Project 16-16-04051).

P.07-024-Wed
Biosynthesis of hispidin by plant type III polyketide synthases in yeast and mammalian cell cultures

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At present there is a growing interest in a fully genetically encodable bioluminescence system. Recently, our research group has established the structure of fungal luciferin (3-hydroxyhispidin)

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and characterized two bioluminescence-related enzymes: luciferase and hispidin-3-hydroxylase from the fungus Neonothopanus nambi. Hispidin-3-hydroxylase converts luciferin precursor, hispidin, plant and fungal secondary metabolite, into luciferin. There are evidences that hispidin itself can be produced from caffeic acid or caffeoyl-CoA by enzymes of polyketide synthase family, however, no genes coding for enzymes of hispidin biosynthesis have been identified. Here, we have experimentally demonstrated activity of several plant type III polyketide synthases in hispidin biosynthesis from caffeoyl-CoA. We compared functionality of thirteen plant type III polyketide synthases in yeasts Pichia pastoris. We chose seven best-performing enzymes and tested their functionality in mammalian cell culture. Five enzymes efficiently converted caffeoyl-CoA into hispidin in both heterologous systems and we can distinguish the best three of them. Assembly of expression plasmids and functional tests were supported by the Russian Science Foundation grant 17-14-01169.

P.07-025-Mon
The improved technology of HER2-specific monoclonal antibody production in plants

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Similar to animal cells, plant cells possess mechanisms for protein synthesis and post-translational modifications that allow to considered them as factories for monoclonal antibody production. Using a transient expression system, we previously developed a technology for the production of the HER2-specific monoclonal antibodies trastuzumab plant biosimilar (TPB) and pertuzumab plant biosimilar (PPB) and showed that their anti-cancer activity in animal models is not inferior to the original therapeutic monoclonal antibodies (mAbs). Here, we suggest two ways to improve this technology. The first method involves the creation of bispecific antibodies that combine the properties of TPB and PPB (Bi-T/P_PB). The resulting antibodies variants differed in stability and output but invariably demonstrated a high level of anti-cancer activity. The second approach is based on the novel promoter of the Kunitz peptidase inhibitor (proKPI) that we have isolated. This promoter effectively directed the expression of genes encoding the TPB light and heavy chains in plant cells. Moreover, we created the proKPI-based vector that encodes the fusion protein consisting of TPB light and heavy chains joined by a 33-aa linker, which is recognized by the *N. benthamiana* endogenous kex2p-like protease. The processed TPB (pTPB) variant showed a high level of anti-cancer activity but also contained a small fraction of the unprocessed form of the polypeptide. The increased level of endogenous kex2p-like protease could result in more effective processing of the pTPB polypeptide. Therefore, we isolated the gene encoding the endogenous kex2p-like *N. benthamiana* protease to achieve its overexpression in plants. We believe that improving the technology of producing mAbs plant biosimilars will make them attractive for cancer therapy because of their economic benefits and high production rates. This study was performed with financial support from the Russian Science Foundation (project No. 16-14-00002).

P.07-026-Tue
Heterologous expression of three minor laccases from *Trametes hirsuta* 072 and their properties

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Laccases (EC 1.10.3.2) are lignolytic enzymes with potential use for various industries. In basidiomycetes they are encoded by multigene families but protein production is limited to a small spectrum of isozymes, usually of major forms. Minor isozymes are either produced in small quantities by native producers, or not produced at all, thus they are almost uncharacterized. Laccase isozymes within the family differ significantly in predicted properties, and are of significant interest for biotechnology. To obtain and characterize minor *Trametes hirsuta* 072 laccases, the *Penicillium canescens* expression system was proposed. For this, recombinant plasmids carrying the cDNA sequences of the three genes *lacC, lacD* and *lacF* under control of the strong promoter of the *P. canescens bgaS* gene were constructed. The plasmids were checked for correct assembly and no errors by restriction analysis and subsequent sequencing, and then integrated into the *P. canescens* genome. Screening of the obtained transformants for laccase production was carried out by ABTS-substrate test under induction conditions of the bgaS promoter. The level of laccase expression was determined by qPCR. The selected recombinant *P. canescens* strains were subjected to cultivation in a liquid medium, the laccase production into cultural broth was estimated by activity measurement with pyrocatechol substrate. Isozymes were purified, the correspondence of proteins to proper isozymes was confirmed by mass spectrometry. Preliminary analysis showed that isozymes differ in their biochemical properties (Mr, pI, temperature and pH optimum). Thus, effective expression systems for 3 minor laccase isozymes LacC, LacD and LacF from *T. hirsuta* 072 were obtained. This work serves a basis for further investigation of the minor isozymes properties and development of enzyme preparations for various fields of the biotechnological industry. This work was supported by a Grant of RFBR 18-04-00983.

P.07-027-Wed
Engineering research of bionanoconjugates for diagnostic test systems of autoimmune diseases

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The rudimentary stages with potential for development of diagnostic test systems for determining the presence of thyroglobulin in the blood of patients with thyroid problems are depicted in this study. The presented development is supplemented by actual nanotechnologies for obtaining bionanoconjugates of gold nanoparticles with antibodies to antigen. The question of disease diagnostics is very important in contemporary medicine. The necessary preventive measures and drug therapy in the incipient stages of a disease can be very effective in fighting for the life and health of a patient. The variety of diagnostic test-systems for viral, infectious and autoimmune diseases are used at the present time. In the modeling of various diagnostic systems, used

conjugates consist of polymer particle that contains on its surface specific bio-ligands capable of affinity binding to a detectable component (antigen, antibody). We took the IgG antibody as a bioligand. The immune activity of this biomolecule depends on how the polymer attaches to antibody and whether the active site of the protein is blocked. Therefore, it was proposed to immobilize gold nanoparticles on the antibody surface in various concentrations to select the optimal conditions for conjugate creation. It is expected that, due to their small size, they will not block the antibodies active sites. Using the chemical reduction of tannin, we obtained gold nanoparticles of size 40 nm. To prepare biononconjugates gold nanoparticles were mixed with antibodies to thyroglobulin in various concentrations. These conjugates were studied using the Transmitting Electron Microscope. The photographs show that nanoparticles on the surface of the protein are so small that they do not block its active site and do not interfere with its normal functioning. The publication was supported of the "RUDN University Program 5-100" and the Ministry of Education and Science of the Russian Federation (the Agreement No. 02.A03.21.0008)

P.07-028-Mon
Bioethanol production by consortium of *Trichoderma reesei* and yeast from cellulose

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Industrial cellulosic ethanol production is still a challenge due to the high cost of cellulase for hydrolysis after using lignocellulosic materials as feedstock. In this study, bioethanol was produced from cellulose by consortium of *Trichoderma reesei* and yeasts (*Candida molischiana* or *Saccharomyces cerevisiae*). Different concentrations of cellulose (Avicel) was hydrolyzed by a fully enzymatic saccharification process using *T. reesei*. The reducing sugar produced during the process was further used as carbon source for bioethanol production using *C. molischiana* or *S. cerevisiae*. *C. molischiana* could utilize both glucose and cellobiose, while *S. cerevisiae* could utilize only glucose. Consortium of *T. reesei* with two yeasts revealed that *C. molischiana* led to efficient bioconversion of reducing sugars to ethanol than that of *S. cerevisiae*. The maximum reducing sugar, glucose, and ethanol yields were 42%, 26%, and 20%, respectively, from 20 g/L Avicel. At higher Avicel concentration of 50 g/L, the maximum concentrations of reducing sugar, glucose, and ethanol were 10.9 g/L, 8.57 g/L, and 5.95 g/L, respectively, with consortium of *T. reesei* and *C. molischiana*.

P.07-029-Tue
Use of 7-methyl-2′-deoxyguanosine for the preparation of 2′-deoxynucleosides

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Enzymes are widely used in industry for carrying out various transformations and producing useful substances and materials. Enzymatic transglycosylation - a transfer of the carbohydrate moiety from one heterocyclic base to another - is being actively developed and applied for the synthesis of biologically active nucleosides. This reaction is catalyzed by nucleoside phosphorylases (NPs), which are responsible for reversible phosphorolysis of nucleosides (Nuc) to yield the corresponding heterocyclic bases and monosaccharide 1-phosphates. Nuc + pi ↔ Sug-p + Base Nucleoside area is one of the most fruitful part of medicinal chemistry. About 100 nucleoside-based drugs were developed on their basis. Two main methods are employed for the preparation of nucleoside analogues. One of them is based on the modification of natural compounds. In another one, heterocycle or monosaccharide are modified followed by the formation of *N*-glycosidic bond. Enzymatic methods for the formation of a glycosidic bond complement chemical procedures and, in several cases, have obvious advantages. We found that 7-methyl-2′-deoxyguanosine (7MedGuo) is an efficient and novel donor of the 2-deoxyribose moiety in the enzymatic transglycosylation for the synthesis of purine and pyrimidine 2′-deoxyribonucleosides in excellent yields. 7MedGuo + Ura ↔ 7MeGua + dUrd. We have studied the stability of 7MedGuo at various pH values, different temperatures, as well as a reagents' ratios (7-Me-dGuo: Base: phosphate) in order to find optimal enzymatic transglycosylation conditions. Using the developed methodology several practically important purine and pyrimidine 2′-deoxyribonucleosides were prepared in high yields. This work was supported by Russian Science Foundation (project No. 16-14-00178)

P.07-030-Wed
Functional and structural characterization of a novel low molecular weight asparaginase

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Bacterial asparaginases (ASNAse) from *Escherichia coli* and *Erwinia chrysanthemi* are high molecular weight tetrameric enzymes (140 kDa) considered as gold biopharmaceuticals largely used in hematological malignancies as acute lymphoblastic leukemia (ALL). In ALL the neoplastic cells are unable to synthesize adequate amounts of asparagine (Asn) and are dependent of extracellular sources. The ASNAse administration depletes Asn from serum, resulting in the death of the neoplastic cells by Asn starvation. Meanwhile, several side effects are associated with their administration, including immunological reactions, neurotoxicity and coagulation abnormalities. The high molecular weight of these enzymes (140 kDa) and a secondary glutaminase activity are frequently related to the side effects and the search for novel enzymes with better characteristics is important. Recently, using molecular biology techniques we characterize a novel asparaginase named ASNAseM. Size exclusion chromatography experiments revealed a monomeric enzyme presenting low molecular weight (~ 45 kDa) and circular dichroism spectroscopy analysis revealed an alpha/beta structure containing ~ 34% of beta sheets and 16% of alpha helix. Thermal analysis also showed that ASNAseM is thermal resistant, maintaining the secondary structure in temperatures higher than 50°C. Through biochemical assays we determined the kinetic parameters as the catalytic efficiency over asparagine as ~ 1.08×10^4 M^{-1} s^{-1} and that the enzyme presents allosteric behaviour with $S_{0.5}$ to Asn determined as ~ 0.35 mM. Additionally, biochemical approaches revealed that the enzyme is free from glutaminase activity. Finally, preliminary cytotoxicity assays using HUVEC (normal cells) and MOLT4 (leukemic cells) revealed that the ASNAseM induced apoptosis in leukemic cells. Together the data presented here indicates that ASNAseM is a promising enzyme to be used as a biodrug in ALL. Financial Support: FAPESP.

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P.07-031-Mon
Enhancement of biohydrogen production by uncouplers in new green microalga *Parachlorella kessleri*

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Green microalgae are suggested to produce biohydrogen (H_2) in photosynthetic reactions using water as electron source and the sunlight as energy source; the responsible enzyme is [Fe]-hydrogenase. The present work was aimed to study H_2 production properties in green microalga *Parachlorella kessleri*, newly isolated from Armenia, in the presence of uncouplers: cyanide *m*-chlorophenylhydrazone (CCCP) and 2,4-dinitrophenol (DNF). The effect of uncouplers on H_2 production ability of *P. kessleri* has been investigated in the presence or absence of sulfur (S). It is known, that S-deprived conditions cause a reversible inhibition of photosynthetic activity of some green algae, and enable the separation of O_2 and H_2 productions. Both uncouplers used showed positive effect on H_2 production by *P. kessleri*. The highest H_2 yield of ~2.15 mmol/L was obtained in the presence of 15 μM CCCP and 50 μM DNF. Moreover, the H_2 yield in microalgal culture was ~2.7 fold lower under S-deprived conditions. These results indicated that the O_2 production activity of microalgal culture was not inactivated under S-deprived conditions, and the anaerobic conditions for expression [Fe]-hydrogenase were not created. The duration of H_2 production in the presence of uncouplers was delayed up to 48 h, whereas in the control culture (without uncouplers) H_2 production decreased after 24 h. Moreover, during dark conditions H_2 production by *P. kessleri* was not observed, indicating that H_2 yield in this microalga was mediated by light. Thus, the enhancing effect of uncouplers can be coupled with dissipation of proton gradient across thylakoid membrane in chloroplasts, increasing the accessibility of protons and electrons to [Fe]-hydrogenase. Uncouplers can also intensify the inactivation reactions of the photosynthetic water-splitting complex, which leads to the inhibition of photosystem II activity, resulting in creation of anaerobic conditions and production of H_2.

P.07-032-Tue
Microbial platform to synthesize aromatic chemicals and the derivatives

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A synthetic metabolic pathway suitable for the production of chorismate derivatives was designed in *Escherichia coli*. An L-phenylalanine-overproducing *E. coli* strain was engineered to enhance the availability of phosphoenolpyruvate (PEP), which is a key precursor in the biosynthesis of aromatic compounds in microbes. Two major reactions converting PEP to pyruvate were inactivated. Using this modified *E. coli* as a base strain, we tested our system by carrying out the production of salicylate, a high-demand aromatic chemical. The titer of salicylate reached 11.5 g/L in batch culture after 48 h cultivation in a 1-liter jar fermentor, and the yield from glucose as the sole carbon source exceeded 40% (mol/mol). In this test case, we found that pyruvate was synthesized primarily via salicylate formation and the reaction converting oxaloacetate to pyruvate. In order to demonstrate the generality of our designed strain, we employed this platform for the production of each of 7 different chorismate derivatives. Each of these industrially important chemicals was successfully produced to levels of 1–3 g/L in test tube-scale culture. In addition, by extending chorismate pathway, we successfully achieved maleate production, which is one of significant dicarboxylic acid as well as succinate and malate. A novel synthetic pathway of maleate was constructed in our base strain, and the productivity reached 7.1 g/L. This is the first report about maleate production using genetically engineered micro-organisms.

P.07-033-Wed
Quantitative biochemical characterization and biotechnological production of caspase modulators, XIAPs: therapeutic implications for apoptosis-associated diseases

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Regulating apoptosis is a common and essential therapeutic strategy for cancer and neurodegenerative disorders. Based on bench-side basic studies of apoptotic mechanisms, a variety of studies have been attempted to overcome the pathogenesis of such diseases by activating or inhibiting apoptosis. In general, the biochemical characteristics of the target molecules should be performed along with an understanding of their mechanism of action during the drug development. Among apoptotic regulators, XIAP serves as a potent negative regulator to block apoptosis through the inhibition of caspase (CASP) action. Although XIAP is an attractive target with such apoptotic-modulating property, biochemical and biophysical studies of XIAP are still challenging. In this study, the CASP inhibitors XIAP, 242D and D230 were prepared using the pGEX expression system and biochemically characterized. These inhibitors were expressed in *Escherichia. coli* at a concentration of ≥20 mg/L culture under a native condition with 0.01 mM IPTG induction. Notably, using a simple and rapid affinity purification technique, these CASP inhibitors have purified, yielding ≥5 mg/L culture at approximately 90% purity. We have determined that HtrA2 specifically binds to the BIR2 and BIR3 of XIAP at a 1:1 molecular ratio. Moreover, in vitro cell-free CASP activation-apoptosis assays have demonstrated that these purified XIAP proteins dramatically inhibit CASP action. Therefore, our system is suitable for biochemical studies, such as quantitation of the number of molecules acting on the apoptosis regulation, and provides a basis and insights that can be applied to the development of therapeutic agents for neurodegenerative disorders and cancer.

P.07-034-Mon
Milk exosomes: isolation, proteins, and nucleic acids

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Exosomes are extracellular membrane vesicles with the diameter of 40–100 nm. They are secreted by cells and detected in various biological fluids. Over the past decade, the role of exosomes in many physiological and pathological processes in the body has become clear. The presence of proteins, peptides, DNA, mRNA and, especially, microRNA in their composition is shown. The collected data of the exosome composition and functioning serve as the basis for the development of new non-invasive methods for diagnosing various diseases and means of targeted drug delivery in the body. The study of exosomes from milk is very important in the perspective of the development of new approaches to the exosome isolation from complex biological fluids containing a large number of proteins. We have developed an approach that

combines standard protocol of exosome isolation (ultrafiltration, ultracentrifugation) with additional gel-filtration. According to transmission electron microscopy, this technique allows obtaining homogeneous vesicles. Immunohistochemical staining using antibodies to tetraspanins - the main surface proteins of exosomes was confirmed that the obtained vesicles are exosomes. In addition, the same antibodies were used to create columns for affinity selection of exosomes. Analysis of protein composition in highly purified milk exosomes showed the presence of a small number of proteins. This indicates that some milk proteins, which were previously described as exosomal, are not part of the exosomes, but co-isolate with them. For the analysis of nucleic acids, we obtained RNA samples at different stages of the exosome isolation, carried out reverse transcription and PCR in real time using primers to several main microRNAs of milk exosomes. Quantitative amplification of these microRNA can serve as an effective high-performance method for determining the content of exosomes in preparations.

P.07-035-Tue
Characteristics of kazakh sheep breeds by microsatellite DNA loci

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In this study two breeds of Kazakh sheep were genotyped by 11 microsatellite loci. Edilbay and Kazakh arkharomerinos are the predominant breeds of sheep in Kazakhstan. Blood samples were collected from two farms, "Birlik" for Edilbay and "Kumtekey" for Kazakh arkharomerinos. Both of them are very strictly isolated farms. Of total 60 samples 31 Edilbay and 29 Kazakh arkharomerinos were studied. Nuclear genomic DNA was extracted from peripheral blood by using *QIAamp DNA Mini Kit* (Qiagen, USA) reagents. The PCR amplification of microsatellite markers were carried out using Thermal Cycler. PCR products attached in the ABI310 Genetic Analyser, *GeneMapper* software was used to determine fragment size. The genotyping included 11 microsatellite loci (McM42, TGLA53, OarFCB20, INRA49, MAF65, McM527, OarCP49, OarAE119, HSC, MAF214 and OarFCB11) which markers located on different chromosomes. All markers were obtained from the National Center of Biotechnological Information (Gene Bank NCBI, USA). Statistical analysis was realized using *GenAlEx 6.5* software. A total of 114 alleles were obtained from the 11 microsatellite loci. The results showed that all investigated loci were complete (100%) polymorphic in both populations. In Edilbay population the observed heterozygosity ranged from 0.138 (OarFCB11) to 0.655 (OarFCB20), while expected heterozygosity from 0.461 (OarFCB11) to 0.798 (McM42). And in Kazakh arkharomerinos sheep the observed heterozygosity varied between 0.600 (TGLA53) and (OarCP49), while expected heterozygosity from 0.743 (INRA49) to 0.873 (OarFCB11). The mean observed and expected heterozygosity indicated 0.621 and 0.743 respectively. The average values of F_{is}, F_{it} and F_{st} were 0.161, 0.261 and 0.122 accordingly. Within populations the negative values of F_{is} and F_{it} were found at loci OarFCB20 (0.141 and 0.072). Further, this results would be compared with the others native sheep breeds.

P.07-036-Wed
Recombinant VP3 protein of infectious bursal disease virus (IBDV) can elicit protective immune response in chickens

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Infectious bursal disease virus (IBDV) causes a contagious immunosuppressive disease in chickens that is one of the main problems in the poultry industry. It leads to high mortality rates. Young chickens are protected by maternal antibodies, but two weeks after birth it is necessary to control their immune status. Among many ways to create new diagnostic tools and vaccines for industrial poultry, generation of recombinant antigens is believed to be the most promising. We decided to obtain recombinant IBDV antigens. Previously we reported a recombinant VP3 protein obtained in soluble form with the yield 40 mg/L of bacterial culture. The aim of this work was to optimize the cultivation conditions for high yield production of the recombinant VP3 protein in *E. coli* and to test it in IBDV antibody screening assay. We increased the product yield using a fed-batch fermentation process for producing high-density bacterial culture with IPTG induction. The final yield of VP3 in *E. coli* BL21(DE3) Gold cells with pETminVP3 plasmid was 600 mg/L. The recombinant VP3 accumulated in the cytoplasmic fraction in soluble form. The recombinant VP3 was purified by metal-chelate chromatography. The virus challenge test was carried out by pathogenic IBDV virus «72/50-M» strain. To assess the IBDV antibody screening assay, 10-day chickens were parenterally injected with recombinant VP3. Vaccination blood samples were collected, and serum was investigated for the presence of antibodies against the IBDV virus with BioCheck ELISA kit. We showed a 4-fold and 10-fold increasing in antibodies titers 14 and 28 days after vaccination respectively. To assess the immunogenic activity, chickens were infected with a pathogenic strain of the IBDV virus at the age of 40 days (30 days after vaccination with recombinant VP3). Survival in vaccinated group was 12/12, while survival in not-vaccinated group was 2/10. This work was supported by the Russian Science Foundation (Project 16-16-04051).

P.07-037-Mon
Playing on both sides of the mirror: engineered amino acid oxidases for chiral biocatalysis

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The increasing demand for enantiomerically pure compounds from the chemical, pharmaceutical and agrochemical industry renewed the interest on the well-known flavoenzymes D-amino acid oxidase (DAAO) and L-amino acid oxidase (LAAO). Enantioselective biocatalysis, a competitive approach in comparison to classical asymmetric synthesis, requires strict enantioselective

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enzymes. DAAO turned out to be a versatile biocatalyst and a robust scaffold to evolve novel variants active on natural and unnatural amino acids. For this reason, this enzyme was widely exploited in biotechnology. On the other hand, biotechnological exploitation of LAAOs turned out to be an awkward task because of the difficulty in their recombinant overexpression. The recent characterization of an L-amino acid deaminase from *Proteus myxofaciens* (PmaLAAD) allowed to close the circle and to extend the application of chiral biocatalysis to the production of pure D-amino acids. PmaLAAD is specific for large hydrophobic natural L-amino acids (e.g., L-Phe, L-DOPA) and, to a lesser extent, on unnatural substituted alanines. Based on the 3D structure of the enzyme and following sequential rounds of site-saturation mutagenesis, enzyme variants with an improved activity on L-1-naphthylalanine were isolated. The evolved enzyme variants were used in biotransformation reactions on the L-enantiomer and on the racemic amino acid mixture, in the latter case in combination with the M213G DAAO variant. The combined use of evolved variants of DAAO and PmaLAAD represented a competitive and versatile biocatalytic system for α-keto acid production, deracemization and stereoinversion of amino acids of biotechnological relevance.

P.07-038-Tue
Optimization of fermentation condition for α-amylase production

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The study is concerned the growth conditions of *Bacillus amyloliquefaciens* MDC 1974 isolated from soil samples (Microbial Depository Center of SPC "Armbiotechnology" NAS RA) for maximum production of thermostable α-amylase. The strain was identified as *Bacillus amyloliquefaciens* by 16S rDNA phylogenetic analysis. The sequence of 16S rDNA gene was submitted to GenBank under the accession number MF953984.1. With consumers growing increasingly aware of environmental issues, industries find enzymes as a good alternative over other chemical catalysts. Among all groups of hydrolytic enzymes, amylases have been used extensively because of their potential of application in the biotechnology-based industries. α-Amylase can be produced by plant or microbial sources. Due to the advantages that microbiological production offers, to α-amylases of microbial origin was paid great attention by researchers. Strains of *Bacillus* genus are the main sources of α-amylases having the industrial application. Five incubation temperatures (30, 35, 40, 45 and 50 °C), two incubation times (24 and 48 h) have been studied. The growth media contains salts (KH_2PO_4 0.05 M, $MgSO_4.7H_2O$ 0.005 M and $CaCl_2$ 0.001 M), different carbon (glucose, starch and wheat flour (1% w/v)) and a nitrogen (yeast extract (0.5% w/v)) sources. The fermentation was carried out at 7.0 pH in 500 ml Erlenmeyer flasks containing 100 ml growth media by laboratory shaker (Innova 43 Shaker "New Brunswick", USA), 200 rpm. For optimization of each parameters, three sets of independent experiments were carried out and the average values were reported. According to the obtaining results the starch was chosen as a best carbon source. Maximum specific activity of α -amylase (9.2 U/mg) was obtained at 40 °C after 48 h of incubation. The total activity yield was 3770 U. Thus, the α-amylase of *Bacillus amyloliquefaciens* strain will have industrial application.

P.07-039-Wed
Optimization of laccase production by fungi co-cultivation

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In this work production of fungal laccase was optimized using solid state fermentation and kiwifruits peels as support-substrate. Fungi *Irpex lacteus* (MUM 94.08) and *Trichoderma viride* (EXF-8977) were cultivated separately and with each other on kiwi fruits peels, which was pre-treated with base to neutralize organic acid and distilled water, with and without soil for period of 21 days at 25° C by taking samples on 7, 14 and 21 days. Extracellular laccase activity was measured spectrophotometrically using 0.5 mM 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) as substrate, concentration of protein was estimated with Bradford's assay as well as amylase and protease enzyme activities were detected under same conditions. Subsequently, purification and optimization of culture conditions for laccase production was studied with the effect of temperature, pH and moisture on production. Comparison of laccase activity among the designed fungi and combination shows increasing of enzyme production. *Trichoderma viride* (EXF-8977) produces on kiwi peels 518.56 Ul^{-1}, co-cultivation on same condition produce 947.45 Ul^{-1} of laccase. *Irpex lacteus* (MUM 94.08) produces on substrate 196.02 Ul^{-1}, while mixed with soil is able to produce 1366.2 Ul^{-1}. However, co-cultivation of fungi on peels and soil give us 2295.81 Ul^{-1}. According to the results co-cultivation of these two strains on kiwifruits peels and soil increase laccase production.

P.07-040-Mon
A modified E3 ubiquitin ligase CHIP of *Arabidopsis thaliana* for the target degradation of GFP

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Over 5% of *A. thaliana* genes are involved in the ubiquitinylation process. More than a thousand genes are E3 ubiquitin ligases (UL) that perform specific degradation of target proteins. Modification of one of these genes for the recognition of a specific target makes it possible to regulate the level of selective protein degradation in cells using the ubiquitin-proteasome system (UPS). To this end, we have modified the gene of E3 UL Chip for the recognition of GFP and used it for the selective degradation of the GFP-expressing transgenic plants of *N. benthamiana*. We have analyzed two variants of the modified gene of E3 UL Chip, lacking 100 or 140 amino acids at the N-terminus, and fused in the reading frame with the anti-GFP monobody gene. For transient expression in plant cells we have created vectors based on plasmid pCXSN in which the gene encoding Ch100mb or Ch140mb was placed under the control of the strong constitutive 35S promoter of the cauliflower mosaic virus. The resulting vectors were used to transform the A. tumefaciens strain C58. Fluorescence microscopy analysis of GFP degradation was carried out on the 6th day after the infiltration of the leaves of the transgenic *N. benthamiana* expressing GFP with agrobacterium carrying the Ch100mb or Ch140mb genes. No changes in the level of GFP fluorescence were identified in the control samples - in the zone without infiltration, in the infiltration zone with agrobacterium containing the empty vector pCXSN or the pCXSN-E3 vector of UL Chip. In both infiltration zones with

agrobacterium carrying the vectors pCXSN-Ch100mb or pCXSN-Ch140mb, almost complete attenuation of the GFP fluorescence was observed. At the same time, qPCR analysis confirmed that there were no changes in GFP expression in all samples, indicating that the amount GFP was only regulated post-translationally. Thus, we have created the system which allows us to perform the selective degradation of plant proteins via the UPS. Supported by RFBR #15-04-09365.

P.07-041-Tue
The new ability of mycelial fungi *Aspergillus nidulans* to acetylate 11alpha-hydroxyprogesterone

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It is known that biotechnological transformation of steroids using enzyme systems of microorganisms is often the only possible method to modify the molecule in the industrial production of steroid drugs. Mycelial fungus *Aspergillus nidulans* is one of the most well-known eukaryotic model systems. Despite this *A. nidulans* strains have been little studied as steroid-transforming microorganisms. We studied the ability of the *A. nidulans* VKPM F-1069 wild type strain (syn. FGSC A4, ATCC 3863, 12996, 26451, CBS 112.46, NRRL 194) and its auxotroph strain - *A. nidulans* 031 with argB2 and pyrG89 mutations (syn. FP-308.1; AN031; CBS 129193) to transform progesterone (PG) for the first time. It has been established that PG acetoxylation is a two-stage process. In the first stage, the hydroxylation of PG occurs into 11alpha position, then the formed 11alpha-hydroxy-PG is modified into 11alpha-acetoxy-PG. Thus, the acetylated product can be obtained both from PG and from 11alpha-hydroxy-PG. It was shown that argB2 and pyrG89 mutations had no effect on the regio- and stereospecificity of hydroxylation and the ability of the strain to acetylate 11alpha-hydroxy-PG, but influence the ratio of final products and provides an increase in 11alpha-acetoxy-PG. The formation of products was confirmed by the ^{1}H NMR, ^{13}C NMR, 2DNMR, high-resolution mass spectrometry analysis. For 11alpha-acetoxy-PG these characteristics were compared with those for 11alpha-acetoxy-PG chemically synthesized from 11alpha-hydroxy-PG. The potential of mycelial fungi, incl. genus *Aspergillus*, for the biocatalytic esterification of 11alpha-hydroxy-PG, wasn't previously described in available literature. These data can serve as a basis for development of the medical drugs synthesis technology, alternative to chemical synthesis, where *A. nidulans* strains can be used as biocatalysts for the biotechnological modification of steroids. This study was supported by a Grant of RFBR No. 18-34-00653.

P.07-042-Wed
Study of some metals effect on α-amylase activity of *Bacillus amyloliquefaciens* MDC 1974 strain

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α-Amylase (EC 3.2.1.1; α-(1,4)-d-glucan glucanohydrolase) is an endo-type enzyme that hydrolyzes starch by cleaving α-1,4-glucosidic linkages in a random fashion. Most of the α-amylases are metalloenzymes, which require calcium ions (Ca^{2+}) for their activity, structural integrity, and stability. α-Amylase has potential application in a wide number of industrial processes and can be produced by different species of microorganisms. Among them, those from *Bacillus* have been intensively studied and are important in industry. The aim of the work was to study the effect of some metal ions on purified to homogeneity α-amylase activity of *Bacillus amyloliquefaciens* MDC 1974 strain (GenBank, MF953984.1). The reaction for determination of effect of different metal ions on enzyme activity was carried out in the reaction medium containing 0.5% starch, 0.5 mM $CaCl_2$, 50 mM HEPES, pH 7.2 and 5 mM salts of metals, at temperature 60°C. The duration of reactions was 20 min. All metals were used in the chloride or sulfate forms. The amount of reduced groups was determinate by Dinitrosalicylic acid (DNS) method. Obtained results indicated that 80% of α-amylase activity was lost when the enzyme was incubated with 10 mM EDTA, thus, indicating the enzyme was a metalloenzyme. The enzyme activity was not retained after incubation with 10 mM $CaCl_2$. It is shown that Cd^{2+} (residual activity – 39.7%) and Cu^{2+} (residual activity – 43.7%) had strong inhibitory effect, while Mg^{2+} (115.4%), Fe^{2+} (129.4%) and Fe^{3+} (166.9%) enhanced the enzyme activity in studied concentrations. On the other hand, K^{+}, Na^{+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, Ba^{2+}, Pb^{2+}, Co^{2+} did not show any effect on α-amylase activity. Thus, Cd^{2+} and Cu^{2+} are strong inhibitors of studied α-amylase.

P.07-043-Mon
Study of the influence of cells-producers of prokaryotic and eukaryotic origin on the functional activity of the transgenic GDNF

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The aim of this research is to study the effect of prokaryotic and eukaryotic cells on the functional activity of the transgenic GDNF. The study was conducted on PC12 cells, whole and dissociated root dorsal ganglion and neuroblastoma SH-SY5Y. Cell cultures were cultivated in the presence of modified forms of the neurotrophic factor GDNF: GDNF with pro-sequence, GDNF with deletion prepro-sequence mGDNF. Pro - and mGDNF was expressed in two different systems: prokaryotic *E. coli* and eukaryotic - HEK293. The number of neural processes during using the mGDNF derived from transgenic HEK293/mGDNF is greater than during the cultivation in the presence of the mGDNF expressed in *E. coli*. But proGDNF that was synthesized in the prokaryotic system showed better results compared to proGDNF that was synthesized in the eukaryotic system. In general a greater number of sprouts were observed during cultivation in the presence of the mGDNF expressed in the

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eukaryotic system compared to other forms of protein. A significant increase of β3-tubulin expression in the case of cultivation in the presence of mGDNF was confirmed by qPCR. As a result of the action of MPP toxin, the number of living cells was higher during co-cultivation with proGDNF obtained from *E. coli*. An isolated from transgenic eukaryotic cells factor, which showed a good result in maintaining the viability of cells, was mGDNF. The results of the study indicate that proGDNF is necessary to maintain cell viability, at the same time GDNF has increased neuroinductor properties. Significant neuroinductor properties of the mGDNF ensure its use if the population of neural cells reduction is necessary, for example, in cases of traumatic injuries or neurodegenerative diseases. Cells-producers are important for the formation of the studied forms' biological activity of a neurotrophic factor. And, evidently, it is preferable to use GDNF, expressed from eukaryotic cells, due to its higher neuroinductor properties.

P.07-044-Tue
A novel approach for fractional photodynamic therapy utilizing a photosensitizer with an additional 2-pyridone module

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Photodynamic therapy (PDT) is considered a promising approach for the treatment of several types of cancer. Generation of cytotoxic singlet oxygen, which has a short lifetime, increases the probability of selective action. Singlet oxygen generation in a photosensitized manner within cancer tissues during PDT is self-limiting, as the hypoxic conditions within cancers is further diminished during the process. In order to minimize photoinduced hypoxia, the light may be introduced intermittently (fractional PDT) to allow time for the replenishment of tissue oxygen. However, such applications extend the time required for effective therapy. In the current study, we show that a photosensitizer with an additional 2-pyridone module for trapping singlet oxygen would be useful in fractional PDT. Therefore, the endoperoxide of 2-pyridone is generated along with singlet oxygen in the light cycle. The endoperoxide undergoes thermal cycloreversion to produce singlet oxygen in the dark cycle, regenerating the 2-pyridone module. The photodynamic process can thus continue in the dark as well as in the light cycles. We validated this working principle *in vitro* with HeLa (human cervical cancer cell line) cell culture studies with cell viability / cytotoxicity assays (MTT). HeLa cells were incubated with varying concentrations of the compound and illuminated with a red light source (λ = 655 nm LED array, 324 μmol/m^2/s photon flux) for 10 min in every one hour, which was repeated 24 times. It was found that even low doses of this novel drug results in a significant decrease of the HeLa cell viability (CC_{50} value: 8.6 nM). Since both intrinsic tumor hypoxia and PDT-induced hypoxia are considerable issues against the wider applicability of PDT, such bifunctional photodynamic therapy agents may prove to be very promising in the future.

P.07-045-Wed
Multimeric expression of acid-cleaved alpha-fetoprotein receptor binding peptide

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Alpha-fetoprotein (AFP) and its receptors (RECAF) are well known tumor-related biomarkers used in cancer diagnostics and tumor-targeted drug delivery systems. RECAF is known to be expressed by the majority of tumor cells, while its expression is downregulated in non-fetal normally functionalized cells. It is common knowledge that the part of AFP, namely KQEFLIN heptapeptide is essential for RECAF binding. In this study, genetic constructs encoding recombinant peptide multimers, consisting of as few as 1 and as high as 14 extended 23 amino acid RECAF-binding sites (PWGVALQTM*KQEFLIN*LVKQKPQITD) of native AFP linked via Asp-Pro cleavage sites have been prepared and cloned in pET 28a+ plasmid via NcoI and XhoI first, SalI and XhoI 2–14th repeat. The expression analysis of transfected *E. coli* BL21(DE3) strains revealed an increase in productivity upon expression from 2 to 5 monomers, respectively. Strains transformed with 6 and more repeats were found to express mostly 3 membered multimer. The most stable multimers were purified and cleaved at Asp-Pro sites to monomers with formic, acetic and hydrochloric acids in different concentration, time, pH and temperature regimes both with chaotropic agent or not. The cleavage process was analyzed by Tricine SDS-PAGE, RP-HPLC and optimized for each multimer. The most effective cleavage agent was found to be ≥70% formic acid, however, it induce several monomer modifications, which cause difficulties during purification. The major products of formic acid hydrolysis were purified. Further, it is planned to characterize all the products by MALDI-TOF MS and both by cellular and cell free RECAF binding assays to evaluate its potency as vector for tumor-targeted drug delivery systems or and an affinity ligand for RECAF purification.

P.07-046-Mon
Enhancing 3-hydroxypropionic acid production in combination with sugar supply engineering by cell surface-display and metabolic engineering of *Schizosaccharomyces pombe*

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3-hydroxypropionic acid (3HP) is the important chemical for building bio-sustainable society. Here, we describe metabolic engineering of fission yeast *Schizosaccharomyces pombe* for 3HP production via malonyl-CoA pathway from glucose and cellobiose. Genes encoding malonyl-CoA reductase (MCR) of Chloroflexus aurantiacus was dissected into two functionally distinct fragments (MCR-C and MCR-N) and the activity between MCR-C and MCR-N was balanced. To increase the cellular supply of malonyl-CoA and acetyl-CoA, we introduced genes encoding endogenous aldehyde dehydrogenase (atd1), acetyl-CoA synthase from *Salmonella enterica* (acsSE*), and endogenous pantothenate kinase (ptk1). The resultant strain produced 1.0 g/L of 3HP from 50 g/L consumed glucose. We also engineered sugar supply by displaying beta-glucosidase on its cell surface. When 50 g/L of cellobiose was used, the engineered strain efficiently consumed cellobiose and produced 3HP at 3.5 g/L. Finally, this

strain produced up to 11.4 g/L of 3HP under fed-batch condition from cellobiose with a yield of 13% (g-3HP/g-sugar).

P.07-047-Tue
Metabolic design of *Escherichia coli* for muconic acid production

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Adipic acid(AA) is a versatile bulk chemical to be used for raw materials such as nylon 6.6. Currently, AA biosynthesis from bio-resources has received a lot of attention in recent years as environment-friendly and renewable AA production process. Muconic acid(MA), also known as 2,4-hexadienoic acid, is expected as a biosynthesis precursor of AA. There are Several studies on MA biosynthesis using *Escherichia coli* introduced foreign genes. In those studies, MA is synthesized from intermediate products of the shikimate pathway. In this work, the MA synthesis pathway was introduced into the strain whose shikimate pathway was enhanced, and optimized for that strain. In our previous studies, we designed the metabolic pathway of *E. coli* to enhance the shikimate pathway and to produce chorismate derivatives in high yields. Firstly, we selected the MA synthesis pathway from three candidates. Secondly, the selected pathways were enhanced by overexpressing *aroC* encoding chorismate synthase, or *aroD* encoding 3-dehydroquinate dehydratase. Finally, an effect of overexpressing fusion proteins by gene-level fusion method was investigated which containing chorismate synthase and isochorismate synthase, or 3-dehydroquinate dehydratase and 3-dehydroshikimate dehydratase.

P.07-048-Wed
Metabolomics and proteomic analysis of serpentinite-associated bacterium *Rhodococcus* sp. S10

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Iron is an essential trace element in regulation of vital metabolic processes in living organisms. It is well known that iron can considerably modulate the structure of microbial communities in different environments. Microorganisms adapted to geochemically extreme conditions through synthesis and secretion of specific molecules that can chelate iron from the environment and make it available for bacteria. These secreted secondary metabolites and proteins are highly interesting for functional studies because of their direct contact with a mineral surface. Here we report the results of metabolomics and proteomic studies of *Rhodococcus* sp. S10 isolated from serpentinite minerals. We showed that *Rhodococcus* sp. S10 can produce siderophores in CAS agar assay. To study the metabolomic profile of *Rhodococcus* sp. S10 the HPLC analysis was performed. It was demonstrated that *Rhodococcus* sp. S10 grown in a minimal medium under iron-depleted condition produced several types of siderophores. Growth of *Rhodococcus* sp. S10 in the presence of 100 mM ferric chloride inhibited the siderophores synthesis. Proteomic analysis of the extracellular proteins secreted in the minimal medium simultaneously with accumulation of siderophores by *Rhodococcus* sp. S10 showed approximately 600 protein spots on the 2D gels and their localization predominantly in the range of pH 3 to 6. Thus, comprehensive analysis of metabolites and proteins excreted by *Rhodococcus* sp. S10 under iron-limited conditions will reveal the mechanisms of adaptation to geochemically extreme conditions and their potential role in the serpentinite biomineralization. This work was performed within the Program of Competitive Growth of Kazan Federal University and supported by RFBR (grant no. 16-34-60200) and the scholarship of the President of the Russian Federation for young scientists and graduate students.

P.07-049-Mon
Sugarcane molasses as an alternative cheap carbon source for polyhydroxybutyrate production by *Halomonas elongata* 2FF under non-sterile conditions

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Polyhydroxyalkanoates (PHAs) are natural biodegradable lipid biopolymers synthesized as intracellular carbon storage material by numerous *Bacteria* and a few *Archaea* representatives. The accumulation of PHAs in microorganisms is favored by growth under nutrient imbalance (*i.e.*, excess of C source and limitation of P and N). The requirement for excess of (relatively expensive) C source alongside maintenance of sterile conditions may raise the costs of PHA production. Here we evaluate the ability of *H. elongata* to produce polyhydroxybutyrate (PHB) using sugarcane molasses (SM) as cheap C source under non-sterile (NS) and saline conditions. The 16S rRNA gene-based identification indicated that the 2FF strain isolated from a hypersaline lake pertained to *Halomonas elongata*. After 48 h of growth in mineral M1 medium containing 1 and 2% (SM), at 5% NaCl, and under NS conditions, *H. elongata* 2FF accumulated lipid granules as evidenced by fluorescence microscopy using Nile Red dye staining. Furthermore, no contamination of culture was observed during 6-days incubation at 37°C. The quantification of extracted PHA was evaluated by crotonic acid assay and the chemical structure was revealed by FTIR, Raman, and ^{1}H-NMR spectroscopy. The FTIR and Raman spectra of extracted polymer were similar to that of standard PHB. The ^{1}H-NMR analyses showed same H chemical shifts for the extracted PHA and the standard PHB. Moreover, partial *phaC* gene coding for Class I PHA synthase in *H. elongata* 2FF strain was successfully cloned and sequenced. Overall, we concluded that *H. elongata* 2FF produced PHB using SM as alternative C source under saline and NS conditions. In perspective, optimization of culture conditions alongside testing of various cheap C sources and subsequent assessment of PHA productivity will be considered to achieve a cost-effective PHB production by *H. elongata* 2FF. We acknowledge the grant from CNCS–UEFISCDI, project PN-III-P4-ID-PCE-2016-0303.

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P.07-050-Tue
Ligands in the active site of carbohydrate oxidase from *Microdochium nivale*

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The carbohydrate oxidase from *Microdochium nivale* is a flavoenzyme with bicovalently linked FAD molecule. The enzyme is preferentially active towards disaccharides such as cellobiose and lactose. The enzyme activity is attractive for application in industrial processes. We investigated binding of various saccharide substrates in the active site of the enzyme using single crystal diffraction analysis. We observed multiple modes of enzyme:ligand interactions. Moreover, we have observed two additional ligand binding sites on the protein surface that have not been observed previously. Surprisingly, high resolution crystal structures of old crystals (more than 6 months) shows specific FAD degradation that has not been observed before. Nevertheless, the old enzyme is capable of substrate binding and is very likely to be still active. Analysis of all available crystal structures of carbohydrate oxidase:ligand complexes together with crystal structures of functionally related proteins revealed the key residues in the active site that modify the enzyme specificity. This publication was supported by MEYS CR (LM2015043 CIISB), by the ERDF fund (CZ.02.1.01/0.0/0.0/16_013/0001776), and by GA CTU (SGS16/246/OHK4/3T/14).

P.07-051-Wed
Bio-active polycaprolactone nanofibers as a potential fluoxetine filter

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Fluoxetine is a widely used serotonin reuptake inhibitor for the treatment of postpartum depression. Adverse effects such as colic, fussiness, and drowsiness have been reported in some breastfed infants of fluoxetine using mothers. In this study, bio-active polycaprolactone nanofiber was developed for the removal of fluoxetine from the milk. The efficiency of the fluoxetine filter was tested by using spiked goat milk samples. Goat milk is used instead of breastmilk, as its content is nearly similar to the breastmilk. Fluoxetine was added to the goat milk in 25, 50, 75 and 100 ng/mL concentrations. Filter is incubated with the spiked milk samples for fluoxetine removal. Lactose, creamatocrit and protein levels were also measured to determine the milk composition before and after the incubation. Within-run repeatability was tested with the same branded goat milk for ten days. Fluoxetine levels were determined with ELISA method following incubation. The fluoxetine filter effectively removed fluoxetine from milk and did not significantly change the milk composition. As a conclusion, different types of bio-active nanofibers can be developed for the sensing of chemicals found in the breastmilk.

P.07-052-Mon
Purification and properties of insulin receptor – related receptor ectodomain expressed in mammalian cell culture

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The Insulin receptor-related receptor (IRR) was originally discovered due to its high homology to the other family members (insulin receptor and insulin-like growth factor 1 receptor), however, no endogenous ligands of a peptide or protein nature have been found for IRR. We determined that IRR can be activated by mildly alkaline extracellular media and has typical features of the ligand-receptor interaction, including its specificity and dose dependence. Since pH-sensitive properties of IRR are determined by its ectodomain; therefore, we chose as option study the soluble an extracellular portion IRR. Based on the CHO-K1 cell line, an effective system of constitutive heterologous expression of IRR ectodomain was developed, which provides a yield of 0.6 mg of recombinant protein per liter of conditioned media. We determined optimal conditions for cultivation of the cell line were selected with the replacement of the nutrient medium for serum-free. A purification protocol has been developed that allows obtaining IRR ectodomain with a purity of more than 99%, according to the results of analytical gel filtration and gel electrophoresis. The identity of the protein was confirmed by the Western blot analysis and MALDI mass spectrometry. It was also shown that approximately 25% of the mass of recombinant IRR ectodomain is comprised carbohydrates. The analysis of IRR ectodomain by circular dichroism, reproducible conformational changes have been shown upon changes of the solution pH. The spectra suggest that the structure of the protein corresponds predominantly to stacking with β-sheet, and at a wavelength below 260 nm the absolute ellipticity of the ectodomain IRR at alkaline pH is lower than at neutral. The obtained spectra correlate with the conformational changes observed in insulin receptor ectodomain, IRR close homolog, upon its activation by insulin. This work was financially supported by the Russian Science Foundation (grant N_ 14-50-00131).

P.07-053-Tue
Multimodal magnetic-luminescent nanocomplexes based on upconversion nanoparticles for theranostics

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The development of synthesis technology made it possible to obtain new non-toxic luminescent nanoparticles with anti-Stokes photoluminescence, also known as upconversion nanoparticles (UCNPs). Such particles are able to convert near-infrared

radiation (NIR) (975 nm), falling into the "transparency window" of biological tissues, into the photons of the visible and ultraviolet spectral range, avoiding the autofluorescence of the tissues and significantly improving the sensitivity of detection. The unique optical properties of anti-Stokes nanoparticles allow us to consider them as a universal platform for multimodal nanocomplexes, whose biocompatibility and biological inertness remain under activation by light. Such complexes provide a unique functional system for a deep photodynamic and photothermal therapy, as well as for multimodal diagnosis of tumors. We prepared magnetic-luminescent UCNP, suitable for *in vivo* applications, by embedding magnetite in polymer shell based on an amphiphilic copolymer poly(maleic anhydride-alt-1-octadecene) for UCNP surface hydrophilization. Multimodal nanocomplexes were also obtained by incorporating magnetic and UC nanoparticles simultaneously in preformed polystyrene particles. Third approach was based on magnetite encapsulation into polymer shell on the UCNP surface formed by NIR-induced photopolymerization using UCNP luminescence as internal source for initiator activation. The enhanced permeability and retention effect (EPR), which was increased due to application of the external magnetic field, enabled accumulation of magnetic-luminescent nanocomplexes in tumor. Nanocomplexes were injected intravenously while neodymium magnet was attached to the tumor. Efficacy of nanoparticle accumulation in the tumor increased more than two times. We developed a new strategy for photothermal therapy using magnetic-luminescent nanocomplexes.

P.07-054-Wed
Generation of 3D in vitro breast cancer models by different techniques and their biological characterization

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The aim of this study was to generate multicellular 3D breast cancer spheroids using different culture techniques and to characterize their biological properties in order to be used as models for cancer research purposes. Triple negative breast cancer cell line MDA-MB-231 was used to generate spheroids by hanging drop and liquid overlay (single and multiple spheroids) techniques using different cell densities (500 – 8000 cells/spheroid). The morphology and cellular viability were evaluated up to 7 days through LIVE/DEAD assay. Spheroids' diameters were also measured over time. Hypoxia (Nrf2 and Hsp70) and proliferation capacity (PCNA) were assessed by Western blot analysis. Reduced glutathione (GSH) content and the level of lactate dehydrogenase (LDH) were analyzed by colorimetric methods. The breast cancer spheroids' morphology was characterized by a high compaction of cells. Therefore, the diameter of spheroids generated by hanging drop and liquid-overlay-single spheroid techniques was dependent by cell density (~ 500 μm for 5000 cells) and decreased over time, while those generated through liquid-overlay-multiple spheroids technique had various diameters (150 - 550 μm). The biological evaluation showed that breast cancer spheroids develop a hypoxic center over time with low cellular viability and high release of LDH in a time-dependent manner, the highest level of LDH being obtained for the liquid-overlay-multiple spheroids technique. Furthermore, a decrease of PCNA expression and a significant increase of Hsp70 and Nrf2 ones in correlation with the increase of GSH intracellular level were observed in breast cancer spheroids generated by both methods. In conclusion, through both techniques similar breast cancer 3D models were generated, expressing characteristics of multicellular spheroids – spherical and ellipsoidal morphology with a necrotic center induced by hypoxia. Acknowledgements to PN-III-P2-2.1-PED-2016-0904 project.

P.07-055-Mon
Heterologous expression of bacterial phytase from *Pantoea* sp. 3.5.1 in *Pichia pastoris*

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Phytase catalyzes the stepwise removal of phosphate from phytic acid. Microbial phytases are the most commonly used feed additive for monogastric animals. However no single phytase can perform effectively under various conditions of digestive systems and dietary compositions. That's why there is a constant search of new phytase-producers and efficient systems for phytase expression. The methylotrophic yeast *Pichia pastoris* has been used for several years as effective expression system of heterologous protein production. The aim of this study was to clone and express phytase from *Pantoea* sp. 3.5.1 in *P. pastoris*. The sequence of histidine acid phytase gene of *Pantoea* sp. 3.5.1 (*agpP*) was used. For the correct expression in yeast the codon-optimization of the nucleotide sequence was performed. Cloning of bacterial gene into integrative yeast vectors pPINK-HC and pPINK-LC was carried out by three-way ligation of a vector, a phytase gene and a signal sequence of *Kluyveromyces maxianus* inulinase gene. Genetic constructs containing *agpP* gene under the control of AOX1 promoter were obtained and transformed into *P. pastoris* cells by electroporation. The expression of bacterial phytase in yeast culture media was detected by immunoblotting and analysis of phytase activity. After screening for the highest phytase activity, one colony was selected for shake flask expression. After 48 h of methanol induction, culture showed an extracellular phytase activity of 0.82 U/mL. Recombinant phytase was isolated and purified by affine chromatography. The study of effect of temperature on the stability of the enzyme showed increase in thermal stability of recombinant phytase in comparison with the native enzyme, which might be associated with glycosylation of the protein by yeasts. This work was performed in accordance with the Russian Government Program of Competitive Growth of the Kazan Federal University and supported by Russian Science Foundation (project no. 16-16-04062).

P.07-056-Tue
Antimycoplasmal activity of the enzyme L-lysine-α-oxidase

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The concentrate of cultural liquid of *Trichoderma harzianum* Rifai being producers of the enzyme L-lysine-α-oxidase was gained after cultivation in the equipment of Experimental technological installation of the G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms (Pushchino, Russia). The concentrate has an activity 0.54–0.56 U/mL. The influence of this concentrate on the growth of mycoplasmas has been studied for the first time: two species of the family *Mycoplasmataceae*: *Mycoplasma hominis* (Mh) and *Mycoplasma fermentans* (Mf) and one species of the family *Aholeplasmataceae*: *Aholeplasma laidlawii* (Al). For the growth of mycoplasmas was used broth (Difco PPLO Broth, Becton, Dickinson, USA) with adding of 20% horse serum, 2% fresh yeast extract, 1% arginine or 1% glucose

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(dependent on kind of mycoplasmas), and 0.005% phenol red indicator. 10-fold dilutions in physiological saline were used for serial titration, The material from all dilutions was seeded into 0.3% agar (BBL Mycoplasma Agar Base, Becton, Dickinson, USA) with the same additives as in the broth. After 3 days, the number of colonies in the last two samples from several dilutions was counted and the average colony count was calculated. The culture titer was expressed in the average number of the colonies, multiplied by cultivation dilution. For the experiment the culture with the previously known titer was taken. It was shown that the cultural liquid of *Trichoderma harzianum* Rifai with the activity of L-lysine-α-oxidase 0.54–0.56 U/mL inhibits growth of *Mycoplasma hominis* after a preliminary contact. The degree of growth inhibition depends on the seed dose of mycoplasma and the content of *Trichoderma* culture fluid. The publication was prepared with the support of the "RUDN University Program 5-100" and the Ministry of Education and Science of the Russian Federation (the Agreement No. 02.A03.21.0008).

P.07-057-Wed
New laccases from different isolates of white-rot fungus *Steccherinum ochraceum*

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Steccherinum ochraceum is a white-rot basidiomycete with wide ecological amplitude. It occurs in different regions of Russia occupying different climatic zones. *S. ochraceum* colonizes stumps, trunks and branches of various deciduous (seldom coniferous) trees at different decay stages, but also could be found on living trees. The phylogenetic diversity of *S. ochraceum* strains isolated from different substrates in different regions of Russia was assessed by analyzing the nucleotide sequences of β-*tub* and *tef1*α genes and the ITS1-5.8S-ITS2 region of rRNA gene. The strains were grouped into three main clades, based on the concatenated dataset of all nucleotide sequences. This grouping correlated with lignocellulose-converting enzyme activity profiles of the strains. Laccases are enzymes belonging to the family of blue multicopper oxidases and one of the main fungal ligninolytic enzymes. They oxidize a wide range of phenolic and some non-phenolic compounds, performing four-electron reduction of the molecular oxygen to water at the active center. The physico-chemical and biochemical properties of laccases from all three mentioned above *S. ochraceum* clades were studied. The molecular masses of all laccases were 63 kDa and the isoelectric points were 3.0. The spectroscopic characteristics were typical for classic "blue" laccases. Azure B decolorization test showed, that the redox potentials of studied laccases were lower than that of laccases from *Trametes* spp. fungi (750–780 mV), that allowed considering *S. ochraceum* laccases as medium-redox potential. The substrate specificity of the laccases was examined using various phenolic and non-phenolic substrates. Different substrate specificity of laccases of *S. ochraceum* strains from different phylogenetic clades was demonstrated. Main differences in affinities were observed for such substrates as pyrocatechol and ABTS. This work was supported by Grant of Russian Foundation For Basic Research 16-04-01184-a.

P.07-058-Mon
The production of recombinant-protein additives and the development on their basis of serum-free media for the cultivation of eukaryotic-cell producers

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The recombinant proteins are major part of modern biotechnology and biopharmacy. The development of new cultural media for growing and cultivation of the cells produced recombinant proteins is one of the important task of bioindustry. Here we focused our attention on the three main protein components of serum-free media: human serum albumin (HSA), human transferrin (TGF) and human insulin. Albumin performs a number of important functions, including the transport of a number of important low-molecular compounds, such as hormones, fatty acids, bile acids, vitamins, cholesterol and growth factors, maintaining the buffer capacity and osmotic pressure of the nutrient medium. Transferrin is involved in the receptor-mediated transport of iron ions into cells, and the hormone insulin takes part in carbohydrate metabolism. Most of the serum-free media used today include albumin and transferrin, obtained from the blood of cattle and humans. The use of these supplementary proteins significantly increases the risks of contamination with various infectious agents, including non-enveloped viruses such as hepatitis A virus and parvovirus B19 or agents causing Creutzfeldt-Jakob disease, because during their production, the detection or inactivation of such agents is not currently are effective. The replacement of HSA, TGF and insulin in the serum-free nutrient media used to produce modern therapeutic drugs, their recombinant analogues will eliminate the risk of contamination with biologically active impurities. This work is supported by grant RFMEFI60717X0177.

P.07-059-Tue
Verification of myelin basic protein recognition by CDR3 obtained from LMP1-immunized mice IgG

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Multiple sclerosis (MS) – chronical inflammatory disease of the central nervous system (CNS). The inflammatory process during MS is commonly associated with T-cell component of immune system, but the role of B-cells and autoantibodies should not be neglected. Recently researchers focused on B-cells and BCR structures in an attempt to clarify the etiology of this pathology. In our study, we turned our mind to the phenomenon of cross-reactivity between viral proteins and autoantigens as possible trigger mechanism of MS development. Previously we identified sequences of complementarity-determining regions (CDRs) of anti-myelin antibodies from mice immunized with Epstein–Barr virus (EBV) latent membrane protein 1 (LMP1). These antibodies specifically recognized myelin basic protein (MBP). In this study, we have shown the binding between MBP and soluble peptide corresponding to CDR3's sequence obtained from LMP1-immunized mice. However, CDRs as a part of immunoglobulin have another conformation, which differs from its independent form. For further verification of its binding ability, we chose Llama single-domain antibody system as the framework for investigated CDR3 in a format of full-sized antibody. These antibodies lack

light chain and consist of heavy chains only. We developed genetic constructions by insertion of CDRs with known specificity in VHHs with different frameworks to demonstrate specific recognition of MBP. We also change the length of CDR3 by removing side amino acid residues to analyze its influence on antibody affinity. In our study, we learned the effect of amino acid substitution in CDR3 on antibody affinity and specificity. Finally, we confirmed anti-MBP reactivity of antibody from LMP1-immunized mice. This study was supported by Russian Science Foundation grant #17-74-30019.

P.07-060-Wed
Development of novel immunomodulating biologicals for suppression of the activity of the endogenous human interferon-gamma

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Human interferon-gamma (hIFNγ) is a pleiotropic cytokine endowed with multiple activities such as antiviral, antibacterial, antiproliferative, antitumor, immunoregulatory and gene activity regulation. The upregulation of its production, however, is related to the aetiology of certain autoimmune diseases (as multiple sclerosis, alopecia areata, autoimmune uveitis, myasthenia gravis) and also the post transplant atherosclerosis. Currently, no therapy can effectively treat any of these diseases. We aim at development of a new generation of recombinant proteins with potential application as drugs for treatment of autoimmune diseases and graft atherosclerosis. The recombinant proteins are derivatives of the hIFNγ endowed with the same affinity to the IFNγ receptor as the natural hIFNγ, but unable to trigger the intracellular signal transduction pathway. These proteins are expected to decrease the proinflammatory action of hIFNγ by competing for its receptor. Among the generated library two of the mutants showed promising features - a single point mutant K88Q and a double mutant bearing an additional substitution at the N-terminus. The two proteins demonstrated 100 (single mutant) to 1000 (double mutant) times lower hIFNγ activity in comparison to the wild type and a distinct dose-dependent competition as measured on WISH cells. Both mutants were purified and their conformational and structure dynamics was studied by means of hydrogen/deuterium exchange associated with mass spectrometry. The obtained results show that the structures of both mutants are almost fully identical to that of the wild type protein, which is a prerequisite for a proper ligand-receptor complex formation. We conclude that both mutant analogues are potential candidates for immunomodulating biologicals for autoimmune therapy based on selective suppression of the endogenous hIFNγ activity. Acknowledgments to: Grants RILA 01/14/2014 and H 11/20/2017 from Bulgarian Science Fund.

P.07-061-Mon
Effect of rotating magnetic field on the activity and catalytic properties of native and immobilized laccase

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In biotechnological processes, enzymes are important biological catalysts that are used by engineers for the increased effectivity and specificity of bioprocessing. A novel approach to the biocatalytic processes focuses on the application of various types of magnetic fields. For example, the rotating magnetic (RMF) field may be used to affect enzymes processes or to control the enzymatic reactions. The aim of this study is to analyse the effect of RMF on the activity and catalytic properties of native laccase. Moreover, this kind of magnetic field is applied to improve of the enzymatic process with the application of immobilized laccase on the functionalised ferromagnetic nanoparticles. In the case of this experimental work, the RMF with the various frequency (1–50 Hz) and the magnetic induction (6–19 mT) was tested. Moreover, the exposure time of enzymatic process to RMF was analysed. The exposure to the RMF of a native laccase significantly increased their activity at 10, 40 and 50 Hz (15, 18, 19 mT) and at 30 Hz (17 mT) for the immobilized enzyme, respectively. The higher stability (50% at pH 4.0 for 50 Hz magnetic field and 1 hour exposure) for the native enzyme was observed. The immobilized laccase under the RMF showed the better reusability in all analysed frequencies than control. The results show that the activity, stability and optimum pH of the native and immobilized laccase were significantly affected by RMF. This suggests that the RMF may be applied as the tool in order to modify the processes catalysed by enzymes. We are grateful for the financial support of the National Science Centre Poland within the PRELUDIUM (Grant No. 2016/21/N/ST8/02343).

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P.07-062-Tue
Novel 7-methoxytacrine-tacrine dimmer inhibitors targeting *Anopheles gambiae* acetylcholinesterase

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Vector-borne diseases are among major causes of illness and death in many tropical and subtropical countries. In 2015, 214 million new cases of malaria transmitted by *Anopheles gambiae* occurred globally. For the insect control were used pesticides such as carbamates (CA) and organophosphates (OP) targeting enzyme

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acetylcholinesterase (AChE). Acetylcholinesterase insecticides work through covalent modification of serine in the active site, thus disabling its catalytic function and incapacitating insects. However, because this serine is also presented in the AChEs of other vertebrates, the CA and OP are toxic to fish, birds and mammals. Moreover, the increasing resistance to the long-term used pesticides leads us to develop new environmentally safe insecticides. The aim of our study was to prepare recombinant AChE from *Anopheles gambiae* mosquito. Template DNA was optimized for production in Bac-to-Bac® HBM TOPO® secreted expression system. The insect cells *Spodoptera frugiperda* Sf9 were used as a host of baculovirus vector containing honeybee melittin (HBM) secretion signal, which was responsible for the production of the cloned gene product into the extracellular media. Supernatant with highly active recombinant protein was obtained and used for evaluation of fourteen newly prepared 7-methoxytacrine-tacrine dimmers. Insecticidal effect (inhibition activity) of our compounds was evaluated using modified Ellman's method. The assay was held in parallel with human AChE to investigate off-target toxicity. We have found two compounds, which seemed to be potent insect-specific inhibitors. These inhibitors showed about two orders lower IC_{50} values for insect AChE than for human enzyme. The work was financially supported by the Ministry of Health of the Czech Republic (grant AZV/16-34390A), MH CZ - DRO (University Hospital Hradec Kralove, No. 00179906) and University of Hradec Kralove (VT2201-2017).

P.07-064-Mon
Expression of bacterial phytase from *Pantoea* sp. 3.5.1 in *Yarrowia lipolytica*

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The dimorphic yeast *Yarrowia lipolytica* has been used for several years as effective expression system. They are characterized with a high level of heterologous protein production, a lesser degree of glycosylation and are unpretentious in cultivation conditions. The aim of this study was to create the recombinant strains of *Yarrowia lipolytica* for the secretion of bacterial phytase, and optimization of production conditions. Integrative yeast vector pINA1296, which contains a strong hybrid promoter hp4d and a secretion signal was used to clone the sequences of histidine acid phytase gene of *Pantoea* sp. 3.5.1 (*agpP*). For the correct expression in yeasts the codon-optimization of the nucleotide sequence of the gene was carried out. *Y. lipolytica* strain Po1 g was used for transformation by electroporation. Transformants were selected on the leucine drop-out medium. Integration of the bacterial phytase gene into the Y. lipolytica genome was confirmed by genotyping. For further work four recombinant yeast strains were selected. The expression of bacterial phytases in yeast culture media was detected by immunoblotting and analysis of phytase activity. Phytase production on rich media (YPD and YP2D4) and industrial semisynthetic medium was studied. On all tested media phytase activity appeared on the 3rd day of cultivation and reached its maximum on the 7th day. Maximal phytase activity was observed on YP2D4 media. This work was performed in accordance with the Russian Government Program of Competitive Growth of the Kazan Federal University and supported by the Russian Foundation for Basic Research (project no. 16-34-60191).

P.07-065-Tue
Biomaterials modified with a synthetic AEBSF inhibitor with properties inhibiting the growth and biofilm formation by *Staphylococcus aureus* strain

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Staphylococcus aureus is one of the most common aetiological factors causing the occurrence of infections within implanted prostheses. Late detection of infection or improper choice of infection treatment can have dangerous consequences for the health and lives of patients. Therefore, in the field of biomaterial engineering, new solutions are still sought, which would allow to increase the aseptic potential of implanted prostheses. In this work, as an innovative solution, the AEBSF inhibitor - synthetic inhibitor of serine proteolytic enzymes, has been proposed as compound that can limit the adhesion of pathogenic microorganisms to the surface of prostheses, can affect the biofilm formation and can limit their multiplication. The experiments carried out included determination of the antimicrobial potential for native synthetic inhibitor and a polyester graft prosthesis modified with an AEBSF inhibitor in suspension cultures of *Staphylococcus aureus* ATCC® 25923™. Moreover, analysis of bacterial biofilm formation on the unmodified and modified prosthesis surface was performed (staining with 2,3,5-triphenyltetrazolium chloride and then analysed by SEM photomicrography). Thanks to the conducted experiments, it was observed that both the native AEBSF inhibitor and immobilized on the surface of the polyester vascular prosthesis inhibit the proliferation of *Staphylococcus aureus* cells. The presence of immobilized inhibitor molecules on the surface of the prosthesis also has the effect on slowdown of the biofilm formation by *Staphylococcus aureus* ATCC® 25923™, in the comparison to the unmodified prosthesis. This work was partially supported by National Science Center (2014/15/N/NZ7/04092).

P.07-066-Wed
The application of biosensors based on photobacteria in the toxicity analysis of new polyethylenimine sorbents

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This work presents the toxicity analysis of the polymer materials based on the cross-linked polyethyleneimine (PEIs) with agent – diglycidyl diethylene glycol (DDG). The sorbents were obtained by cryogenic polymerization(-20°). The sorbents are applied to clean water areas from pollution and microflora overgrowth. Toxic effect has been analyzed by a bioluminescent method using as a test object photobacteria *Photobacterium phosphoreum* in free form and immobilized in PVA cryogel form. Standard toxicity analysis (10, 15, 30 and 60 min) didn't allow to identify significant toxic effect of tested sorbents. However, there is an intense toxic effect of some sorbents during the prolonged incubation (22 h). We researched the stability of sorbents during their incubation in salt solutions and the effect of crosslinking agent (0.9–120%) concentration on the sorbent density. It is established, that the toxic effect is caused by the sorbent destabilization in salt solutions, accompanied by an intense pH shift to the alkaline side. The speed of destabilization and pH shift depended on the concentration of the crosslinking agent: the maximum destabilization was observed for sorbents with lower percentage of DDG. It is shown that the inhibition of luminescence can be

caused by both the action of sorbents and the effect of pH shift in the alkaline side. The possibility of photobacteria adhesion and introduction were also studied. According to the luminescent analysis it was detected that sorbents are effectively populated by photobacteria. A direct indication of the photobacteria's adhesion on sorbents was obtained from electron microscopic studies. Photobacteria on sorbents formed colonies. Therefore it was established, that the toxicity of sorbents and the adhesion of photobacteria on it depend on a polymerization process, a structure and concentration of a crosslinking agent and a stability of a rheological parameters of the sorbent in salt solutions.

P.07-067-Mon
Rational design of formate dehydrogenase from the thermotolerant yeast *Ogataea parapolymorpha*

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Formate dehydrogenase (EC 1.2.1.2, FDH) is widely used for coenzyme regeneration of NADH or NADPH in different biocatalytic processes. The gene of a novel NAD^+-dependent formate dehydrogenase from the thermotolerant methylotrophic yeast *Ogataea parapolymorpha* DL-1 was cloned in our laboratory. Nucleotide sequence of the *opafdh* gene was modified at N-terminus with additional Gly codon. It was shown that recombinant wild-type OpaFDH had the lowest values of Michaelis constants (K_M) both for NAD^+ and formate among all described FDHs from yeast and bacteria. The enzyme also demonstrated high thermal stability, which was lower only in comparison with the FDHs from bacteria *Pseudomonas* sp. 101 and *Staphylococcus aureus*. According to the multiple amino acid alignment Tyr286 residue placed in front of conserved motive PQP was selected and substituted by rational design for improving thermal stability of wt-OpaFDH. Four plasmids encoded mutant forms of OpaFDH with change of Tyr286 with Glu, Asp, Ser and Ala were obtained, mutans were expressed in *E. coli* cells, purified and characterized. Substitution with Glu and Asp resulted in 7- and 15-fold increases of thermal stability, respectively. Thermal inactivation kinetics and differential scanning calorimetry were used to study thermal stability. Mutation Cys230 (analog of Cys255 in PseFDH) to Ala increased chemical stability by 100-fold. Double mutant OpaFDH C230A/Y286D showed both increase of thermal and chemical stability. This work was supported by Russian Foundation for Basic Research, grant 17-04-01469.

P.07-068-Tue
Obtaining and characterization of a single-chain variable fragment alkaline phosphatase fusion proteins and specific polyclonal antibodies for detection of IFN-beta and interleukin-10

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Interferon-β 1b (IFN-β) is used as first line treatment for multiple sclerosis (MS). The incidence of MS has been increasing worldwide. One of the proved effects of IFN-β longitudinal MS treatment is an increase in the number of interleukin-10 (IL-10) secreting cells. Development of new effective test systems for detection of IL-10 and IFN-β is of current importance. The aim of our research is the development of method for IL-10 and IFN-β detection using single-chain variable fragment (scFv) conjugates with alkaline phosphatase (AP) and specific polyclonal antibodies. ScFv were chosen due to their obvious advantages, such as small size, possibility of easy obtaining in high concentrations in bacterial systems of expression. Besides, they can be genetically fused to the marker protein, that allows to use them for one-step immunodetection of target molecules. ScFv specific to IFN-β and IL-10 were obtained from combinatorial libraries of cDNA V-genes of murine IgG using phage display technology. AP coding DNA sequence was subcloned into plasmid vectors *pCANTAB-scFv(IL-10)* and *pCANTAB-scFv(IFN-β)*. *E. coli* BL21(DE3) cells were transformed by obtained plasmid vectors. ScFv(IFN-β)-AP and scFv(IL-10)-AP synthesis was induced by adding IPTG. Proteins of interest were accumulated in the periplasmic space of *E. coli*. Functional activity of scFv(IFN-β)-AP and scFv(IL-10)-AP was confirmed with dot blot analysis and ELISA. The possibility of long-term storage of scFv(IFN-β)-AP and scFv(IL-10)-AP as a periplasmic extracts at -20°C without loss of their functional activity was shown. Polyclonal antibodies with high specificity and affinity to IFN-β and IL-10 were produced via immunization of laboratory mice. Application of polyclonal antibodies for antigen capture and scFv-AP for antigen detection is promising for the development of new sandwich ELISA system for the determination of human IL-10 and IFN-β levels.

P.07-069-Wed
Site-directed mutagenesis of the recombinant alpha-amino acid ester hydrolase from *Xanthomonas rubrilineans*

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Alpha-amino acid ester hydrolase (EC 3.1.1.43; AEH) catalyzes the synthesis and the hydrolysis of the amide bond in semi-synthetic β-lactam antibiotics and displays the preference for the

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substrates with α-amino group. Kinetically controlled synthesis of penicillins and cephalosporins proceeds through the acylation of β-lactam nucleus, penam or cephem respectively, by α-amino acid esters. Crystal structures of AEH from *Xanthomonas citri* and *Acetobacter turbidans* were solved, as well as mutant structure and enzyme complexes with substrates. AEH displays high synthetic activity for ampicillin but its properties for amoxicillin synthesis with side-chain *para*-hydroxyl group should be improved. The AEH gene from *Xanthomonas rubrilineans* (XrAEH) was cloned and expressed in *Escherichia coli* cells earlier, and its model 3D structure was simulated. Based on model 3D structure, computer docking with some beta-lactams was conducted, that revealed several perspective amino acid substitutions which could contribute to the improved substrate specificity of XrAEH towards some beta-lactams. Calculation results of modeling were checked by site-directed mutagenesis experiments with further characterization of the mutants obtained.

P.07-070-Mon
Probiotic properties of strains of *Bacillus subtilis*

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A significant problem of biotechnology is the development of new probiotics for poultry as an alternative to the use of antibiotics. From the rhizosphere of potato, we isolated two strains of *B. subtilis* GM2 and GM5 with high antimicrobial activity. Sequences of the genomes GM2 and GM5 were entered in to the NCBI database (NKAK00000000.1 and NKJH01000001.1). Annotation of DNA sequences in the program "Antismash (Version 4.0.0rc1)" was carried out and the genes responsible for the synthesis of antibiotics and secondary metabolites were identified. We investigated important probiotic properties of the bacteria. It was shown that the bacterial spores were resistant to 1–10% of bile and a wide pH range. Strains GM2 and GM5 possess proteolytic and phytate-hydrolyzing activities, showed pronounced antagonistic properties against fungi and pathogenic enterobacteria. Genes responsible for the synthesis of cyclic lipopeptides of iturin A, bacillomycin L, surfactin and the dipeptide antibiotic bacilysin, were detected in the genome of GM2 strain. Genes responsible for the synthesis of fengycin and surfactin were identified in the GM5 strain genome. The lipopeptide fraction from *B. subtilis* GM5 showed higher inhibitory properties. To assess the probiotic effectiveness of bacteria, we conducted an experiment on cross broiler chickens "Cobb-500". When *B. subtilis* GM2 was consumed, the weight gain of each chick was 4.83% higher, while GM5 strain produced 11.47% weight gain in comparison with the control. Upon the addition of GM2 and GM5 to the feed Feed conversion ratio was higher by 5.49% and 1.83% relative to the control group chickens. Thus, GM2 and GM5 strains have a stimulating effect on the growth of broiler chickens and can be potentially used as probiotics in poultry. The work is performed in accordance with the Russian Government Program of Competitive Growth of Kazan Federal University and was supported by grant from Russian Scientific Foundation (project No. 16-16-04062).

P.07-071-Tue
Comparative study of thermal stability of formate dehydrogenases from different sources

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Formate dehydrogenase (FDH, EC 1.2.1.2.) catalyzes the reaction of formate ion oxidation coupled with reduction of NAD^+ to NADH. FDH is an enzyme of high practical interest because it is used for cofactor regeneration in fine organic and chiral synthesis with oxidoreductases. The genes encoded FDH from several bacteria Pseudomonas sp. 101 (PseFDH), Moraxella sp. C1 (MorFDH), Mycobacterium vaccae N10 (MycFDH), Staphylococcus aureus (SauFDH), methylotrophic yeasts Candida boidinii (CboFDH), Ogataea parapolymorpha (OpaFDH), bakery yeast Saccharomyces cerevisiae (SceFDH), plant *Arabidopsis thaliana* (AthFDH), soya Glycine max (SoyFDH) and moss Physcomitrella patens (PpaFDH) were successfully cloned and expressed in E. coli cells. Analysis of thermal inactivation kinetics revealed, that thermal stability of free forms was different and depended on source. PseFDH, SauFDH, OpaFDH showed high thermal stability, MorFDH, CboFDH and AthFDH were in the middle, PpaFDH, SoyFDH and SceFDH were less stable, compared to others. We also studied thermal stability with differential scanning calorimetry of apo- and holo- forms (complexes with NAD^+ and complexes with NAD^+ and inhibitor azide) of formate dehydrogenases. It was found that binding of the enzyme in ternary complex resulted in high increase of thermal stability and effect was higher as less stable was free enzyme. Also the influence of pH and ion strength on thermal stability of formate dehydrogenases from different sources was studied. It was shown that increase of phosphate concentration provided high stabilization effect. The highest value of such effect was observed for the enzymes from plants. It means that the structure of plant enzymes are more sensitive to additives and can be more compact in case of forming complexes or in the solvents with high ionic strength. Parts of this work were supported by Russian Science Foundation (grant 16-14-00043) and Russian Foundation for Basic Research (grant 17-04-001469a).

P.07-072-Wed
The effects of rotating magnetic field on the activity and catalytic properties of horseradish peroxidase

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The influence of electromagnetic field on enzymes has been extensively studied in recent years. It has previously been observed that the various kinds of magnetic fields influenced on the enzymes activity e.g. α-amylase, alkaline phosphatase, acetylcholinesterase or superoxide dismutase. Horseradish peroxidase (HRP) is widely used in organic synthesis and molecular biology. Despite the fact that HRP has been studied for more than a century, the knowledge about the influence of different kind of electromagnetic fields on the activity of this commonly occurring enzyme is still poor. The aim of this study was to analyse the effect of rotating magnetic field (RMF) on the catalytic properties of HRP. The obtained results showed that the exposure of HRP on the RMF at a range of frequencies 20–50 Hz (16.5–19 mT), reduced its activity of about 10% and about 20% at frequencies from 1 to 10 Hz (6–15 mT). Exposure of HRP to RMF also induced changes in the optimum pH of peroxidase. During HRP exposure to the RMF at 50 Hz (19 mT) and 1 Hz (6 mT), a constant profile of enzyme activity in the pH range from 4.0 to 7.0 was found. For the control, the highest activity was obtained at pH 4.0, after exceeding this value was observed a dramatically reduced enzyme activity about 75% the initial activity (pH 7.0). The results showed that the exposure of HRP at RMF can lead to reduction the activity of the enzyme, but increased its stability over a wider range of pH. It can suggest that the RMF can influence on catalysis process without change of enzyme structure. We are grateful for the financial support of the National Science Centre Poland within the PRELUDIUM (Grant No. 2016/21/N/ST8/02343).

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P.07-073-Mon
Three-dimensional growth of endothelial cells by cocultured cancer cells in nanofibrous scaffold

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Angiogenesis is a critical element for local growth and metastasis of malignant tumors and one of the factors involved in neovascularization is vascular endothelial growth factor (VEGF). In this study, we developed a three-dimensional (3D) *in vitro* coculture model of endothelial and cancer cells to investigate effects of cancer cells on a growth of endothelial cells. A two-layer coculture system was developed using an electrospun poly(*e*-caprolactone) nanofibrous scaffold (NFS). Endothelial cells which were top-seeded on nanofibers in the absence of VEGF were able to adhere to nanofibers and changed to small, shrink and round shape in a time-dependent manner. Most of the endothelial cells were found to be dead after 5-day of culture. The change of endothelial cell morphology was abrogated by addition of VEGF and coculture of cancer cells in the lower layer of NFS. Moreover, the morphology of endothelial cells was steadily maintained under chemical hypoxia by exposing cancer cells to cobalt (II) chloride (CoCl2). Secretion of VEGF and expression of HIF-1*a* were time dependently increased in cancer cells and further increased by addition of CoCl2. The maintenance of morphology of endothelial cells cultured with cancer cells was blocked by VEGF neutralizing antibodies. These results suggest that coculture of endothelial and cancer cells in NFS was similar to the naturally occurring cross-talk between these two types of cells and coculture system with NFS can be used to study cancer angiogenesis.

P.07-074-Tue
Develop TNF-α vaccine for inflammatory diseases

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Cytokines are secreted proteins released by cells that have specific effects for cellular interactions and communications. Inflammatory cytokines including transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), platelet-derived growth factor (PDGF), and the interleukins, (IL)-1 and IL-8 have been implicated in the pathogenesis of various autoimmune diseases, such as rheumatoid arthritis (RA), inflammatory bowel diseases (IBD), and lung fibrosis. TNF-α is among the "master regulators" of the inflammatory (immune) responses in many organ systems. The introduction of TNF-α blocking therapy in 1998 began a new era in the treatment of chronic inflammatory human diseases, including RA, ankylosing spondylitis (AS), plaque psoriasis, psoriatic arthritis (PsA), IBD, and ulcerative colitis. Anti-TNF-α antibodies are currently being used clinically with high demand. However, therapeutic antibodies are expensive and require frequent injection. Thus, to develop TNF-α vaccine has its strengths toward clinical application. In this project, we are aiming to develop TNF-α DNA vaccine against inflammatory diseases, which will minimize the frequent injection of therapeutic antibodies.

P.07-075-Wed
Characterization of the enzymes and catabolic pathway for the detoxification and assimilation of L-azetidine-2-carboxylic acid by *Aspergillus nidulans*

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L-azetidine-2-carboxylic acid (AZC) is a natural occurring analogue of proline produced by the roots of the *Liliaceae*, acting as a plant protectant against infections and consumption. Despite its ecological and economic importance, the mechanisms for the resistance to and detoxification from AZC remain largely unknown. We have obtained evidence that AZC, is not only

non-toxic for the filamentous ascomycete *Aspergillus nidulans,* but it can also be utilized as nitrogen source. The products of *azhA* and *ngn*2 genes, encoding a putative hydrolase and acetyltransferase, respectively, are necessary for the resistance of *A. nidulans* to AZC. Our results show that for the utilization of AZC as a poor nitrogen source by *A. nidulans,* a functional AzhA and a fully active GABA catabolic pathway are necessary, indicating that AZC is metabolized via its conversion to GABA. Expression of both *azhA* and *ngn2* genes is induced by AZC while *ngn2* is additionally subjected to Carbon Catabolite Repression. Consistently, GFP-tagged versions of AzhA and Ngn2 are diffusely distributed at the cytoplasm, only in the presence of AZC. Heterologous expression of *azh*A complements the inability of a wild type yeast strain to utilize AZC as sole nitrogen source, indicated that AzhA hydrolase is necessary and sufficient for this process. Finally, phylogeny analyses indicate a strong conservation of AzhA sequence in many fungal species of the Ascomycota phylum, while a wider analysis showed a scarce presence of AzhA homologues in bacteria, archaea and eukaryotes.

P.07-076-Mon
Physicochemical properties of exopolymer from *Rhodococcus rhodochrous*

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Microorganisms are known as a rich source of compounds synthesized in response to some environmental stress factors. Among these substances, especially valued due to wide biotechnological applications, are extracellular polymeric substances (EPS). Bacterial EPS are considered as the alternative to harmful synthetic polymers because of their non-toxicity and biodegradability. Therefore, it is highly important to search new natural polymers with possible industry potential. Our studies were conducted on physicochemical properties of the exopolymer R_s-202 received by ethanol extraction from culture broth of *Rhodococcus rhodochrous* strain. Chemical composition of exopolymer was tested by the series of spectrophotometric measurements including the total sugar content (phenol-sulphuric acid method) and the total protein content (Bradford method). The molecular weight analysis was performed by a gel permeation chromatography. The monosaccharides content was identified quantitatively by high performance liquid chromatography. Bacterial exopolymer was analysed by the Fourier transformation infrared spectra and the X-ray photoelectron spectroscopy. *Rhodococcus rhodochrous* cells and the structure of exopolymer were estimated by scanning electron microscopy (SEM). The study results indicate that exopolymer R_s-202 was found to be 62.86% of polysaccharide and 10.36% of protein with the molecular weight of about $1.3x10^3$ kDa. The monosaccharide components of the studied exopolymer were mannose, glucose and galactose (12:6:1). X-ray photoelectron spectroscopy and Fourier transform infrared revealed that R_s-202 is a negatively charged polymer and contains functional groups (hydroxyl, carboxyl, amine, amide) preferred for the flocculation process. This work was partially supported by the National Science Centre (2012/07/B/ST5/01799).

P.07-077-Tue
Expression enhancement of antibody fragment in *E. coli* by designing mutations from database

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Antibody, which is a key protein for diagnostics and target-detection, has the function of molecular recognition. The antibody fragment with the function of molecular recognition has been utilized for designing the small antibody such as single chain Fv (scFv) and bivalent scFv dimer (diabody); however, the small antibodies comprised of Fv are structurally instable and some are hardly prepared by using *E. coli* expression. In this study, we attempted to mutate rare amino acid residues in the frame work of Fv to enhance the expression of small antibodies. Incremental mutations of the rare residues with low appearance frequency to those with higher frequency resulted in generating appropriate variants for enhancing the expression; while the binding affinity for target irregularly varied. Here, we employed small-scale iterative libraries in the low-frequency residues to increase the thermostability and expression amount without inactivation of binding affinity. In this study, we applied the PMab-2 diabodies which are hardly expressed as active form by means of *E. coli* expression. Firstly, we analyzed the influence of mutation on rare residues on expression amount and binding affinity. The mutations for the residues within 10% frequency decreased the expression amount of diabody together with inactivation of binding affinity. Secondly, in order to improve the expression amount of PMab-2 diabodies without inactivation of binding affinity, we applied small-scale iterative libraries in the low-frequency residues. On the mutation for the residues within 5% frequency, we made the library where the wild-type or most common amino acid appeared at the residues with the frequency of 5% to 10%, and the variants with comparable binding affinity to wild-type diabody were selected. The small-scale library was iterated to mutate the residues within 20%, and we found some new variants enhanced the expression amount with the comparable binding affinity to wild-type.

P.07-078-Wed
Gluconic acid production with recombinant strains activated gluconic acid production pathway and provided endogenous oxygen source

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Gluconic acid that is the third most widely produced commercial organic acid, is produced by chemical, electrochemical, catalytic oxidation, and biotechnological methods. The most important problem of the biochemical conversion of gluconic acid production is the dissolved oxygen concentration limitations. The use of oxygen for glucose oxidation along with aerobic respiration by the cells during gluconic acid fermentation makes a crucial parameter the concentration of dissolved oxygen for the high efficiency. *Vitreoscilla*, a gram-negative aerobic bacteria, can grow under conditions of limited oxygen. The first bacterial hemoglobin was isolated from *Vitreoscilla* bacteria at oxygen-poor environments and was called as *Vitreoscilla* hemoglobin (VHb). In the all organisms that have cloned VHb gene (*vgb*), it was observed that there is a significant increase in the synthesis of the many metabolites and recombinant proteins that are regulated by

oxygen. Particularly, it was determined that VHb possess the ability to increase respiration and growth in the bacteria and fungi. *Escherichia coli* is capable of synthesizing the apo-glucose dehydrogenase enzyme (GDH) for the gluconic acid production but not pyrroloquinoline quinone (PQQ), which is required for formation of the holoenzyme. PQQ synthesis in *E. coli* cells requires all *pqq* operon containing six genes from *Klebsiella pneumoniae* bacterium. The main idea of this study is that a bioprocess such as gluconic acid production which consumes too much oxygen would require to stabilize dissolved oxygen concentration by VHb thus increasing the efficiency. It is foreseen that the limitation of oxygen in the environment will be overcome with VHb, providing ease of aeration and mixing would decrease the costs, the absence of further oxidation enzymes in bacteria would increase gluconic acid production.

P.07-079-Mon
Nonsense-mutation sup35-74 affecting the [PSI+] prion properties in yeast *Saccharomyces cerevisiae*

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Prionization is the abnormal protein aggregation which results in appearance of infectious conformations. In mammals prionization of PrP leads to various neurodegenerative diseases. However about 10 non-toxic prions are described in yeasts, which are a convenient model for studying the mechanisms of prionization. [PSI^+] is one of the most studied yeast prion. The [PSI^+] prion is formed by the Sup35 protein, which functions as a translation termination factor eRF3. The aggregation of the Sup35p, as well as mutations in the essential *SUP35* gene, results in reduced efficiency of translation termination process that promotes nonsense suppression. Most of the mutations influencing [PSI^+] induction, propagation and stability are located in the prion forming domain of Sup35p. In our study we characterized effects of spontaneous *sup35-74* mutation on [PSI^+] properties. This nonsense-mutation results in a premature termination of Sup35p synthesis at the beginning of M-domain and formation of truncated product approx. 15 kDa. Full-length Sup35p is also produced due to readthrough of the stop-codon in *sup35-74* allele. We showed that diploid [PSI^+] strains bearing *sup35-74* allele are viable while haploid [PSI^+] strains are incompatible with this mutation. The presence of *sup35-74* together with wild type *SUP35* gene in diploid [PSI^+] strain results in changes in the Sup35p aggregate size and thermostability. One possible explanation of this result is the sequestration of truncated Sup35-74p into [PSI^+] aggregates. The *sup35-74* mutation was also found to induce *de novo* appearance of the [PSI^+] prion. Together, truncated proteins produced in strains with nonsense mutations in the *SUP35* gene provide a unique model for study [PSI^+] prion formation. The study was funded by St. Petersburg State University (1.50.1041.2014) and supported by research resource center "Molecular and Cell Technologies" of St. Petersburg State University.

P.07-080-Tue
Preparation and characterization of wild-type and mutant D-amino acid oxidases from *Hansenula polymorpha* and *Trigonopsis variabilis*

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D-amino acid oxidase (EC 1.4.3.3, DAAO) is the enzyme of high fundamental and practical interest. It plays important role in regulation of many important processes in living cell and DAAO is widely used in practice. Here we report our results of work with wild-type and mutant DAAOs from yeasts *Hansenula poly*morpha (HpDAAO) and *Trigonopsis variabilis* (TvDAAO). The enzyme from the yeast *Trigonopsis variabi*lis (TvDAAO) is used in biocatalytic two-enzyme process of preparation of 7-amino cephalosporanic acid from cephalosporin *C*. The main drawback of the enzyme is low operation (chemical) stability due to oxidation/modification of cysteine residues. Previously we have reported about site directed mutagenesis of Cys108. Amino acid changes in this position were combined with substitutions in other positions providing increase of chemical stability as well as thermal stability and improvement of catalytic properties. Now we carried out site-directed mutagenesis of Cys298 which is placed in oxygen channel of active site. Four mutant TvDAAOs with changes in position 298 were prepared, expressed in *E. coli*, purified and characterized. It was shown that half time inactivation period increased at least two times. Analysis of genome of *Hansenula polymorpha* (*Ogataea parapolymorpha* DL-1) revealed presence of tentative genes of DAAO. Two genes (HpDAAO1 and HpDAAO2) were cloned and expressed in *E. coli* cells. Analysis of catalytic properties showed that ones are close to ones for DAAO from *Candida boidinii*. Thermal stability of HpDAAO1 and HpDAAO2 was lower in comparison with wild-type TvDAAO. Parts of this work were supported by Russian Science Foundation (grant 16-14-00043) and Russian Foundation for Basic Research (grant 17-04-001487a).

P.07-081-Wed
In-vitro antioxidative and antimicrobial activity of *Gracilaria* sp. via secondary metabolites profile

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Algae are an important source of bioactive molecules used in the nutrition of living things. Fatty acid, carotenoids, protein and polysaccharide compounds which are used as natural raw materials in drug, food and cosmetics industry can be obtained from algae cells and by extraction methods. In recent years, besides the antiviral, anticancer, antifungal, antibacterial, antimicrobial effects, enzyme inhibition and other pharmacological effects of the metabolites have been investigated. As a result of this fact, the market share of the environmentally friendly natural products is rapidly increasing. In this study, the antioxidative and antimicrobial activity of the macroalga extracts of *Gracilaria sp*. was investigated. The extractability of these compounds from the algal biomass was investigated under soxhlet conditions by the influence of solvent systems using non-polar to polar solvents in a sequential extraction procedure. The solvent was removed from

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extracts and the samples were freeze dried. The extracts were re-dissolved in appropriate solvent to investigate their fatty acid, carotenoid and phenolic acid profile by GC and HPLC analysis under gradient conditions. The antioxidant capacity and total phenolic content of extracts were evaluated using TEAC and the Folin-Ciocalteu method respectively. Finally, the antimicrobial activities of the extracts were investigated by means micro-broth dilution method against *E. coli* and *S. aureus* species. As a result, the major carotenoids and phenolic acids were determined as β-carotene, β-cryptoxanthin, catechol and rutin for *Gracilaria sp*. Also the strain is a reassuring source for EPA and DHA fatty acids. Promising results were obtained in the case of antimicrobial activity studies as up to 98% inhibition of growth. Within the results, it can be declared that the macro-algal compounds proved the applicability of these strains in industrial applications with further standardization techniques.

P.07-082-Mon
Why is G6PD enzyme activity measured as zero?

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Glucose-6-phosphate dehydrogenase (G6PD) enzyme is the first and key enzyme of the hexose monophosphate shunt. In this enzymatic reaction, G6PD reduces nicotinamide adenine dinucleotide phosphate (NADP) to its reduced form (NADPH) while oxidizing glucose-6-phosphate (G6P) and producing a proton. Conventional test used to quantitatively measure G6PD activity are spectrophotometric methods based on measuring the amount of NADPH generated. This test is a standardized protocol in centralized laboratories. Currently available methods can measure G6PD enzyme activity as zero. But, since the enzyme is housekeeping enzyme, the activity of G6PD enzyme cannot be zero. We aimed to clarify by using an enzyme biosensor that the cause of G6PD enzyme activity was measured as zero in our study. We needed optimization and characterization studies in order to improve the optimization of the working conditions of G6PD enzyme activity. Enzyme concentration, pH, temperature, presence of activator and inhibitors, cofactor presence were studied in optimization studies. In the characterization studies, the use of the analogues of the substrates of the G6PD enzyme was studied. Triple electrode system used in the study. The components of our enzyme biosensor consist of gold electrode, reference electrode and counter electrode. Proton production from a chemical reaction catalyzed by G6PD was measured to reflect G6PD activity in a blood sample. We determined that the activity of G6PD enzyme is not absolutely zero at the end of the study. The improved enzyme biosensor serves as a specific results to G6PD and G6P interaction. This new procedure can be suggested as an alternative to G6PD enzyme activity measure methods. Enzymatic Biosensors show high performance characteristics with broad detection ranges, short measuring times, and good specificities. Thus, we provide new approaches to specific enzyme biosensors that are fast, accurate and easy to use for the determination of G6PD enzyme activity.

P.07-083-Tue
Yeast isogenic system as a tool for study protein interaction with DNA structures

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Protein-DNA interaction is the fundamental part of life. There are many techniques for detection and evaluation of the protein DNA binding from in vitro to in vivo assays. Here we presented application of yeast isogenic system for in vivo transactivation studies in chromosomal context. This variable system is applicable for analyses of influence of DNA structures to protein-DNA binding in vivo. We used a panel of *S. cerevisiae* haploid isogenic strains, except for the different p53 target site with different DNA structures located upstream of the luciferase reporter gene. The targeting of p53 target sequence of interest by the replacement of the ICORE cassette, using transfected single strand oligonucleotides, was performed following the *Delitto Perfetto* technique. Correct targeting events were isolated exploiting the counter-selectable and the reporter selection markers of the ICORE cassette and confirmed by colony PCR across the modified locus and Sanger DNA sequencing. yLFM isogenic derivative yeast strains constructed for this study were transformed with different plasmids for the expression of wild-type human p53 or p73 proteins. Our results show that transactivation *in vivo* correlated more with the relative propensity of a response element to form cruciforms than to its expected *in vitro* DNA binding affinity. Structural features of target sites are therefore an important determinant of its transactivation function.

P.07-084-Wed
Ultrahigh-throughput screening techniques for investigation of bacterial interactions in microbiota community

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Intimate interactions between different bacteria in microbiota community represent one of the most important "hot spots" of common microbiology. Nevertheless, classical microbiological techniques suffer from a shortage of productivity of analysis. Consequently, bacterial interactions between rare and slow-growing species are often ignored or misinterpreted. The development of next generation ultrahigh-throughput screening (uHTS) techniques is of particular interesting as it allows direct detection of biological activity of even minor components of specific microbiota communities. Moreover, uHTS techniques allow investigating the dynamics of microbiota community that is very important in complex mixtures of different species with highly distributed growth parameters and sensitivity to the external influence of environmental conditions. Utilization of uHTS techniques based on cultivation of bacterial species in droplets of microfluidic double emulsion (MDE) enabled us to perform highly sensitive single-cell selection of predesigned activity and the exploration of pairwise interactions between different bacteria unperturbed by other microbiota species. Thus, individual droplets of MDE serves as isolated microcontainers for cultivation and analysis. uHTS of MDE with encapsulated bacteria, enable us to isolate culturable bacterial strains and predict effectors inhibiting growth of model pathogenic bacteria. This work was supported by grant RFMEFI60716X0145 from the Ministry of Education and Science of Russia.

P.07-085-Mon
Expression, purification and initial biochemical studies of signaling receptor CD160

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The immune system's response to ongoing infections is essential to restore normal health. The response may be directed against pathogens or to own cells with abnormal structure or function. In regulating the immune system response an important role is played by inhibitory receptors presented on lymphocytes, that are activated due to the interaction of lymphocyte with an antigen presenting cell. A lot of signaling receptors have been widely characterized, but some of them are still under investigation. One example is member of the immunoglobulin superfamily glycosylphosphatidylinositol-anchored membrane glycoprotein CD160. The regulatory functions of the CD160 receptor are complexed. Due to binding to appropriate ligands, it participates in inhibition or enhancement of T cell responses. The increased expression level of CD160 was observed for example in patients with atherosclerosis, chronic lymphocytic leukemia, and chronic viral infections, such as HIV or HCV. Furthermore, CD160 is a ligand for HVEM (Herpes Virus Entry Mediator), a protein found on the surface of tumor cells. Due to the interaction of these two proteins, $CD4^+$ T cell proliferation is inhibited. The functions and structure of CD160 have not been fully understood. The efficient method of expression and purification of human CD160 and its characteristic is necessary to plan further experiments which may help to understand the function of this receptor. In order to obtain protein, we cloned it into two different expression systems (bacterial and yeast). Expression of His-tagged CD160 was observed in *E. coli* as well as in *P. pastoris*. Purification was performed using affinity column followed by size exclusion chromatography. The oligosaccharide chains added by yeasts were identified by enzymatic deglycosylation followed by SDS-PAGE analysis. CD, DSC and DLS studies were performed to characterize CD160. This work was supported by Polish National Science Center grant no. 2016/21/D/NZ1/02777.

P.07-086-Tue
Encapsulation of taro lectin (*Colocasia esculenta*) in liposomes: a nutraceutical production with antitumoral potential

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Our research group has previously isolated tarin, a promising globulin displaying lectin activity, from taro (*Colocasia esculenta*). Prior evidence indicates that tarin is able to induce *in vitro* and *in vi*vo proliferation of total hematopoietic cells, suggesting that this compound could be a novel therapeutic immunomodulator. Herein, we propose the optimization of tarin effects by encapsulation in liposomes. Tarin, purified to homogeneity from a taro extract by affinity chromatography, was loaded onto the aqueous core of small unilamellar vesicles from hydrated-phospholipid films, according to a modification of the protocol described previously by Ferreira et al. (2016). Liposome formulations presented round-100 nm shaped vesicles, stable for 6 months at 4°C. Cytotoxicity assays were performed against bone marrow cells exposed for 24 h to free or encapsulated tarin, using resazurin as a cell viability indicator. None of the compounds displayed cytotoxic effects against bone marrow cells. Optical microscopy images from bone marrow cells cultured in the presence of free or encapsulated tarin (20 μg/mL) presented large, elongated cells, with a different morphology from those observed in the control wells. Flow cytometry revealed a transitory increase in erythroid progenitors (TER119) in the bone marrow culture after 7 days of tarin treatment. Encapsulated tarin at 50 μg/mL inhibited 65% and 41% of human glioblastoma cells (U87MG) and human breast adenocarcinoma (MDAMD231), respectively. Encapsulated tarin demonstrated improvements in pharmacological properties when compared to free tarin, indicating potential in the food industry as a nutraceutical addictive, as well as in the pharmaceutical industry, as an adjuvant associated to chemotherapeutic drugs.

P.07-087-Wed
Comparative analysis of the bull microbial community by microbiology and genetic analysis traditional methods of microbiology and T-RFLP

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The study of rumen microorganisms, their role in digestion and the metabolism of substances in the ruminant's multicameral stomach is of great interest to both scientists and practitioners (veterinarians, cattle breeders, etc.). The recent development of metagenomic methods makes it possible to study the diversity of microorganisms without limitations of traditional microbiology methods (i.e. bypassing the stage of cultivation). Here, a comparative study of the microflora of the bull's rumen was carried out using classical microbiology methods and terminal restriction fragment length polymorphism (T-RFLP) analysis. In general, the obtained data on rumen microbiocoenosis using both methods were comparable. However, the number of colonies grown on enterococcus agar exceeded the number of all lactic acid bacteria on MRS-agar by 10-folds. The taxonomic affiliation of colonies with enterococci-agar using the sequencing method showed that 32% of the colonies belong to the genus Bacillus, 50% - Aerococcus (Lactobacillales), 18% - Enterococcus (Lactobacillales). Thus, the results of the investigation of the microflora of the bull's rumen with the help of T-RFLP-analysis turned out to be more accurate. Thus, the results of the T-RFLP analysis of the rumen microbial community are more accurate, suitable and reproducible as compared to the microbiology. The T-RFLP analysis is especially useful is the studied cases due to the relatively small (about 300) number of bacterial species in the rumen as compared to other ecosystems (soil, water). An additional advantage of the T-RFLP analysis is the possibility to determine quantitatively an uncultivated microbial community of the rumen of ruminant animals. This work was supported by the Russian Foundation for Basic Research (grant 18-016-00207).

P.07-088-Mon
Selection of the optimal strain-producer of subtilisin-like proteinase of *Bacillus pumilus*

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Today there is a continuous increase in the prices of feed for farm animals, so manufacturers are forced to look for new ways

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to reduce the cost of feeding rations and increase the digestibility of nutrients from cheaper components. The use of an enzyme protease becomes topical in these conditions. The main condition for the successful carrying out of the technological process of obtaining the enzyme is the selection of a highly effective strain. We tested a number of recombinant strains for obtaining subtilisin-like proteinase (AprBp) *B. pumilus*: MRB044, *B. subtilis* MRB045, *B. subtilis* MRB046 (containing optimized LIKE system), *B. subtilis* MRB073 (containing the constitutive promoter P_{DegQ}, *B. subtilis* pCS9 (containing multicopy plasmid pCS9, constructed on the basis of the vector pCB22) and *B. pumilus* laboratory streptomycin resistant strain. The dynamics of growth and activity of the strains were studied. It was shown that the maximum activity of the AprBp was at 22–28 h of growth in all strains. We also studied the specific activity of all strains. The most effective strain was *B. subtilis* pCS9. We also developed optimal conditions for growth and cultivation. Purification of the subtilisin-like proteinase was carried out on a column of carboxymethylcellulose ("Sigma"). SDS-PAGE in 12.5% was performed, the protein fraction after chromatography showed the presence of a protein with a molecular weight of 28 kDa, which corresponds to the molecular mass of the AprBp. Physicochemical, biochemical and enzymatic properties of individual enzymes will be established to assess the possibility of their use the subtilisin-like proteinase as feed additives. This work was performed in accordance with the Russian Government Program of Competitive Growth of Kazan Federal University. The experiments on selection of the optimal strain and purification of serine proteinase were supported by the Russian Science Foundation (Project No. 16-16-04062).

P.07-089-Tue
Optimization of pectinase production from sugar beet pulp as carbon source by *Piriformospora indica*

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Pectinases, as a large group of enzymes, catalysis break down of pectin to smaller molecules including galacturonic acid, and they are widely produced by many microorganisms. Nowadays, abundant waste from agriculture and fruit processing industries including sugar beet pulp (SBP), apple and citrus pomace as rich source of pectin are employing to induce pectinase production by many microorganisms. The aim this study was optimization of pectinase production of *Piriformospora* indica fungus by using sugar beet pulp (SBP) as a carbon source. *P. indica* was cultured in Kaefer medium supplemented with SBP, then biomass and spore production, enzyme activity and total protein content were measured for 10 days. One unite of pectinase activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol galacturonic acid per min under the assay condition. According to the results, maximum biomass production and pectinase activity were detected 6.125 g/L and 3.2 U/mL in the presence of SBP at 6th day, respectively. Furthermore effect of different concentrations of glucose and ammonium sulphate and their interaction on enzyme activity was investigated. Based on the results by increasing the amount of them alone the activity was increased but in the case of their interaction with sugar beet pulp the activity was decreased. Next, by using Response Surface Methodology (RSM), pectinase production was 19.4 U/mL which increased by 5.84 times compared to non-optimized conditions. Then, the enzyme were purified by 80% ammonium sulphate and dialysis and the enzyme activity and specific activity were 12.64 U/mL and 55.46 U/mg, respectively. The pectinase purification was confirmed by SDS-PAGE and silver nitrate staining, and its molecular weight was estimated 50 KDa. In conclusion, because of noticeable pectinase production of *P. indica* by using sugar beet pulp as a suitable and cheap substrate, economically is valuable and moreover cause to decrease environmental pollution.

P.07-090-Wed
The implementation of the CRISPR-Cas system for *Kluyveromyces lactis* genome editing

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Kluyveromyces lactis are amongst the most frequently used and well-studied non-conventional yeast. It serves as an expression platform for recombinant proteins including secreted proteins and produces industrially relevant enzymes. Gene targeting efficiencies in *K. lactis* are low and differ greatly depending on the integration locus and the design of the homology cassette. In this study the implementation of the CRISPR-Cas system as a tool for highly efficient genome editing was used for improvement of gene targeting efficiency and introduction of mutations in *K. lactis* genome without additional selection markers. The endonuclease Cas9 (human-codon optimized and fused to a SV40 nuclear localization signal at its N and C-terminus) was expressed either under the control of the constitutive *PGK1* promoter, or inducible *LAC1* promoter in the plasmid which also included the gRNA cassette(s) and pKD1 stabilizing element for expression in *K. lactis*. The gRNA was expressed as a chimeric guide RNA from either of *S. cerevisiae* or of *K. lactis* the *SNR52* promoter and both gRNA expression cassettes terminated by the *S. cerevisiae SUP4* terminator. The high targeting efficiency was reached by introducing mutation(s) in one essential and four non-essential genes even though no selection marker was used for integration at the site of modification. In case of co-transformations of the CRISPR-Cas9 system and donor cassettes, the donor DNA fragments efficiently integrated by homologous recombination. When the donor DNA fragment was not used the Cas9 endonuclease double strands brakes were repaired by NHEJ and introduced indels. The efficient CRISPR-Cas9 system generated for *K. lactis* genome editing was also successfully applied for *S. cerevisiae*.

P.07-091-Mon
Electrochemical detection of aspartate transaminase using a multienzyme-modified biosensor

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AST and ALT are considered to be two of the most important tests to detect liver injury, although ALT is more specific for the liver than is AST and is more commonly increased than is AST. Sometimes AST is compared directly to ALT and an AST/ALT ratio is calculated. This ratio may be used to distinguish between different causes of liver damage and to distinguish liver injury from damage to heart or muscle. We aimed to design a biosensor for the quantitative determination of AST enzyme in a short time and an affordable cost. Polymerization of the biosensor active layer developed for the AST assay was immobilized with UV, BSA, gelatin, malate dehydrogenase, AST and glutaraldehyde. L-aspartate changing in the range of 1.5–15 mM and α-oxoglutarate 25–100 mM were investigated in the reaction medium. The results were compared with spectrophotometric results. Optimization of the bioactive layer used for the designed biosensor were performed. The response current in the range of 0.2–1.2 V was realized in the cyclic voltammogram where the scanning speed

was 0.02V/s. In bioactive layer optimization, 0.06 g of BSA, 0.45 g of gelatin and 2.5% of glutaraldehyde were determined. Bland Altman analysis comparing the averages of biosensor and spectrophotometric results and Pearson correlation analysis in which correlations were evaluated. The AST biosensor we designed and 10 µl of the Randox AS1267 enzyme kit were used at reasonable cost and in a short period of time 1 minute 12 s. We think that it could be suitable for clinical use as a point of care testing. Correlation analysis revealed a strong and significant correlation between the two methods when the r-value was 0.999 and the *P*-value was <0.01. In addition, despite the use of 500 µl of kit per sample by spectrophotometric method we can obtain results for more than one sample with 10 µl AST enzyme immobilized on Au-electrode with the biosensor we designed.

Host–pathogen interactions

P.08-001-Mon
Phenotypic memory in bacteria with minimal genome

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Individual cells within clonal microbial cultures exhibit marked phenotypic heterogeneity. Under stress conditions this phenotypic heterogeneity become more explicit. But why bacteria need such multiplicity? Good model for studding this question is bacteria of class Mollicutes. They feature significant genome and metabolic pathways reduction, are widespread and cause various diseases from pneumonia to diseases of the urogenital tract. In this study, we demonstrated the bacterial proteomic phenotypic memory using cell culture-based infection model for examining the host-pathogen interactions. As a model object we used bird's pathogen *Mycoplasma gallisepticum* and chicken erythroblast HD3 cell line as a host cells. It was shown using a combination of microscopy, two-dimensional differential gel electrophoresis, MALDI-MS, LC-MS, RT-PCR and genome sequencing that this heterogeneity is manifested only on proteomic level without changes in genome sequence and level of transcripts. Interesting, these proteomic changes persisted after prolonged cultivation of mycoplasma in nutrient medium and then returned to the initial state through consecutive state changes (attractors). As the switches of these attractors we assumed two global regulators YebC/PmpR and SpxA operate. We observed the step-by-step operation of these regulators during interaction of bacterium with the host cell. We constructed *spxA* and *yebC*/*pmpR*-overexpressing strains of *M. gallisepticum* for more detail understanding the role of these regulators. In the future, this work can shed light on the understanding of the bacterial memory phenomenon, what mechanisms it provides and for what it is needed. This work was supported by RSF grant No. 14-24-00159.

P.08-002-Tue
Porphyromonas gingivalis lipopolysaccharide (Pg-LPS) regulates mineralized tissue associated genes of hPDL-MSCs

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The aim of this study is to assess the effects of *Porphyromonas gingivalis*-lipopolysaccharide (Pg-LPS) on the cell survival and mRNA expressions of mineralized tissue associated genes of human periodontal ligament mesenchymal stem cells (hPDL-MSCs). hPDL were isolated from teeth extracted for orthodontic reasons. Cells were characterized for MSCs properties and hPDL-MSCs were treated with different concentrations of Pg-LPS (0, 0.1, 1, 10, 100, 1000 and 10.000 ng / ml). To monitor the effects of Pg-LPS on the hPDL-MSCs viability, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay was performed at 8, 16, 24 and 48 h. Cells were treated with 1000 ng/mL Pg-LPS for further gene expression experiments. Total RNA isolation was conducted at 16, 24 and 72 h from hPDL-MSCs and cDNA synthesis was done. Type I collagen (COL I), bone sialoprotein (BSP) and osteocalcin (OCN) mRNA expressions were evaluated using qRT-PCR. Results demonstrated that while 100, 1,000 and 10,000 ng/mL concentrations of Pg-LPS reduced hPDL-MSCs survival at 24 h, there was no statistically significant effect in other concentrations and at other time points. Pg-LPS increased COL I and BSP mRNA expressions at 8, 16 and 72 h and it stimulated OCN mRNA expression at 72 h. Findings of this study suggested that the response of hPDL-MSCs which play important role in the periodontium homeostasis is significantly regulated by periodontopathogen lipopolysaccharide (Selcuk University Scientific Research Project -5102027).

P.08-003-Wed
BolA protein as a new player in *Salmonella typhimurium* virulence

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The intracellular pathogen *Salmonella* Typhimurium emerged as a major cause of foodborne illness, representing a severe clinical and economical concern worldwide. The capacity of this pathogen to efficiently infect and survive inside the host depends on its ability to synchronize a complex network of virulence mechanisms. Therefore, the identification of new virulence determinants has become a hotspot issue in the search for new targets for drug development. BolA-like proteins are widely conserved in all kingdoms of life. In *E. coli*, this transcription factor has a determinant regulatory role in several mechanisms that are tightly related with bacterial virulence. Therefore, in the present work we used the well-established infection model *Galleria mellonella* to evaluate the role of BolA protein in *S.* Typhimurium virulence. We have shown that BolA is an important player in *S.* Typhimurium pathogenesis. Specifically, the absence of BolA leads to a defective virulence capacity that is most likely related with the remarkable effect of this protein on *S.* Typhimurium evasion against cellular response. Furthermore, it was demonstrated that BolA has a critical role in bacterial survival under harsh conditions. Hence, we provide evidences that BolA is a determinant factor in the effectiveness of *Salmonella* to survive

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and overcome host defences mechanisms, which is as important progress to the understanding of the pathways underlying bacterial virulence.

P.08-004-Mon
Isolation and characterization of bacteriophages infecting uropathogenic *Escherichia coli* strains

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Urinary tract infections (UTIs) caused by *Escherichia coli* are among the most common bacterial infections in humans. Despite effective antibiotic therapy, these infections tend to recur. The prevalence of UTIs caused by antibiotic-resistant UPEC is steadily increasing, highlighting the need to find an alternative for the control of pathogenic bacteria. In this study we characterized *Escherichia coli* isolates obtained from patients with urinary tract infection and supplemented with eight collection strains. Strains were differentiated by nonconventional phylogrouping (quadruplex PCR), K1 antigen detection and two locus MLST - CH (*fumC*/*fimH*) typing. Phylogenetic grouping revealed that group B2 was the most common type (n = 15). PCR-based detection of *neuC* (K1-specific gene) identified two clinical strains as K1 positive. A remarkable level of haplotype diversity identified by CH typing among 28 clinical isolates was observed (n = 21). *fumC*/*fimH* allele combination 40/30 was found to be most common (n = 6). Few representative strains were chosen as a hosts to isolate bacteriophages from sewage infecting *E. coli*. Four phages (vB_EcoP_KMB13, vB_EcoP_KMB14, vB_EcoP_KMB15 and vB_EcoP_KMB16) were then isolated which were able to lyse 53% of a subset (36) of the *E. coli* strains. The phage genome sequences were obtained, annotated and this resulted in classification of vB_EcoP_KMB13 and vB_EcoP_KMB14 as the Podoviridae family and vB_EcoP_KMB15 and vB_EcoP_KMB16 were classified as the Siphoviridae family. New isolated phages still need to be characterized in terms of interactions with different bacterial hosts, but they represent a promising candidates for phage therapy targeted specific for infections caused by uropathogenic *E. coli*.

P.08-005-Tue
Protein complexes as a mechanism of adaptation in bacteria with a reduced genome

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M. gallisepticum is a simplistic organism with significantly reduced genome. Adaptation mechanisms based on differential gene expression are generally reduced in mycoplasmas due to the loss of majority of respective proteins (*i.e.* transcription factors). Alternate adaptation pathways may involve reorganization of protein complexes without significant changes in abundance of participating proteins. To explore this possibility we tested model mycoplasmal intracellular infection of eukaryotic cells (chick erythroblast cell line HD3). To study protein complexes we used protein correlation profiling (PCP) as main approach with verification by tandem affinity purification (TAP) for complexes of interest. PCP included fractionation of complexes by centrifugation in sucrose gradient with subsequent native electrophoresis of each gradient fraction. Label-free quantitative LC-MS was used to evaluate abundance profiles of proteins along the gel lane which allowed to determine changes in complex stoichiometry based on profiles likelihood. We found difference in composition of protein complexes in control mycoplasma laboratory strain and mycoplasma cultivated after being extracted from host cells. Besides, we observed the formation of novel non-canonical complexes in post-infectional mycoplasma. This study was funded by Russian Science Foundation (14-24-00159).

P.08-006-Wed
Prediction and validation of the structural features of Ov58GPCR as an immunodominant antigen of *Onchocerca volvulus*: implications in onchocerciasis control efforts

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Onchocerciasis, also called river blindness is a devastating yet neglected tropical disease that creates a remarkable stigma, generates and perpetuates poverty, and is a major impediment to socioeconomic development/progress of affected communities. It is the second leading infectious cause of blindness worldwide, affecting about 15.5 million people including 12.2 million with onchocercal skin disease (OSD), 1.025 million with vision loss and an additional 172 million people in need preventive chemotherapy. Currently, Ivermectin (IVM) is the only drug used for mass treatment against the disease. Ivermectin, however has limited effects on adult parasites, so treatments must be expanded over at least 12–15 years, corresponding to the reproductive lifespan of the adult worm when exposed to drug pressure, hoping no further infection occurs within this time. The WHO's ambitious goal of eliminating the disease by 2025 will not be achieved without the development of new tools. Measures to certify disease elimination and surveillance are of dire need for health systems in countries where onchocerciasis is a public health problem. Here, we report that (i) *O. volvulus* antigen Ov58 is a G-protein coupled receptor (GPCR) conserved in nematodes, (ii) synthetic peptides predicted in the extracellular domain (ECD) of Ov58GPCR elicit positive responses to sera from onchocerciasis patients, (iii) synthetic peptide cocktails discriminate sera from infected, treated patients and normal African controls, (iv) polyclonal antibodies against the recombinant ECD revealed a single band on blots of *O. volvulus* total extracts, corresponding to the expected size of the endogenous native antigen, (v) Ov58GPCR is transcriptionally activated in both the larvae and adult parasite, (vi) humoral immune responses depict declines with IVM treatment compliance. Hence, we conclude that the ECD of Ov58GPCR harbors antigenic features of interest in the context of onchocerciasis control efforts.

P.08-007-Mon
Interplay between L-A and M viruses in yeast

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Totiviridae family dsRNA viruses are commonly spread among fungi and protozoa kingdoms. Special kind of these viruses - L-A - are found among *Saccharomycetales* and other yeasts. Some yeast strains together with L-A helper virus carries smaller satellite

dsRNA of so-called M virus. Yeast hosting both viruses engages in killer phenotype. L-A genome encodes major capsid protein Gag and a fusion protein Gag-Pol. Gag-Pol protein displays RNA dependent RNA polymerase activity, is crucial for L-A and M virus maintenance in a cell. Satellite (M) dsRNA genome encodes a secreted protein toxin. L-A spreads by direct cytoplasm contacts occurring during cell division and cell-cell mating. Extracellular stage of L-A virus remains unidentified. Presence of L-A virus in yeast does not cause slower cell growth or other detectable phenotypic changes. Global gene expression analysis of yeast focusing on the impact of L-A dsRNA should reveal molecular process engaged in the maintenance of L-A virus. To obtain relevant RNA-seq results, an adequate method for excluding L-A virus from yeast is required. Our group developed unique, controllable and safe strategy allowing elimination of L-A viruses. Over-expression of modified L-A capsid protein Gag results in complete L-A virus elimination from the yeast. Furthermore, research performed with overexpression of various type of L-A virus Gag-Pol proteins revealed complex interactions between different L-A and M viruses. The collected data provides interesting observations into the evolutionary relations between various types of L-A viruses and suggests possible mechanisms involved in maintaining helper and satellite viruses. This research was funded by Research Council of Lithuania, grant #SIT-07/2015.

P.08-008-Tue
Secreted and surface-associated proteins of *Mycoplasma gallisepticum*

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Mollicutes or mycoplasmas are bacteria that lack a cell wall and have significant genome reduction. *Mycoplasma gallisepticum* is a cause agent of avian chronic respiratory disease. Mycoplasma infections can lead to acute inflammatory episodes, but most are chronic, suggesting that these bacteria have developed strategies to avoid host's immune system response. In this work we focused on analysis of secreted (SP) and surface-associated proteins (SAP) of M. gallisepticum. For isolation of SP, washed bacterial culture in exponential phase was incubated for 2 h in growth media without horse serum. For SAP, bacterial cells were treated with proteinase K. All samples were analyzed using LC-MS. To exponentially access relative protein abundances, the modified protein abundance index was calculated. Two biological replicates were obtained. Proteins that were statistically significantly (P-value <0.05) enriched in secreted and surface-associated fractions compared with total proteome were accepted like positive results. There were 24 and 20 proteins, respectively. All SP have predicted signal peptide. SP were divided into four groups: IgG-binding proteins, serine proteases and two groups of proteins with uncharacterized function. Some genes are clustered in operons. There were three groups among SAP: variable lipoprotein family proteins, putative peptidases DUF31 and proteins with uncharacterized function. Identified proteins may be missed partners of known IgG-binding proteins - putative peptidases DUF31 system. Three SP contain mitochondrial localization signal and one of them is predicted like mitotic checkpoint regulator. Some proteins were identified for the first time and these results can help to elucidate molecular mechanisms both of infection and escape host's immune system response to mycoplasmas. This work was funded by the RSF grant 14-24-00159 "Systems research of minimal cell on a *Mycoplasma gallisepticum* model".

P.08-009-Wed
Heat stress is superior to biotic stress caused by flagellin

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Recognition of microbe-associated molecular patterns (MAMP) is crucial for plant defense against pathogen attack. The best described MAMP is peptide from bacterial flagellum (flg22), which is recognized by receptor flagellin sensing 2 (FLS2) in *Arabidopsis thaliana*. In our work we focus on biotic stress responses triggered by flg22 under heat stress conditions. It is important to study plant reaction to multiple stress conditions because plants are often exposed simultaneously to abiotic and biotic stresses. We observed that high temperature inhibits flg22-induces reactive oxygen species burst in four-week-old *Arabidopsis thaliana* leaves. This effect is time dependent and transient. In addition, effect of elf18, which is the other MAMP, was similar. Heat stress decreases transcript level of *FLS2* and other genes activated in flagellin signaling pathway. The plants defend against the pathogen attacks on the level of cell wall by increasing callose production. We observed that heat stress decreases also flg22-induced callose production. It is known, that flg22 caused endocytosis and degradation of FLS2, but also at later time flg22 elicited recovery and *de novo* synthesis of FLS2. We used stable *Arabidopsis thaliana* transformant FLS2:GFP with natural promotor to envisage its possible role in biotic stress in heat stress pretreated plant. Altogether our data show inhibition effect of heat stress on the events triggered by recognition of flg22 in *Arabidopsis thaliana*. This work was supported by Czech Science Foundation grant PPPLZ L200381652.

P.08-010-Mon
Diversity of genes encoding hevein-like antimicrobial peptides in cereals

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Studies of antimicrobial peptides (AMPs), the most important components of the plant defense machinery, are of great importance for our understanding of plant immunity. Previously, we isolated AMPs named WAMPs from *Triticum kiharae* seeds. They belong to a new family of hevein-like antimicrobial peptides with a characteristic 10-cysteine motif. WAMPs display potent antimicrobial activity against fungal and bacterial pathogens. According to their primary and 3D-structure, these peptides are similar to the chitin-binding domains of class I chitinases, plant defense proteins. It was shown that WAMPs specifically inhibit fungalysin, a secreted metalloproteinase of the *Fusarium* fungi, and the amino acid residue at position 34 affects the degree of enzyme inhibition. The present work deals with analysis of *wamp* gene diversity in cereals including both cultivated and wild-growing plants: *Hordeum vulgare*, *Secale cereale*, *Elytrigia repens* and *Echinochloa crus-galli*. Using primers designed on the basis of earlier determined wheat WAMP cDNA sequence and DNA as a template, we isolated *wamp* genes from all species studied. Their expression was confirmed in plant seedlings. The discovered *wamp* genes encode precursors of a similar structure, consisting of a signal peptide, a mature peptide region and a C-terminal prodomain. The mature peptide sequences are highly conserved pointing to the important role of WAMPs in plant defense. Barley homologues, with a series of deletions in the mature peptide

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domain, represent the only exception. In WAMP homologues, at position 34 of the mature peptide Ala and Val were detected. Using heterologous expression in *E. coli* cells, the recombinant WAMPs were produced. In vitro antimicrobial assays showed that WAMP homologues differ in antifungal activity. The results obtained expand our knowledge on the biodiversity of genes encoding WAMPs in cereals.

P.08-011-Tue
Peculiarities of BCG vaccine performance in B10.M (H2f) mice

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Pathogenesis of tuberculosis (TB) infection is a multistage, complex sequence of events involving a plethora of cell populations and molecules. Earlier, it was demonstrated that generally vaccination with bacillus Calmette-Guerin (BCG) prior to Mycobacterium tuberculosis infection significantly prolongs survival time of genetically resistant and susceptible inbred mice. The B10.M (H-2f) mouse strain appeared to be an interesting exception: these mice exhibited intermediate resistance to primary infection but their survival time even slightly decreased if intravenous challenge was applied after BCG vaccination. In the present study, we addressed: (i) possible immune mechanisms behind this paradoxical phenotype, and (ii) assessed whether the "fail-of-vaccination" phenotype is expressed after challenging mice via respiratory tract. Our experiments confirmed previously reported defect in B10M mice: after intravenous challenge with 2 x 106 CFU of M. tuberculosis H37Rv post-infection life span and cachexia levels were identical in non-vaccinated mice and mice vaccinated with 5 x 107 BCG CFU. Moreover, vaccination did not change mycobacterial loads at weeks 4 and 8 post challenge, the ratio of various cell types, and cytokine profiles in the lung tissue. We also applied a more "natural" TB model based upon aerosol infection (result 600 CFU) to B10.M and B10/Sn control mice; the latter have the same genetic background but differ by the H2 haplotype (H-2b) and readily respond to BCG vaccination in the intravenous challenge model. We still accumulate immunologic data concerning vaccination effect in these to mouse strain, but it is already clear that lung cells of infected B10.M mice produce significantly lower amounts of IFN-γ in response to mycobacterial antigens compared to B10/Sn cells, and that this phenotype does not depend upon BCG vaccination. This result suggest that immune response in infected lungs of B10M mice might be less Th1-biased.

P.08-012-Wed
RNA aptamers for inhibition of the effector protein HopU1 of phytopathogenic bacteria *P. syringae*

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It is known that phytopathogenic bacteria are capable of suppressing both basic and induced plant immunity due to the secretion of the virulence factors or effector proteins inside the plant cell. It is established that the main players in the pathogenesis of gram-negative bacteria are the effector proteins secreted by bacteria via the third type transport system (TTS) into the cells of the host plant. One of the key effector proteins in the pathogenesis of P. syringae is HopU1. HopU1 is an ADP-ribosyltransferase interacting with RNA-binding proteins, thereby suppressing plant immunity. It acts as a "saboteur" that ruins plant immunity at the posttranscriptional level. In this regard, we hypothesized that the suppression of the functions of this effector protein should lead to an increase in plant resistance to the phytopathogens that use the effector proteins of the third transport system. In order to suppress HopU1 we have proposed the utilization of the RNA aptamers specifically binding this protein. The approach of the utilization of RNA aptamers to suppress the function of proteins is known for a long time, but has never been tested on plants. First we have expressed a heterologous recombinant effector protein HopU1 of P. syringae bacteria. Using the SELEX method we have selected RNA aptamers for HopU1 protein. Next we have characterized the selected RNA aptamers, described their secondary structure and evaluated the dissociation constants (Kd) of the RNA-aptamer-HopU1 complexes by the MST method. The values of Kd for aptamers H1 and H2 were Kd (H1) = 6.71 +/- 0.69 nM and Kd (H2) = 6.41 +/- 0.48 nM, respectively, indicating a high degree of binding. In our future work we plan to obtain transgenic plants constitutively expressing selected RNA aptamers and use them for phytotests. We expect that such plants will have increased resistance to pathogenesis by P. syringae This work was supported by a grant from the Russian Foundation for Basic Research No. 16-04-01002

P.08-013-Mon
The diversity of putative antimicrobial peptides revealed in wheat by high-throughput next-generation transcriptome sequencing

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Antimicrobial peptides (AMPs) are important components of the plant immune system. Diverse in structure, biological activity and the mode of action they offer a rich repertoire of biologically active molecules for practical applications: in agriculture to produce disease-resistant crops and in medicine to develop next-generation antimicrobials. This study is focused on AMP mining in transcriptomes of the wheat species *Triticum kiharae* Dorof. et Migusch, a synthetic allopolyploid, which is highly resistant to pathogens. For the first time RNA-seq analysis of healthy, infected with *Fusarium oxysporum*, treated with elicitor, and treated with elicitor followed by infection with *Fusarium oxysporum* wheat seedlings, as well as *de novo* transcriptome assembly and annotation, are presented. To identify pAMPs a hidden Markov model based on conserved cysteine motifs characteristic to different AMP families was created followed by searching, quality controlling and sorting out the results. Using the developed pipeline 563 unique putative AMPs belonging to different families – defensins, thionins, hevein-like peptides, LTPs, snakins, and cysteine-rich peptides with novel cysteine motifs, were predicted. Furthermore, other cysteine-rich peptides with defensive role, such as Bowman-Birk inhibitors and Proteinase inhibitors II, as well as RALF peptides involved in defense signalling were identified, together with other CRPs, such as MEG Ae1 and Pollen Ole e1, whose role in immunity is still unclear. For the first time three types of hevein-like peptides that differ in the number of cysteine residues and the length of the C-terminal prodomain were discovered in wheat. The antimicrobial activity of a number of predicted AMPs was proven by in vitro assays of recombinant peptides. Thus, the developed pipeline of AMP search disclosed an amazing

complexity of AMP array in wheat. This work was supported by the Russian Science Foundation (grant no. 16-16-00032).

P.08-014-Tue
Outer surface protein C (OspC) from *Borrelia* is capable of fibrinogen binding

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Pathogenic bacteria differ widely in their strategies for evading immune surveillance. In the case of *Borrelia burgdorferi sensu lato*, which is the causative agent of Lyme disease, such a strategy relies on producing a multitude of surface proteins showing high serological divergence among pathogenic strains and capable of escaping the immune response. In addition, some of the surface proteins recruit host factors to perturb the host homeostatic system or cover the spirochete with an "invisibility cloak". Outer surface protein C (OspC) is one of the most abundant surface lipoproteins produced by the spirochete during the early stages of infection and its DNA coding is much more variable than of other genes from these bacteria. A few specific functions have already been proposed for OspC, such as binding of host complement factors, interaction with tick immunoprotective salivary protein Salp15, plasminogen recruitment, or protection from phagocytosis by macrophages. Our report adds a new target to the OspC interaction network. We observed that recombinant OspC proteins from different human-infectious *Borrelia* species are capable of fibrinogen binding with high affinity. We also propose a fibrinogen binding site of OspC, present some structural features of the complex in solution, and evaluate the influence of the OspC-fibrinogen complex formation on fibrinogen polymerization. Finally, we discuss the implications of our findings in the context of *Borrelia* survival at the early stage of the mammal host infection. This work was supported by grant no. UMO-2015/17/B/NZ1/00873 from the National Science Center (Poland).

P.08-015-Wed
Arginine sensing and transport is uncoupled in *Leishmania donovani* parasites

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Visceral leishmaniasis is a deadly disease caused by *Leishmania donovani*. During infection, parasites up-regulate macrophage nitric oxide synthase and arginase activity, both of which use arginine as a substrate. These elevated activities depleted macrophage arginine pools, a situation that invading *Leishmania* cannot tolerate as this is an essential amino acid. *L. donovani* imports exogenous arginine via a mono-specific amino acid transporter (AAP3) and utilizes it primarily to provide precursors for trypanothione biosynthesis. The depletion of arginine from promastigote and amastigote growth media induced a rapid up-regulation in AAP3 expression and activity, as well as a few other genes by activation of a signaling cascade of arginine deprivation response (ADR) pathway. Arginine is a positively charged amino acid with a guanidine cap at the distal end. Based on previous arginine transport analysis data from our group we postulated that some of best known arginine transport inhibitors have conserved guanidino groups in their side chains and some of these transport inhibitors were also structural analogues of arginine. To understand whether arginine sensing and transport are coupled; we used these arginine transport inhibitors (canavanine, N-Methyl L-arginine acetate (NMLAA) and pentamidine) to check their effect on ADR. *L. donovani* promastigotes and THP-1 infected amastigotes were analyzed in this study. We identified that pentamidine and canavanine downregulated ADR response; While NMLAA had no effect on ADR both in promastigotes and intracellular amastigotes. Additionally, pentamidine and canavanine also mimicked the presence of arginine thus downregulating ADR response in arginine starved promastigotes and intracellular amastigotes. Our data shows that show that arginine sensing and transport are uncoupled. The arginine sensor responds to both arginine deprivation and sufficiency by either activating or deactivating ADR by modulating the expression of AAP3.

P.08-016-Mon
The protective capacity of anti O:4 antigen antibodies against *Salmonella* infection is influenced by the presence or absence of O:5 antigen

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O-antigen antibody such as anti O:4 antigen IgG induces protective immunity against *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) infection. Although *S.* Typhimurium belongs to the group O:4, it can be classified into two serological variants, factor O:5 positive strain (O5+) and factor O:5 negative strain (O5-). In this study, to determine whether anti O:4 antigen IgG induces protective immunity against both O5+ and O5- *S.* Typhimurium infection, we compared *S.* Typhimurium x3306-oafA (O5+) with x3306-pBR322 (O5-) using mouse model. High dosage of anti O:4 antigen IgG induced protective immunity against both O5+ and O5- *S.* Typhimurium infection. However, low dosage of anti O:4 antigen IgG was able to induce protective immunity against O5- *S.* Typhimurium infection only. The affinity of O:4 antigen with O:4 antigen IgG was found to be stronger in the O5- strains compared to O5+ strains. Next, macrophages infected with O5- strains treated with anti O:4 antigen IgG showed enhanced pathogen uptake compared to counterparts infected with O5+ strains treated with anti O:4 antigen IgG. Moreover, these macrophages produced elevated levels of nitric oxide, indicating that O5- strains treated with anti O:4 antigen IgG enhanced more pronounced macrophage activation than O5+ strains treated with anti O:4 antigen IgG. We suggest that, although anti O:4 antigen IgG has the potential to protect against *S.* Typhimurium infection, the effects of anti O:4 antigen IgG in protection against Salmonella infection might differ depending on the presence of O:5 antigen.

P.08-017-Tue
Modulating host metabolic sensors for targeting TB

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An effective host immune response is important to control *Mycobacterium tuberculosis* (*Mtb*) growth, and to contain its latent persistence. Using a chemical genetics approach we have demonstrated that activating AMP-activated protein kinase (AMPK) and Sirtuin 1 (SIRT1) axis, regulator of whole-body energy metabolism, by FDA approved drugs / supplements could control inflammation and *Mtb* infection. This indicates the deep engagement of *Mtb* pathogenicity with host cell immunometabolic machinery. We hypothesize that the functional connections

between immunity and pathways controlling metabolic signaling could be harnessed to advance the host-directed therapy (HDT) pipeline, leading to the development of clinically relevant anti-TB therapies. This strategy also provides an opportunity of targeting persisters, demonstrated by less prevalence of latent TB among individuals taking some of these metabolic drugs.

P.08-018-Wed
Consequences of gut microbiota manipulation by antibiotics in animal model of early immune dysregulation: phenotype analysis of behaviour and biochemical correlates

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Host-microbe interactions are now thought as essential for maintaining host health. Additionally, microbiota and activation of host′s immune system play significant role in the development and regulation of CNS. Furthermore, it was presented that certain microbial proteins can mimic several key neuropeptides involved in the regulation of behaviour. The role of the gut microbiome in the bi-directional interaction on the gut-brain axis and the role in a set time-frame for normal synaptogenesis in the development seem crucial. Finally, infectional insult during perinatal period may be responsible for the development of neuropsychiatric disorders such as schizophrenia in later life. According to these facts, our study was aimed at describing behavioural and biochemical alterations in rats exposed to early inflammatory stimulation. This was based on the subchronic administration of lipopolysaccharide to neonatal rat together with effect of microbiota modulation realised by antibiotic cocktail. The 10-days modulation of gut microbiota was started in adults at PD 60 in drinking water. The microbiota from faeces was analysed before and at the end of experiment together with analysis of neurotransmitters from plasma and brain tissues. Shift in the composition of the bacterial diversity in faeces of studied animals was preliminary assessed by DGGE analysis and then determined by the Ion Torrent next-generation sequencing technology. To evaluate changes on low- and high-molecular level, the collected biological samples were analysed using advanced metabolomic and proteomic strategies. Significant changes in the fingerprints of bioactive molecules among the tested groups were found. The values were discussed with relation to results obtained in behavioural tests, where impairment of behavioural profiles (locomotion and sensorimotor gating) and weight gain deficit were documented. Supported by GACR project P304-12G069, AZV MHCR 17 - 31852 A and NPU - LO1611.

P.08-019-Mon
Tick-borne encephalitis virus induces translational shut-off in human cells of neural origin

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Tick-borne encephalitis virus (TBEV) is a member of the genus *Flavivirus*. It can cause serious infections in humans which may result in encephalitis/meningoencephalitis. In order to study the virus-host interactions *in vitro*, our laboratory has previously described a new experimental model: human medulloblastoma cell line (DAOY HTB-186) which is derived from the neurons located in the cerebellum, one of the most affected areas during tick-borne encephalitis. Based on our transcriptomic data and literature, we hypothesized that viperin, an interferon-stimulated gene involved in antiviral defence, could play a significant role in immune response of DAOY cells against TBEV. Therefore, we analysed the viral titres in DAOY cells over-expressing viperin infected by two TBEV strains – the milder strain Neudoerfl and the more severe strain Hypr. In both cases, no effect on virion production was observed. Surprisingly, over-expression of viperin or other selected genes in already infected cells resulted in decreased protein levels proportionally to the viral titre used. Interestingly, no change in mRNA levels was detected, suggesting a viral-induced inhibition of protein production on the translational or post-translational level. Subsequent analysis of *de novo* protein synthesis (metabolic labelling using Click chemistry) revealed that the more severe TBEV strain Hypr reduced the rate of protein synthesis by 40% in comparison to mock-infected cells at 48 h post infection. Surprisingly, the effect of translational shut-off was detected also in the case of several housekeeping genes. Further experiments are in progress in order to describe the exact mechanism of the observed virus-induced translational shut-off. This study was supported by the MEYS CR project Postdok_BIOGLOBE (CZ.1.07/2.3.00/30.0032), the Grant Agency of the Czech Republic (15-03044S, 18-27204S), and Czech research infrastructure for systems biology C4SYS (LM2015055).

P.08-020-Tue
Gene expression pattern of HeLa cells producing recombinant PGLYRP1 infected with *Chlamydia trachomatis*

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Human peptidoglycan recognition proteins (PGLYRPs) are components of the innate immunity that possess antibacterial activity. We obtained HeLa cell lines that stably produce recombinant human PGLYRPs into the cultivation media. Previously, we showed that recombinant PGLYRPs inhibit the growth of chlamydia. The aim of the work was to study the change in gene expression in recombinant PGLYRP1 producing cell-lines in response to infection with *C. trachomatis* elementary bodies. Two cell lines produced native (active) and defective (inactive)

PGLYRP1 were used in the experiment. We performed transcriptome analysis of cells infected and non-infected with *C. trachomatis*. Cells were collected for the analysis at three time points corresponding to different stages of infection: (i) 1 hour (the elementary bodies penetrate into the cell), (ii) 48 h (chlamydia are inside the cells), and (iii) 72 h (the second round of infection) after infection. Total RNA was isolated. Samples were prepared using the Illumina TotalPrep RNA kit Amplification Kit. Measurement of the gene expression level was carried out by RNA-microarray using Illumina Expression BeadChip Kit. The primary data processing was performed using the GenomeStudio software. Search for differentially expressed genes were carried out using the Student's *t*-test, the library limma in part of the Bioconductor package. We performed a functional analysis of groups of differentially expressed genes (in terms of GO-Gene Ontology and KEGG) with WebGestalt. We found that expression levels of the genes in infected cells that produce active PGLYRP1 did not differ from non-infected cells. In the case of cell line producing defective PGLYRP1, about 30 differentially expressed genes related to various groups of metabolic processes were found after infection with *C. trachomatis*. Thus, we suppose that PGLYRP1 binds to chlamydial elementary bodies and affects chlamydial development in the cell.

P.08-021-Wed
The involvement of the Kunitz peptidase inhibitor-like protein in plasmodesmata gating control in the Solanaceae plants

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Plasmodesmata (PD) in intact mature leaves are limited in their ability to pass macromolecules into neighbouring cells. Such important process as PD control under stress conditions is determined by a set of protein factors, which are regulated not only at the level of gene transcription but also at the stage of mRNA translation. Recently, we identified a pathogenesis-related gene encoding the Kunitz peptidase inhibitor (KPI)-like protein (KPILP) in plants of the Solanaceae family. This is the first example of the matryoshka gene with a nested alternative open reading frame controlling maternal gene expression in stress conditions. Here, we investigated the possible participation of *Nicotiana benthamiana* KPILP (NbKPILP) in the stress-induced increase of PD gating. We showed that the NbKPILP-specific signal sequence is responsible for its cell wall (CW) localization. GFP-tagged full-length NbKPILP displayed the typical PD-specific punctate accumulation and co-localized with the mRFP-tagged Tobacco mosaic virus (TMV) movement protein (MP). The deletion analysis allowed us to determine the PD localization signal in the N-terminal half of NbKPILP. Experiments with a vector encoding two tandem copies of GFP (2xGFP) as a tracer of cell-to-cell movement showed the capacity of NbKPILP to dilate the PD. This property of NbKPILP was also confirmed in the complementation experiments whereby NbKPILP compensated *in trans* loss of the MP_{TMV} gating function. An infectious copy of crTMV with a frameshift mutation in the MP gene [crTMV(MPfs):GFP], which lacks the ability to move from cell to cell, in the presence of NbKPILP formed multicellular GFP-expressing clusters. We concluded that NbKPILP is a CW-localized protein that participates in mature PD gating control under stress conditions. This study was performed with financial support from the Russian Foundation for Basic Research (project No. 17-29-08012).

P.08-022-Mon
Immunolabeling of cell-surface abnormal prion protein in viable cells

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Prion diseases are fatal neurodegenerative disorders in humans and animals. The key event in the pathogenesis of these disease is the conversion of host-encoded normal cellular prion protein (PrPC) into its pathogenic isoform (PrPSc) and its accumulation in the central nervous system. The conversion of PrPC into PrPSc appears to occur at the cell surface or along the endolysosomal and endocytic recycling pathways, however the molecular events in the conversion process and the roles of cellular factors in this process remains poorly understood. Due to the lack of definitely PrPSc-specific antibodies, immunochemical differentiation of PrPC from PrPSc created a barrier for the evaluation of the conversion process. In attempt to identify the candidate molecules facilitating PrPSc formation, the optimal conditions to label cell-surface PrPSc in viable cells were investigated. Using an approach based on a recent study describing amino-proximal anti-PrP antibodies react natively with cell surface PrPSc in its physiological context, we evaluated several amino-proximal anti-PrP antibodies and optimized the immunocytochemical conditions for immunolabeling aggregated PrPSc on the cell-surface of prion-infected GT1-7 cells. Sucrose density gradients analysis of prion-infected GT1-7 cells solubilized with 1% Lubrol WX indicates that most of cell-surface immunolabeled-PrPSc were enriched in fractions between detergent-resistant membrane fractions and Lubrol soluble fractions, possible detergent partial-resistant domains.

P.08-023-Tue
Thionins from black cumin (*Nigella sativa* L.) as novel insectotoxins

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Plants are known to be donors of different biologically active compounds, most of which are components of innate immunity to a biotic or abiotic environmental stress factor. Protein and peptides with defense properties (mostly antimicrobial, signal, and hydrolase enzyme inhibitors) are the main and required molecules associated with the disease and pest resistance level. Wild plants are good sources of diverse biologically active polypeptides with significant tolerance to insects and microbial pathogens. In this work, we discovered a family of the thionins from black cumin (*N. sativa* L.) seeds, which were previously shown to display potent antimicrobial (antifungal and antibacterial) features and antitumor effects, to increase the insecticidal action toward mealworm (*Tenebrio molitor*) and flesh fly (*Sarcophaga carnaria*) larvae by direct injection into the hemolymph (oral toxicity). We detected LD_{50} values of the lost larvae at an average concentration of 25–30 μM. We additionally discovered a possible association between thionin cytotoxicity and the observed insectotoxicity. The results of cytotoxicity assays toward the Sf9 commercial insect cell line were provided followed by staining by trypan blue and detection with optical microscopy. There was no cell lysis or plasmolysis thionin concentrations up to 36 μM, and thus, the results are not dependent on insect cytotoxicity, but can be associated with an intracellular target. This

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P.08-024-Wed
Comparative analysis of secondary metabolites with antimicrobial activities from mosses and liverworts

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Plants have evolved efficient systems to counteract microbial phytopathogens, including production of various antimicrobial secondary metabolites. Bryophytes (mosses, liverworts and hornworts) are the ancient group of land plants with many unique antimicrobial compounds, both intracellular and secreted. Antimicrobial potential of many bryophyte-derived compounds is well-known but there is no comprehensive data about their nature and composition. To begin analysis of bryophytes' secondary metabolites with antibacterial and antifungal activity we examined natural compounds from mosses *Physcomitrella patens*, *Ceratodon purpureus* and *Sphagnum fallax* and a liverwort *Marchantia polymorpha*. Specifically, we aim to determine chemical composition of the most biologically active against plant pathogens bryophyte compounds from water-, methanol- and hexane-soluble extracts. In addition, we analyze bryophytes' extracellular peptidomes and secreted enzymes. Overall, our study has a unique potential to help identify metabolites suitable for further development of new plant-derived biotechnological strategies to control plant pathogens and improve crop quality and yield. This study is supported by the Russian Foundation for Basic Research grant 18-016-00146a. KFU is supported by the Russian Government Program of Competitive Growth.

P.08-025-Mon
TAR RNA-dependent mechanism of Ku-mediated transcription regulation of HIV-1 genes

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The Ku protein plays a key role in the DNA double-strand break repair by non-homologous end-joining mechanism. In addition, it participates in transcription, telomere maintenance, V(D)J-recombination and some other processes. Ku is a participant of human immunodeficiency virus-1 (HIV-1) replication at the stages of integration and transcription although the exact mechanism of Ku-dependent transcriptional regulation is unclear. We identified RNA structural motifs important for the interaction with Ku. The highest Ku affinity was detected toward a hairpin RNA structure containing bulk bulge close to the loop. TAR RNA forms a hairpin at the 5'-end of HIV mRNA and has a Ku preferred structure. TAR RNA lacking the bulge had significantly lower affinity towards Ku. We constructed a set of reporter vectors containing firefly luciferase gene either under the control of wild-type HIV LTR or its deletion mutants: lacking the region coding for the whole TAR RNA or the region corresponding to the bulge of TAR RNA. CRISPR/Cas9 depletion of Ku led to significant decrease in luciferase expression from HIV LTR and only slightly influenced the mutant constructs. Overexpression of Ku protein in Ku-knockdown cells partially restored luciferase expression from WT LTR. TNF-a mediated induction of HIV transcription initiation had no significant impact on Ku-dependent regulation. Tat is an HIV trans-activating protein participating in activation of transcription elongation that binds to TAR RNA and recruits P-TEFb complex to the promoter for the phosphorylation of RNAP II. Ku overexpression reduced Tat-mediated activation of elongation and Ku depletion increased the effect of Tat protein. Moreover, the effect of Ku depletion on transcription from LTR promoter was abolished by Tat expression. These data demonstrate the role of TAR RNA in the positive regulation of transcriptional elongation from LTR promoter by Ku. This work was supported by the RSF grant 17-14-1107.

P.08-026-Tue
Human adenovirus type 26 uses αv integrin for infection of human epithelial cells

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Human adenovirus vectors based on type 5 (HAdV5) are the most commonly investigated adenoviral vectors for gene therapy and vaccination. Due to activation of the host innate immune responses as well as preexisting immunity to AdV5 development of new strategies to evade undesired anti vector host immune responses is needed. One approach is the search for other types of adenoviruses that occur at low prevalence in human populations, such as human adenovirus type 26 (HAdV26). HAdV26 belongs to subgroup D and its natural tropism is still unknown. While HAdV26 immunogenicity in vivo is rather well described, basic biology of this virus is less studied. Hence, the main objective of this study was to investigate transduction efficacy and cell entry of HAdV26 in epithelial cells. As a cell model we used A549 and SK-OV-3 cells which have different expression of known adenovirus receptors: CAR, CD46 and αv integrin. We showed that HAdV26 transduces SK-OV-3 cells 4 times better than A549 indicating that SK-OV-3 cells might express molecule/s that HAdV26 uses as a receptor. Thus, we downregulated CAR, CD46 and αv integrin in A549 and SK-OV-3 cells and subsequently measured transduction efficacy and binding of HAdV26. Downregulation of αv integrin significantly decreased transduction efficacy and binding of HAdV26 in both A549 and SK-OV-3 cells. We obtained the same effect by measuring transduction efficacy of HAdV26 after antibody-mediated blocking of αv integrin Namely, blocking αv integrin in A549 cells efficiently decreased transduction efficacy of HAdV26. These results were further confirmed in A549 cells with increased expression of αv integrin. We stably transfected A549 cells with αv integrin expression plasmid and showed that overexpression of αv integrin increases binding and transduction efficacy of HAdV26 in A549 cells. Based on our results we conclude that HAdV26 uses αv integrin for infection of epithelial cells.

P.08-027-Wed
Virulence factors of *Achromobacter ruhlandii* important for the host-pathogen interaction

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In the era of antibiotic resistance there is very important task to develop new antibacterial drugs. Bacterial virulence factors (VF)

are the candidates for potential targets. So investigation of VF of dangerous strains by genomic approach is the first step of medicine elaboration. *Achromobacter ruhlandii* SCCH3:Ach33-1365 ST36 (Arul ST36), the Russian epidemic strain, was the subject of our analysis, because of Arul ST36 is transmissible, and decreases the lung respiratory function of the cystic fibrosis patients. Type III secretion system (T3SS) and motility factors attracted our attention. Genome of Arul ST36 (Accession Number CP01743) was analyzed by the set of bioinformatic tools. Only type IVb pili were revealed in Arul ST36 in contrast to famous *Burkholderia* pathogen. At least 12 tight adherence (tad) genes are required for IVb pili formation. The tad genes of Arul ST36 genome were organized in operon consisted of 18 genes. Flp prepilin of Arul ST36 had typical structure for Flp subclass. It was 58 aa long and consisted of 3 parts (leader peptide, hydrophobic and C-terminal regions). The leader peptide was 14 aa long. Flp motif ITALEY, crucial for the assembly of pili VIb, was characterized by adjacent E(+5) and Y(+6) residues, and G residue in (-1) position, that was essential for a cleavage of the signal peptide. Conservative F reside was revealed in C-terminal region. T3SS was detected in *Achromobacter* genome for the first time. T3SS of Arul ST36 contained 31 ORFs encoded by the 23920 bp region, and separate *virB1* gene, and belonged to the EscN/YscN/HrcN family defined an extracellular pathogen. 570 candidate effectors of T3SS were revealed in Arul ST36 genome. 9 effectors were established important for the regulation of innate immune response. Analyzed VF of Arul ST36 revealed new targets for the antibacterial drug elaboration and demonstrated the permission of using new drug Ftortiazinon for the suppression of secretion of Arul ST36 effectors.

P.08-028-Mon
BP1253, a phosphoribohydrolase enzyme in *Bordetella pertussis*

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Notwithstanding the existence of a vaccine, the Whooping cough infection caused by the Gram-negative bacterium *Bordetella pertussis* (*Bp*), is re-emerging. This recurrence could be the reflection among others of a waning immunity, epidemiological changes in the circulating strains and lack of herd immunity. New features related to Bordetella pathogenesis and microbiology could be relevant to defeat the infection. BP1253 from *Bp* is a predicted exported protein erroneously classified as lysine decarboxylase. Nevertheless BP1253 contains high sequence homology with some newly structurally characterized "Lonely Guy" proteins. These phytohormone cytokinins-activating enzymes share with BP1253 a nucleotide-binding Rossmann fold, a highly conserved Motif PGGxGTxxE and the catalytic core complying with the peculiar distance among the catalytic Arg and Glu. SEC and MALS analysis revealed a main dimeric form as functional unit. Studies by Biacore demonstrated that BP1253 has the ability to selectively bind monophosphate nucleotides such as AMP and GMP. Time-course and dose-response experiments carried out with an enzymatic assay in the presence of AMP and GMP as substrate, clearly showed that BP1253 is able to cleave the N-glycosidic linkage between the base and the ribose, leading to the formation of free bases. As a matter of fact this phosphoribohydrolase activity is the crucial reaction in producing active cytokinins. Through LC-MS/MS we identified on growth medium of Tohama strain the 6-O-methylguanine as the physiological product of the BP1253, in agreement with the higher activity of the enzyme towards GMP. Moreover, BP1253 turns out to be also prevalently expressed in clinical isolate strains compared to Tohama. Overall these data suggest that the 6-O-methylguanine could have a real impact on virulence during infection and that BP1253 is a cytokinin-activating enzyme showing for the first time the presence of a phosphoribohydrolase activity in *Bordetella pertussis*.

P.08-029-Tue
The structure of rhizospheric and rhizoplanic fungal microbiome of potato in different crop rotation

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The present study analyzed the root-associated fungal microbiomes of potato (*Solanum tuberosum*), cultivated under different agricultural rotation on 3 fields; previously, peas, wheat and potato had been grown on them. DNA of microbes in the Rhizosphere (RS) and rhizoplane (RP) were isolated using "FastDNA® SPIN Kit for Soil@". The fungal ITS fragments 5.8S rRNA genes were amplified and sequenced by the Illumina MiSeq platform. The sequence data were further analyzed using the bioinformatics pipeline QIIME, version 1.9.1. The mean of Shannon index was 5.35 ± 0.37 for the RS of fungal metagenomic data, while that of RP was 4.43 ± 0.3. Fungal communities were classified into 6 phyla, 31 classes, 91 orders, 191 families, and 329 genera. *Ascomycota*, *Basidiomycota* and *Zygomycota* were the 3 most dominant phyla in the potato's RS and RP, accounting for 98–99%. Their frequency did not significantly change between the samples: averagely, the respective constituents of RS and RP for *Ascomycota* was 81.4% and 81.7%; *Basidiomycota* - 9.7% and 9.8%, while *Zygomycota* - 8.5% and 6.7%. The genus *Fusarium* was dominant in majority of the samples but their highest occurrence was seen in the RS and RP of potatoes, whose precursor for the same field was also potato. 30 isolates of Fusarium were obtained from tubers, affected with dry rot as well as latently infected tubers. Isolates with high virulent properties were selected for molecular genetic identification. 9 isolates were identified as *Fusarium oxysporum*; 3–*Fusarium solani*; 3- *Fusarium sambucinum*; 2–*Fusarium* sp. and 1–*Fusarium redolens*. It has been shown that the level of latent infection of tubers increases with the absence of crop rotation and in the cultivation of potatoes. The work is performed in accordance with the Russian Government Program of Competitive Growth of Kazan Federal University.

P.08-030-Wed
The interaction between HIV-1 integrase and Ku70 facilitates postintegrational DNA repair

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The integration of the HIV DNA into the host genome is completed by cellular factors that repair the short gaps flanking the proviral DNA. We have shown earlier, that viral integrase can

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directly interact with Ku70 repair factor, and this interaction is weakened by E212A/L213A substitutions in integrase. The transduction of HEK 293T cells by a single-round HIV-1 vector is found to be decreased in cells stably depleted of the DNA-PK repair components (Ku70, Ku80 or DNA-PKcs). We have established a qPCR-based approach for quantitative measurement of postintegrational gap repair and shown that depletion of any DNA-PK component reduces gap-repair efficiency in our system. The same effects have been detected in presence of specific DNA-PKcs inhibitor Nu7441. Viral vector carrying E212A/L213A integrase substitutions demonstrates decreased gap-repair and a reduced sensitivity to the DNA-PK components depletion while its integrational capacity remains at the wild-type level. We speculate that integrase recruits DNA-PK complex to gap cites and facilitates gap-repair through a direct interaction with Ku70 subunit. This work was supported by RFBR grant 17-04-01178 and RSF grant 17-14-1107 (development of the gap repair approach).

P.08-031-Mon
Adhesive properties and cytotoxicity of Klebsiella spp. for urothelial T24 cells

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Catheter-associated urinary tract infections (CAUTIs) account for 80% of cases of nosocomial urinary tract infections (UTIs). The ability for long-term persistence of UTIs can be attributed by bacterial ability to invade urothelial cells and proliferate inside of them. Mannose-sensitive type I fimbriae are shown to be important for adhesion and invasion of uropathogenic *Escherichia coli*. Using NCBI (https://www.ncbi.nlm.nih.gov/) and ASAP (https://www.genome.wisc.edu/tools/asap.htm) services genomic locus containing genes responsible for expression of type I fimbriae was identified at *K. oxytoca* and *K. pneumoniae*. Strains had the same structural genes of the locus organized in *fim*-operon but their regulatory foundations varied. *K. pneumoniae* had *fim*B recombinase gene placed ahead of *fim*-operon and *K. pneumoniae* had another regulatory gene conservative in the species. Yeast cells were used for detection of fimbriae on the surface of bacteria. Agglutination of the yeast cells by *K. oxytoca* was more distinct than that of *K. pneumoniae* and in both cases it was not inhibited by mannose. The effect of mannose could be masked by mannose-resistant agglutination supported by type III fimbriae characteristic for *Klebsiella*. Despite results of yeast cell agglutination assay, *K. pneumoniae* was more adhesive towards urothelial T24 cells than *K. oxytoca*. It should be noted that both strains did not have a cytopathic effect for T24 cells after 2 h of coincubation and during further 23 h of incubation with amikacin. The fact that bacteria do not damage T24 cells may indicate that *K. pneumoniae* and *K. oxytoca* use urothelial cells for defence, intracellular proliferation and as a source of secondary infection. Differences in the fimbrial regulatory genes may explain dissimilar adhesive properties of *K. pneumoniae* and *K. oxytoca*. The work is performed in accordance with the Russian Government Program of Competitive Growth of Kazan Federal University.

P.08-032-Tue
Interaction forces between the extracellular matrix and variants of borrelial decorin binding protein A probed by atomic force microscopy

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The spirochetal bacterium *Borrelia burgdorferi*, the etiologic agent of Lyme disease (LD), infects mammalian hosts via the bite of *Ixodes* spp. ticks. Unlike in North America, where the infection is caused only by *B. burgdorferi sensu stricto*, in Europe there are a number of *B. burgdorferi* genospecies that are associated with human LD. Different genospecies display different patterns of host specialization and tissue tropism and are associated with distinct spectra of clinical manifestations. It is well established that *B. burgdorferi* modulates its gene expression as it cycles between the tick vector and the mammalian host they infect. Upon host invasion, *B. burgdorferi* has avoid vigorous host immune response in order to colonize and persist in the host. *B. burgdorferi* binds various components of the host extracellular matrix (ECM). Arguably the most well studied borrelial adhesin is decorin-binding protein A (DbpA). DbpA was identified as the adhesin responsible for binding to the proteoglycan decorin. Interestingly, DbpA amino acid sequences are highly polymorphic between the genospecies, with sequence similarities as low as 40–60%. This sequence heterogeneity results in structural variations that contribute to large differences in the adhesion activities of the protein, which ultimately influences the tissue tropism and disease manifestation of the genospecies. Using atomic force microscopy, we measured specific binding forces of single bio-molecular adhesive interactions between variants of borrelial DbpA and several ECM components, and localized the interactions on the surface of *Borrelia*.

P.08-033-Wed
Cross talk between nuclear PML, SUMO and caspase 8 in response to interferon α and TGF-β

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ProMyelocytic Leukaemia protein (PML), an interferon-stimulated gene product, is essential for the formation of nuclear matrix-associated organelles named PML nuclear bodies (NBs). Covalent modification of PML by the Small Ubiquitin-like Modifier (SUMO) is necessary for PML NB function. PML NBs are dynamic structures harboring numerous transiently and permanently localized proteins and have been associated with the regulation of several cellular functions including apoptosis. Due to alternative splicing, many PML isoforms are synthesized from a single gene that are classified into seven groups, designated PML I-VII, they share the N-terminal region, whereas they differ in their C-terminal region. The variability in the C-terminal region confers specific functions to each isoform. Most of PML isoforms (PMLI to PMLVI) contain the nuclear localization signal (NLS) and are localized in the nucleus, whereas PMLVII (cPML) lacks the NLS and is found in the cytoplasm. It has been reported previously that PML-null primary cells are resistant to TGF-β-dependent induction of apoptosis and that cPML is an essential activator of TGF-β signaling. The defects in TGF-β responses are rescued by the restoration of a cPML. Here we show that

interferon (IFN) α potentiates TGF-β-mediated apoptosis. Interestingly, TGF-β induces the nuclear PML conjugation to SUMO and the shift of PML from the nucleoplasm to the nuclear matrix. This shift leads to the recruitment of caspase 8 within PML NBs where PML and caspase 8 were found colocalizing. This process is followed by caspase-dependent PML degradation leading to NB disruption. Our findings add a new complexity to the cooperation between TGF-β and PML NBs in mediating apoptosis.

P.08-034-Mon
Conserved RNA secondary structure of the NS segment as a possible additional influenza viral pathogenicity factor

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Nowadays, RNA secondary structure elements are well known to be vitally important for the life cycle of RNA-containing viruses, including influenza virus. Here, we investigated a predicted hairpin structures in the (+)-RNA of the influenza NS-segment (nucleotide positions 82–148 and 497–564). Both regions are located near the 5′ and 3′ splice sites: the first region within the NS1 open reading frame, while the second – within the NS1 and NEP open reading frames. The secondary structures in these regions differ in various influenza strains. The highly pathogenic H5N1 strain has more stable secondary structure in the second region compared to other strains. Since evolution of the NS gene is strongly associated with host adaptation, it was intriguing to investigate these structures as an additional factor of the pathogenicity of the influenza virus. Based on RNA secondary structure predictions of the NS-segments of more than 25,000 sequences from different influenza A viruses, we chose single nucleotide positions which may be important in hairpin structure formation and stability. We obtained several combinations of mutations in the corresponding regions of the NS-segment by site-directed mutagenesis. Then, we experimentally demonstrated the existence of the previously predicted RNA secondary structures by electrophoresis in the native and denaturing conditions. To directly test the functionality of conserved stem-loop structures, we undertook reverse genetic experiments and obtained viral strains based on A/Puerto Rico/8/1934(H1N1) virus with different secondary structural characteristics on the (+)-RNA NS-segment. According to preliminary data, the infectious titer of the strain containing the stable secondary structures was ten-fold higher in comparison to others. The pathogenicity and other characteristics of novel assembled strains are under further investigation.

P.08-035-Tue
Mapping the inner profile of pores formed by adenylate cyclase toxin of *Bordetella pertussis*

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The whooping cough agent, *Bordetella pertussis*, secretes the adenylate cyclase toxin-hemolysin (CyaA, ACT, AC-Hly), a major virulence factor that plays a crucial role in bacterial colonization of host respiratory tract. CyaA exhibits two distinct and mutually independent biological activities on CD11b expressing myeloid phagocytic target cells. Firstly, one class of CyaA conformers forms small cation-selective pores that can cause colloid-osmotic lysis of target cells. In parallel, other CyaA conformers translocate the adenylate cyclase (AC) enzyme domain of the toxin across the membrane of target cells, thus effectively subverting the bactericidal functions of cells by unregulated catalysis of cytosolic ATP conversion into cAMP, a key signaling molecule. The dual function CyaA toxin thus belongs to the RTX (Repeats-in-ToXin) family of pore-forming toxins. However, it forms notably smaller pores than other pore-forming toxins from the RTX cytolysin family. Here we investigated the specific segments of CyaA involved in the reduction of the pore conductance and diameter. Using planar lipid bilayers made of DPhPG we investigated single pores formed by CyaA and several mutant toxin variants and their pore selectivities. By measuring pore conductance in the presence of non-electrolytes of known diameter, we have studied the overall shape of CyaA pores. Our results suggest that CyaA pores adopt two distinct states with distinct pore selectivities.

P.08-036-Wed
Study of synergistic antibacterial action of host defense peptides with varied antibiotic compounds and their combined action on mammalian cells in vitro

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Innate immune system plays a pivotal role in realization of host–pathogen interactions. Among the key effector molecules of the innate immunity there are host defense peptides (referred also as antimicrobial peptides - AMPs) containing mainly in neutrophils and epithelial cells. Most of AMPs posses a potent activity towards pathogenic microorganisms. The synergy of antibacterial action of AMPs with different antibiotic compounds including other AMPs or proteins of neutrophil granules as well as with clinically used antibiotics (AB) has been reported before. It is known that most of natural AMPs exert some toxic effects for human cells, but the toxicity of the combinations of antimicrobial compounds for the host cells is much less investigated. The aim of this study is an exploration of a combined action of several structurally different AMPs (beta-hairpin protegrin 1 (PG1), linear proline-rich caprine bactenecin, alpha-helical human cathelicidin LL-37, alpha- and beta-defensins) with varied conventional AB towards bacteria and mammalian cells in vitro. Using broth microdilution assay and checkerboard titration approach for fractional inhibitory concentrations determination we have shown that more often the cases of synergistic antibacterial action were stated for combinations of AMPs with AB that act via inhibition of protein or nucleic acids synthesis in bacterial cells. Effects of AMPs – AB combinations on bacterial membranes integrity and bacterial metabolism have been explored. In most cases the toxicity of combinations of AMPs with AB for normal mammalian cells was not increased in comparison with effects of individual substances. But we revealed a synergy for PG1 and doxorubicin toward several types of mammalian cultured tumor cells, and studied a mechanism of their combined action. The obtained data point for the future prospect of combined application of AMPs

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based drugs and AB to combat infections or cancer. This work was supported by RFBR grant 17-04-02177.

P.08-037-Mon
Enzymatic desialylation of lung epithelium increases binding of lectin PAIIL, *Pseudomonas aeruginosa* virulence factor

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Cystic fibrosis (CF) is one of the most common human autosomal recessive diseases. This genetic disorder is associated with increased susceptibility of lungs to bacterial infections, which frequently develop to life-threatening conditions for CF patients. In this regard *Pseudomonas aeruginosa* (PA) is the most dangerous bacterial pathogen. Among PA virulence factors lectins seem to play crucial role in the PA adherence on CF airway epithelium. PA lectin, PAIIL, showing high affinity for L-fucose, is assumed to be namely involved in the bacteria binding. Likely, the low-sialylation of glycoconjugates on CF epithelial cells provides exposed saccharide residues suitable for the PAIIL interaction. To study the PAIIL involvement in the adherence of PA on airway epithelium the mouse model mimicking CF lung conditions was developed. The status of low-sialylation was induced by the intratracheal instillation of neuraminidase, the enzyme cleaving terminal sialic acid. For our experiments PAIIL was recombinantly expressed and coupled with high-performance fluorescent label, DyLight 488. The saccharide binding of the DyLight 488 labeled PAIIL was checked by using a red blood cell agglutination assay. The PAIIL labeling did not alter the lectin saccharide binding affinity. Next, tissue slices of lungs from neuraminidase-treated and untreated mice were mounted on microscope slides and incubated with DyLight 488 labeled PAIIL. Finally, tissue specimens were examined on an inverted fluorescence microscope (Nikon Eclipse TE2000-U). In the neuraminidase-treated group the cuboidal epithelium lining the surface of respiratory bronchioles showed an apparent fluorescence when compared with untreated mice. These results support the assumption that PAIIL mediates the PA binding on CF airway epithelium. Thus, lectin PAIIL is a candidate target in preventing PA lung infections of CF patients.

P.08-038-Tue
The role of zinc in soybean resistance to *Phomopsis longicolla*

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Soybean is among the most important crops for global agriculture, food and nutrient security. The major agent affecting soybean yield and seed quality is the pathogenic fungus *Phomopsis longicolla*, which causes PSD (Phomopsis seed decay) disease all over the world. Favourable factors for PSD spread are frequent rainfalls and high humidity during warm periods of soybean maturation and at this time *P. longicolla* infects all plant organs. Although several soybean cultivars have been described as tolerant to PSD, the search for resistant traits is still ongoing, which is why alternative means of improving plant vigour and seed quality without genetic modifications are of importance. The aim of this research is to investigate the effects of a range of Zn concentrations on PSD resistance in the soybean cultivar Galina. As an essential micronutrient, Zn is required for the function of transcription factors, enzymes, and structural proteins. The hypothesis is that Zn distribution in the roots and leaves, and activation of defence signalling pathways may affect the severity of *P. longicolla* infection. To investigate this, soybean plants were grown hydroponically under different Zn availability (deficient 0.03 μM up to sublethally toxic 10 mM) for 4 weeks prior to start of infection. Distribution of Zn and other elements in the leaves and roots was measured *in vivo* by imaging micro X-ray fluorescence (μXRF) and related to the level of infection. In addition, combined effects of Zn and *P. longicolla* on photosynthetic performance were determined by imaging measurements of chlorophyll fluorescence kinetics (OJIP and Kautsky transients, Q_A reoxidation). The results should contribute to better understanding of the mechanisms of metal-induced plant immunity and the cross-talk between abiotic and biotic stress, as well as to the importance of biofortification of crops for securing maximal yield and food production.

P.08-039-Wed
Type IIA topoisomerase in *Entamoeba* is essential for its encystation

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Topoisomerases are a group of ubiquitous enzymes, associated with all key DNA processes and are often involved in stress response across the biological world. Owing to its indispensable nature for cell survival, it has been extensively explored as a drug target for various diseases including cancer as well as infectious diseases. The genome of *E. histolytica,* the causative organism of amoebiasis, has seven unexplored putative topoisomerases and five of them have orthologs in the reptilian counterpart, *E. invadens*. The stage conversion of *Entamoeba* from a pathogenic trophozoite to an infective cyst is responsible for the host-to-host transmission of the disease. Formation of a tetranuclear stage in the late hours of encystation is one of the key features. However, the exact process behind this phenomenon is still not understood. In this study, we have identified a Type IIA topoisomerase of *Entamoeba* that is highly upregulated both at transcription as well as protein level during the later stages of encystation and various other stress conditions, like heat shock and oxidative stress. Silencing of that gene has shown a significant impact on the transcription of meiosis-specific genes as well as on the viability, and encystation efficiency of *Entamoeba*.

P.08-040-Mon
Mutations of selected amino acids of Mason-Pfizer monkey virus capsid protein and their effects on its life cycle

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Retroviruses belong to the group of enveloped RNA viruses, causing lifelong, sometimes life-threatening infections. To inhibit their life cycle it is important to understand its steps into the detail. The potential target for new antivirals can be protein-protein interaction interfaces within retroviral structural proteins, thus inhibiting the assembly and/or influencing stability of immature and mature retroviral particle. The most important interactions are mediated mostly by amino acids present in retroviral capsid protein (CA), that consists of two domains, N-terminal domain (NTD) and C-terminal domain (CTD), connected by unstructured flexible linker. Based on 3D structure of Mason-Pfizer monkey virus (M-PMV) immature particle, we predicted amino acids in both NTD and CTD that seemed to be critical for interactions in immature and mature retroviral particle, namely N89, G119, E175, D179, Y180 and E187 (numbered from the first amino acid of CA). Then we mutated these amino acids and studied the effect of these mutations on *in vitro* formation of immature-like virus particles and also on selected virus life cycle steps in infected cells. N89 and G119 mutants did not form any immature particles *in vitro* or *in vivo*, suggesting the critical role of these two CA NTD amino acids in CA-CA interactions. Mutations of amino acids present in CA CTD, i.e. E175, D179, Y180 and E187, did not inhibit immature particle formation, but these particle were often unstable and with different morphology in the comparison with wild type virus. Except E187 mutant they did not bud from infected cells, thus confirming the importance of studied amino acids in virus life cycle. Description of interaction interfaces in CA-CA in both immature and mature virus particle, followed by targeted design of molecules blocking these amino acid motifs may lead to finding of new inhibitors of retrovirus life cycle.

P.08-041-Tue
Study of the role of cysteines present in Mason-Pfizer monkey virus capsid protein

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Retrovirus immature particle assembly is driven mostly by the oligomerization of their structural polyprotein Gag. Gag-Gag interactions are mediated by inter and intra protein interactions among their capsid (CA) domains. Mason-Pfizer monkey virus (M-PMV) CA contains three cysteines at positions C82, C193 and C213, counted from the first amino acid of CA. Since C193 and C213 are highly conserved among the majority of retroviruses and their distance enables them to form intramolecular disulfide bond, the study of the role of these cysteines in virus life cycle is of a high interest. To determine the importance of M-PMV CA cysteines, we introduced their mutations in both bacterial and proviral vectors and evaluated the impact of these mutations *in vitro* and also in M-PMV transfected cells. In *in vitro* assembly system we observed the formation of immature virus-like particles and mature cores and in tissue culture cells we focused on intracytoplasmic immature particle formation, their budding, maturation and virus infectivity. Our data revealed that the presence of both C193 and C213 of M-PMV CA is necessary for immature and mature particle assembly and virus infectivity. However, if we mutated only one of conserved cysteines, we did not observe any serious defects in M-PMV assembly and mature core formation. We concluded that the presence of both conserved cysteines is important for keeping proper CA structure and virus particle stability and that their mutations probably cause virus arrest at some process occurring shortly after the virus entry.

P.08-042-Wed
Differential expression of *Fusarium oxysporum* genes upon infection of susceptible and resistant flax genotypes

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Fusarium oxysporum sp. lini is one of the most harmful pathogens of flax, which leads to fusarium wilt and causes more than 20% reduction in yield. Flax varieties differ considerably in their resistance to fusarium wilt. Alterations in the expression of a number of flax genes between resistant and susceptible genotypes under the infection were shown. However, it is unknown whether the expression of *F. oxysporum* genes differs when this pathogen affects flax genotypes with diverse resistance. Our work was devoted to study this issue. Flax seedlings of four flax varieties with different resistance (resistant – Dakota and # 3896, susceptible – AP5 and TOST) were inoculated with isolate #39 of F. oxysporum from the collection of the All-Russian Research Institute for Flax. Forty-eight hours after infection, the roots were harvested and total RNA was isolated. RNA samples were obtained in duplicate for each variety. The concentration and quality of RNA were assessed using Qubit 2.0 fluorometer and Agilent 2100 Bioanalyzer. To prepare DNA libraries for high-throughput sequencing, the TruSeq Stranded Total RNA Sample Prep Kit (Illumina) was used. A total of 8 cDNA libraries were obtained, and their quality was evaluated using Agilent 2100 Bioanalyzer. The libraries were mixed and sequenced on NextSeq 500 sequencer (Illumina) using 80 nucleotides pair-end reads. About 25 million pair-end reads were obtained for each type of the experiment, and 30–40% of reads were mapped on *F. oxysporum* genome, while other reads belonged to flax. The number of reads for each *F. oxysporum* transcript was assessed, and expression analysis was performed using the edgeR software. Fungus genes with differential expression upon infection of susceptible and resistant flax genotypes were revealed that brings the novel knowledge on plant-pathogen interaction. This work was financially supported by the Russian President Grant MK-5828.2018.4.

P.08-043-Mon
Determination of the ability of *Clavibacter* michiganensis ssp. *sepedonicus* strains to infect various in vitro cultures of potato

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Potato is one of the most economically crucial crops in the world and it is exposed to many dangerous diseases caused by numerous bacterial, viral or fungal pathogens. Their presence is usually associated with significant economic losses. Quarantine diseases, including ring rot of potato, caused by *Clavibacter michiganensis* ssp. *sepedonicus* (Cms) (Spickermann et Kotthoff) Davis et al.- one of the most important quarantine potato pathogens - are considered as particularly troublesome for potato cultivation. There are several factors that can stimulate uncontrolled spread of Cms bacteria in the environment. One of them is the lack of effective biological or chemical method of utilization of Cms in potato tissue. Furthermore, the latent form of the disease is extremely significant, as well as absence of information on how potato varieties are infected by different strains of Cms bacteria. In both cases these bacteria put future potato generations at risk. Existing in low concentration in potato tissue, they remain undetected even for a few growing seasons. However, inhibition of the symptoms of infection by Cms tolerant varieties favors the rapid spread of these pathogens in the environment. Therefore, the purpose of the presented research, was to determine the ability of Cms bacteria to infect potato plants *in vitro*. Due to the high level of the disease expression symptoms and the simplicity of multiplication, potato variety in form of *in vitro* plants was used for research. The obtained results allowed for determination of influence of the examined pathogenic Cms strains, causing ring rot, on the level of expression symptoms on the tested *in vitro* plants and the attained result of the molecular test.

P.08-044-Tue
Epstein–Barr Virus LMP1 induces IL-8 expression via regulation of the NF-kB pathway in human gingival epithelial cells

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Periodontitis is a major public health problem that effects over half of the adult population worldwide. Although traditional microbiological research on periodontitis has focused on putative bacteria, emerging evidence implicates an association between Epstein–Barr virus (EBV) and periodontitis. However, the molecular mechanism underlying the role of EBV in the pathogenesis of periodontitis is unknown. This study investigates the effect of EBV-encoded latent membrane protein 1 (LMP1) on IL-8 protein expression in human gingival epithelial cells. We performed RT-PCR and luciferase assays to investigate the effects of LMP1 transfection on IL-8 expression and NF-kB activation in the human gingival cell line Ca9-22. Western blot analysis and enzyme-linked immunosorbent assay were used to detect protein expression. LMP1 induced dose-dependent production of IL-8 and activation of NF-kB. LMP1-induced IL-8 expression was inhibited by dominant negative mutant of IkBa. In addition, mutated LMP1 lacking the NF-kB activation domain failed to induce IL-8 expression. LMP1 activates NF-kB, leading to the induction of IL-8 expression in human gingival epithelial cells. Our findings suggest that LMP1 is involved in the pathogenesis of periodontitis. Thus, in addition to bacteria, EBV should be considered as a target for treating periodontitis.

P.08-045-Wed
Chemical structure of lipopolysaccharides isolated from *Veillonella parvula*

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The human gut microbiota harbours a complex community of microorganisms which influences human physiology, metabolism, nutrition and immune function. To remain immunologically tolerant to these commensal bacteria and preserve this symbiotic relationship, elaborate biochemical mechanisms exist. Bacterial cell wall glycoconjugates such as Lipopolysaccharides (LPS) act as microbe-associated molecular patterns (MAMPS) and are vital for the initiation of immune response to pathogens as well as immunological suppression to symbiotic bacteria. The aim of this research project is the extraction, purification and structural elucidation, through Mass Spectrometry and NMR techniques, of LPS isolated from commensal bacterial species such as *Veillonella parvula*. *V. parvula* has been found to play a vital role in polymicrobial infections specifically in respiratory and oral infections. Conversely, alternative research has shown that its LPS shows a protective behaviour towards the effects of toxic LPS and can influence the susceptibility of children to allergies and autoimmunity. Therefore, *V. parvula* is a key component of a healthy microbiota and since LPS is involved in the interaction between the bacteria and the host, the investigation of the LPS structure is necessary to shed the light on the molecular mechanisms at the basis of the beneficial effects of commensal bacteria.

Biochemistry and medicine

P.09-001-Mon
A predicted unstructured C-terminal loop domain of SIRT1 is required for cathepsin B cleavage

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Previous reports demonstrated that a SIRT1 C-terminal truncation, generating a 75 kDa SIRT1 polypeptide (75SIRT1), is facilitated by cathepsin B at amino acid (a.a.) H533. Here we identified N-terminally intact Sirt1 variants at the 75 kDa range, which appeared to be augmented with ageing in various mice tissues. Variable N-terminally intact SIRT1 variants, were increased in lung, liver, brain, heart and kidney as a function of age, and correlated with increased cathepsin B levels. To examine whether the H533 amino acid is essential for cathepsin B cleavage, we generated SIRT1 H533A (in the P1 site of SIRT1), which did not affect the extent of cathepsin cleavage. Next, a SIRT1 mutant protein lacking a portion (a.a. 528–543) of the predicted C-terminus loop domain of SIRT1 (denoted "DSIRT1"), was generated, and presented relative resistance to cathepsin B cleavage, compared to full-length SIRT1 (flSIRT1) in-vitro and in cell cultures. Moreover, DSIRT1 displayed similar enzymatic activity as flSIRT1, based on analysis of H3K9/14 and RelA acetylation. Cells expressing DSIRT1 were more likely to apoptose under pro-inflammatory stress, given that it possessed lower capacity to bind cytochrome C, compared to 75SIRT1. In all, the data support that the predicted unstructured

15aa loop motif embedded in the C-terminal domain of SIRT1, is susceptible to proteolytic cleavage, leading to the formation of variable N-terminally intact SIRT1 truncated isoforms in various tissues, which may contribute to cell survival under inflammatory insult.

P.09-002-Tue
Spherical particles of tobacco mosaic virus enhance the immunogenic effect of the inactivated rabies vaccine

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Spherical particles of the tobacco mosaic virus (TMV SP) are obtained by thermal denaturation and remodeling of the TMV virions. We have previously described a number of specific and promising properties of TMV SPs such as their high stability and biosafety. Moreover, TMV SPs were demonstrated to adsorb target proteins of different nature on their surface. Complexes of TMV SPs and proteins were shown to dramatically enhance the immune response in mice against the target antigenic protein, supposing the TMV SPs to act both as an antigen-presenting platform and an adjuvant. These facts make TMV SP a promising candidate for a new safe adjuvant of the modern vaccines. We continue the study of the adjuvant properties of TMV SPs testing of their potential to enhance the immunity against the rabies virus. For comparative study of the immune-stimulating (IS) potency of TMV SPs, the standard NIH test was performed with a reference inactivated rabies vaccine based on the vaccine strain "Shchelkovo-51" and the currently licensed adjuvant-free IRV (Rabikan™, Russia). Rabikan™ is highly effective and successfully applied for rabies prevention. For comparison Rabikan™ was adjusted with TMV SPs or Freund's incomplete adjuvant (IFA). According to the NIH test protocol all vaccine variants were applied for intraperitoneal immunization of mice (immunized twice: on days 0 and 7). Then mice were subjected to the lethal challenge against the rabies virus of CVS strain, injected intracerebrally. According to the obtained results 300 μg of TMV SPs enhance the IS potency of the Rabikan™ by 0.2 IU and the IFA enhances it by 0.3 IU, while the IS potency of the intact Rabikan™ was 1.5 IU. This result demonstrates the ability of TMV SPs to efficiently stimulate the immune response (comparable with the IFA) and thus provides further evidences of the high clinical potential of this novel immunostimulatory compound. Funding: Russian Science Foundation (grant No. 14-24-00007).

P.09-003-Wed
Renal ischemia-reperfusion injury in a pig model reveals gender-specific expressed genes as potential new biomarkers of renal injury/regeneration processes driving to chronic kidney disease

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Kidney diseases are a global public health problem, that is reaching epidemic proportions. Renal ischemia/reperfusion injury (IRI) is a major cause of acute kidney injury (AKI) leading to injury of proximal tubule epithelial cells (PTEC). After injury, the kidney can either regenerate or be engaged in remodeling processes driving to fibrosis and chronic kidney disease (CKD). Men are more prone to AKI and CKD than women and it is accepted that androgens participate on that. The molecular mechanisms involved in regeneration as well as in gender-related outcomes upon injury remain to be elucidated. We postulate that the identification of differentially expressed genes in male and female kidney pigs, both in basal and in IRI conditions might unravels genes and pathways useful to understand the different outcomes observed in men and women. We aim to provide new candidate repair regulators able to promote restoration of kidney function. Renal IRI was performed in female and male pigs and mice to identify genes of translational relevance for humans that could be also studied in mouse models. Pre-ischemic, ischemic and post-ischemic kidney tissues from male and female pigs were collected for microarray assays. Moreover, systems biology-based mathematical models for the analysis of microarray data were conducted. The most promising targets that exhibit sexual dimorphism along the injury/regeneration process have been selected and characterized. The mRNA levels, the protein expression and localization in the kidney have been assessed. The early results strongly suggest that the selected targets are potentially androgen-regulated. In order to further study the molecular mechanisms of these targets, *in vitro* IRI models of pig and human PTEC cultured cells are currently under development. An *in vitro* model of kidney organoids where IRI is induced is also being established.

P.09-004-Mon
Does vitamin D receptor play a role in the pathogenesis of Behcet's disease?

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Behcet's disease(BD) is an autoinflammatory disorder with unknown etiology. Vitamin D has a crucial role for immune system and the functions of vitamin D depends on vitamin D receptor(VDR). The VDR effects many genes through vitamin D-VDR ligand and the defects of this pathway may be associated with pathogenesis of autoimmune diseases. In this research rs1544410(G>A), rs2228570(T>C), rs7975232(G>T) and rs731236 (T>C) single nucleotide polymorphisms(SNP) in VDR gene were studied in BD patients in Turkish population. 150 patients with BD and 150 healthy controls (HCs) were included and genotyping of each SNP was carried out by PCR/RFLP. Then as a pilot study, soluble VDR levels were measured by ELISA in 32 BD patients(12 active, 20 remission) and 30 HCs. There are significant differences between patients and HCs in rs1544410, rs2228570 and rs731236 genotypes (respectively; $P = 0.04$, $P = 0.007$, $P = 0.01$). The alleles of rs1544410 and rs2228570 were evaluated with clinical features and patients with ocular lesion had higher percentage of rs1544410 A allele ($P = 0.013$) and patients with oral aphthae, positive pathergy test and arthritis had more rs2228570 C allele than patients without this clinical features (respectively; $P = 0.027$, $P = 0.037$, $P = 0.02$). In the analysis of the VDR expression; mean VDR concentrations were 18.69 ± 8.77 ng/mL for patients in active period; 31.73 ± 11.05 ng/mL for patients in remission period and 32.49 ± 18.12 ng/mL for HCs. Patients in active period had significantly lower serum VDR level than patients in remission period and HCs (respectively; $P = 0.002$, $P = 0.04$). Patients carrying rs2228570 C allele were found to had lower VDR level than patients with TT genotype ($P = 0.033$). In conclusion VDR may have possible role in the pathogenesis of BD and VDR SNPs may be affecting VDR level. Furthermore rs1544410-

rs2228570 SNPs may associated with increased risk of several clinical manifestations.

P.09-005-Tue
Vitamin D3 modulates impaired upstream NF-κB-associated signalling pathways and NF-κB downstream target genes in rat bone marrow after chronic glucocorticoid treatment

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Osteoporosis is the common side effect of glucocorticoid (GC) therapy. NF-κB is considered as integrating link between GC receptor (GR) and RANK (receptor activator of NF-κB)/RANKL (RANK ligand) signalling pathways, involved in bone remodeling. Little is known about GC-evoked disturbances of the NF-κB-associated axes in bone marrow (BM) cells and ways to prevent them by modulating vitamin D_3 (D_3) receptor (VDR) signalling. We studied GC-induced changes in upstream NF-κB-associated pathways – GR, RANK/RANKL and NF-κB downstream target genes – VEGF and TNFα in BM depending on D bioavailability. Female Wistar rats received prednisolone (5 mg/kg b.w.) with and without 1000 IU/kg b.w. of D_3 (for 30 days). Western blotting, RT-qPCR, ELISA, flow cytometry and confocal microscopy were used. Prednisolone caused a decrease in GR protein level and the number of GR^+ BM cells. GC-induced increase in RANKL, RANK protein levels and the number of $RANK^+$ osteoclast precursors (OCPs) in BM, peripheral blood and spleen were seen. In response to RANK activation we found an increase in the level of phospho-NF-κB and decrease in IκB. VEGF and TNFα were shown to be declined after GC treatment while caspase-3 elevated. These changes were accompanied by D-deficiency and down-regulated CYP27B1 and VDR expression. Notably, we observed VDR/RANK co-localization in OCPs. Vitamin D_3 administration elevated GR level and prevented disturbances of the RANK/RANKL in BM, which may be associated with improved D bioavailability and VDR signalling. D_3 lowered pNF-κB and inhibited its translocation to the nucleus contributing to normalization of VEGF, TNFα, caspase-3 levels and OCPs pool. Thus, GC-induced changes in upstream NF-κB-associated signalling pathways and NF-κB downstream target genes are associated with abnormal vitamin D auto/paracrine system in BM and can be ameliorated by D_3 treatment.

P.09-006-Wed
Ferritin modified with methylglyoxal produces reactive oxygen species but otherwise resists functional alteration

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Diabetes mellitus affects about 8. 5 per cent of the world's population and leads to long term complications such as blindness, amputation, neuropathic pain and renal failure. With diabetes, glycolysis overflows and its metabolites convert into alternative products such as methylglyoxal. Simultaneously, a relatively free and reactive iron in the cell called labile iron pool (LIP), is increased. Some studies indicate that methylglyoxal and LIP play an important role in the pathogenesis of diabetic complications, but how these two factors are related is not yet known. This study focuses on the interaction of the main cellular iron storage protein, ferritin, and the most potent glycation agent, methylglyoxal. In these experiments, commercially available horse spleen ferritin was in vitro glycated with methylglyoxal; fluorescence of glycation products, iron uptake, reductive iron release and hydrogen peroxide production were assayed. Glycation of ferritin resulted in detectable structural changes, but the ferroxidase activity, iron release in classical reductive assay or the diameter of pores in ferritin were not altered. However, the interaction of methylglyoxal with ferritin protein shell did increase production of hydrogen peroxide, especially in the presence of iron. In conclusion, ferritin is a stable protein resistant to functional alteration due to glycation, this modification can nevertheless increase oxidative stress in the cell. Possibly this can represent one of the mechanisms through which long-term complications of diabetes develop.

P.09-007-Mon
The role of adiponectin in streptozocin-induced diabetic rat placental development

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A healthy pregnancy is closely associated with normal placental development. Inadequate placental development may cause clinical complications such as intrauterine growth retardation, preeclampsia and diabetes. In order to prevent the adverse pregnancy outcomes, it is important to understand the hormonal changes and altered glucose metabolism that are associated with diabetes mellitus during pregnancy. Adiponectin, an adipokine, regulates glucose and lipid metabolism and its concentration is negatively correlated with diabetes. This study aims to evaluate the role of adiponectin on placental development in diabetic conditions. This study comprises diabetic and control groups. The control group underwent injection of saline. Diabetes was provided by injection of streptozotocin (STZ, 50 mg/ kg). Female rats whose blood glucose value is higher than 200 mg/dL were considered as diabetic. Adiponectin, its receptors (AdipoR1 and AdipoR2), and its downstream molecules (PPARα, PPARγ) were detected in the control and STZ-induced rat placentas on day 14, 16, 18, and 20 of pregnancy by using Western Blot and RT-PCR techniques. Serum concentrations of adiponectin were assessed by ELISA method. We showed that there is an inversely association between adiponectin, PPARs and diabetes. In the present study, we observed that adiponectin and its receptors levels reduced in diabetic conditions. Furthermore, maternal serum adiponectin levels reduced during the pregnancy in diabetic rats. We also showed that the levels of PPARα, PPARγ and RXRα expression were lower in diabetic rats compare to control groups. Based upon those observations above, we believe that adiponectin has a significant role in placental development. In conclusion, pregnancy and diabetes is associated with adiponectin levels.

P.09-008-Tue
Conjugation of small molecule inhibitor to nucleobase promotes a mechanism-based inhibition

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Small molecule compounds phosphonoformic (PFA, foscarnet) and phosphonoacetic (PAA) acids comprise one of the three types of principal inhibitors of viral polymerases. PFA is the most effective small molecule inhibitor of HIV reverse transcriptase identified so far. Sensitivity of various viral polymerases towards PFA is 100- to 1000-fold higher compared to that of mammalian replicative polymerases. However, it is cytotoxic and is used for the treatment of HIV and HIV-associated viral infections in critical cases only. Despite the similar structure, PAA is far less effective against viral polymerases but does not exhibit observable cytotoxicity. To achieve a better affinity, PAA was linked with different positions of natural nucleotide. Here, we focused on novel analogues of nucleoside triphosphates designed so as to be catalytically acceptable by the target polymerases for incorporation into DNA. Three different PAA conjugated compounds were prepared, and they varied significantly in inhibition capacity towards different DNA polymerases, including reverse transcriptases. Nucleobase-conjugated phosphonoacetic acid restricted the processivity of DNA polymerases. Ternary complexes of DNA polymerase with reactive substrates were found to adopt discernible conformations in the presence of different nucleotides, and provided the mechanistic rationale for a switch in the mode of DNA synthesis. Mikalkėnas A, Ravoitytė B, Tauraitė D, Servienė E, Meškys R, Serva S. Conjugation of phosphonoacetic acid to nucleobase promotes a mechanism-based inhibition. J Enzyme Inhib Med Chem. 2018. https://doi.org/10.1080/14756366.2017.1417275.

P.09-009-Wed
Oleuropein aglycone and its metabolite hydroxytyrosol interfere differently with toxic Aβ1-42 aggregation

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Epidemiological studies support the efficacy of the Mediterranean diet not only against cardiovascular and cancer diseases but also against ageing-associated cognitive decline. Several data highlight the role played by phenolic components of extra virgin olive oil, particularly oleuropein aglycone (OleA) and hydroxytyrosol (HT), against amyloid aggregation and toxicity. In this sense, particular emphasis has been given to the pathways involved in the onset and progression of Alzheimer's disease (AD), where Aβ1-42 aggregation is one of the key pathogenic features of the disease. Drug discovery efforts are focused at preventing the formation of toxic aggregates and/or at favouring their disaggregation. In particular, recent data show that OleA interferes with the aggregation path of some peptides/proteins (amylin, tau and transthyretin) skipping the growth of toxic oligomers, even though OleA was found in the brain of OleA-fed Tg mice as HT, its main metabolite. However, there are limited studies exploring at the molecular level how these molecules bind to Aβ1-42 preventing fibril formation and toxicity. We sought to elucidate the molecular and cellular determinants of OleA and HT protection against protein aggregation and/or aggregate cytotoxicity by a set of in vitro experiments carried out using biophysical analysis (ThT fluorescence, FT-IR, Intrinsic fluorescence quenching and TEM images) and cell biology techniques (MTT assay, Ca2+-Flux, ROS production, Immunofluorescence-FRET analysis). Our results besides confirming previous data on the relation OleA/Aβ1-42 aggregation, highlight a modulation of the molecular mechanism of Aβ1-42 aggregation also by HT; in particular, differently to OleA, HT was found to accelerate the protein aggregation path thus skipping the appearance of toxic oligomers. Our data offer the possibility to validate and optimize the use of OleA and/or HT to rationally design novel drugs for possible use in AD prevention and therapy.

P.09-010-Mon
The role of PGC-1β in metabolic functions of adipose tissue in mice during cold exposure

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Peroxisome proliferator-activated receptor gamma (PPARγ) is involved in the regulation of energy homeostasis in metabolically active tissues including white and brown adipose tissues (WAT/BAT). Other transcription factors interacting with PPARγ are engaged in controlling pathways of energy metabolism, namely PPARγ coactivators 1 alpha/beta (PGC-1α/β). PGC-1 coactivators are responsible for regulation of mitochondrial biogenesis and oxidative functions. The aims of this study were to characterize the role of PGC-1β in thermogenic and metabolic functions of BAT and WAT during cold exposure. Experiments were performed on male C57BL/6J mice with adipose tissue specific PGC-1β deletion, which was accomplished using enzyme Cre recombinase expressed under adiponectin promoter. Adipose tissue specific PGC-1β ablated mice and their wild-type littermates were either exposed to cold for 2 or 7 days, or kept at thermoneutral temperature. The levels of gene expression and proteins were quantified using qPCR and Western blotting analysis and concentration of lipid metabolites were determined spectrophotometrically in plasma samples or alcoholic KOH tissue homogenates. The results show that inactivation of PGC-1β in WAT probably does not affect metabolic functions of the tissue. In BAT of mice with PGC-1β deletion, increased weight of tissue and upregulation of the expression of genes connected with regulation of mitochondrial oxidative functions were observed. Levels of PGC-1α gene expression in BAT of cold exposed animals positively correlated with the expression of genes responsible for BAT thermogenesis and fatty acid metabolism, namely uncoupling protein 1 (UCP1). In conclusion, PGC-1β plays unreplaceable role in BAT and compensation of its ablation by increased expression of genes engaged in energy metabolism is not sufficient to maintain fully functional tissue. *Supported by the Czech Science Foundation (14-36804G).*

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P.09-011-Tue
Cell-penetrating peptides inhibiting ERK-dependent activation of HIF-1α reduce survival and induce apoptosis of cancer cells under hypoxia

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Development of a hypoxic microenvironment is a prominent trait of solid tumours. Adaptation of cancer cells to hypoxia promotes cancer progression and is conferred at transcriptional level by Hypoxia-Inducible Factors (HIFs). HIF-1α, the oxygen-regulated subunit of HIF-1 is associated with oncogenesis and represents a valid therapeutic target. We have previously reported that ERKs modify two serine residues inside a small HIF-1α domain termed ETD (ERK Targeted Domain) resulting on its activation. In order to specifically target HIF-1α function, we generated cell-permeable peptides by fusing the HIV TAT-sequence to small (42 amino acid long) ETD domain variants that include wild type and phospho-deficient (ETD-SA), phospho-mimetic (ETD-SE) or constitutively nuclear (ΔNES or ETD-IA) mutant forms. When peptides ETD-wt, ETD-IA or ETD-SE (0.42 μM) were added into the culture medium of hepatoma (Huh7) cells, they accumulated inside the nucleus and caused mislocalization of a large endogenous HIF-1α fraction to the cytoplasm as well as significant inhibition of HIF-1 transcriptional activity. In contrast, peptide ETD-SA was cytoplasmic and acted as a negative control in all experiments. Treatment with the inhibitory ETD peptides blocked biosynthesis of triglycerides and impaired cancer cell migration and colony formation under hypoxia. Furthermore, the inhibitory nuclear ETD peptides reduced cancer cell survival under hypoxia by activating apoptosis as detected by caspase activation, annexin V staining and TUNEL assays. These results offer proof of principle for the development of sequence specific peptide inhibitors that can prevent cancer cell adaptation to low oxygen by selectively targeting the nuclear and ERK-dependent function of HIF-1. A.K. is supported by a fellowship from States Scholarship Foundation (IKY).

P.09-012-Wed
Folate- and PEG-containing cationic liposomes to effective delivery of nucleic acids into KB-3-1 cells

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To solve the problem of selective effective delivery of nucleic acids into cancer cells, we have synthesized PEG-containing lipoconjugate with molecular weight of PEG spacer group approximately 800 Da and included it or folate-containing lipoconjugate (*O*-{2-[*rac*-2,3-di(tetradecyloxy)prop-1-yloxycarbonyl]aminoethyl}-*O'*-[2-(pteroyl-L-glutam-5-yl)aminoethyl]octadecaethyleneglycol) with same length of PEG spacer group into composition of effective cationic liposomes 2X3 (spermine-based cationic amphiphile 2X3: lipid helper DOPE, 1:2). Liposomes F with 2% or 4% of folate-containing lipoconjugate were shown targeted delivery of plasmid DNA into KB-3-1 cells *via* receptor-mediated endocytosis at low N/P ratios. Liposomes P with 2% or 4% of PEG-containing lipoconjugate (800 Da) provided the successful delivery of plasmid DNA into KB-3-1 cells with the efficiency significantly higher than that of Lipofectamine 2000, liposomes 2X3 and liposomes F at different N/P ratios (2/1, 4/1, 6/1). Multifunctional liposomes (M) with 2% of folate-containing lipoconjugate and 2% of 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-750] (ammonium salt) provided the successful delivery of plasmid DNA into KB-3-1 cells with the efficiency comparable with of Lipofectamine 2000, but smaller than that liposomes 2X3, F and P. Cationic liposomes F and P were selected as promising candidates for further *in vivo* transfection studies. This research was supported by the Russian Foundation for Basic Research (grants Nos 13-04-40181-N comfi and 13-04-40183-N comfi) and by Russian Science Foundation (grant No 16-15-10105). Shmendel E.V. is a recipient of Presidential fellowship 1199.2018.4.

P.09-013-Mon
The *Bufo bufo* crude skin secretions as a perspective source of hemostatic system effectors: a preliminary study

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Amphibian crude skin secretions have always considered to be a powerful source of bioactive compounds, but the study of the effects of their skin poisons on diverse parameters of the hemostasis system has not been yet carried out. Adult specimens of *Bufo bufo* (Kyiv region, Ukraine) were used in this research. Crude secretions were obtained by washing of the skin with distilled water after mechanical stimulation of glands, than centrifuged to remove debris, lyophilized and kept frozen at −20°C. Aqueous extract of lyophilized skin secretions was used in the study. Firstly, it was subjected to SDS-PAGE, where six proteins in the range 49–161 kDa were identified. In order to detect active proteases among extract proteins zymography with gelatin (1 mg/mL) as a substrate was carried out. The presence of two proteases with molecular weight 112 and 122 kDa was proved. The skin secretions from studied amphibian did not influence on such coagulation parameters as prothrombin time, thrombin time and activated partial thromboplastin time. *B. bufo* skin secretions in dose-dependent manner induced platelet aggregation in rabbit platelet-rich plasma; its final concentration of 50 mg of total protein/mL caused the same effect as 5×10^{-6} M ADP. The aggregation effect was also observed in platelet fraction purified by gel-filtration method. The method of gel filtration chromatography on Superdex-75 PG column (flow rate – 0.75 ml/min) was used for separation of crude skin secretion proteins. Fractions were collected in 5 ml volume (35 fractions in total) and each of them was used for aggregation assay. The ability to induce platelet aggregation was observed only for one of them. It was shown that the aggregation activity of this fraction remained after it was lyophilized in the second time. The identification of an active component and the elaboration of its mechanism of action are required in further investigations.

P.09-014-Tue
Stress-protective action of Dalargin intranasal form, shown in the dynamics of average weight molecules index distribution in blood serum of laboratory rats subjected to stress ulcerogenesis

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There is convincing evidence pointing to expressed biological effectiveness of intranasal Dalargin application (10^{-9}–10^{-11} M) in case of overcoming various complications of physiological stress in an organism [Minina, Cheretaev, 2016]. Definition of average weight molecules (AMW) [Nikolskaya, 2012] widely used as an integral marker of the biochemical state of the body at various pathologies and stress. The aim of this work was to test the presence of stress-protective action Dalargin intranasal form by studying the dynamics of average weight molecules (AWM) in blood serum of laboratory rats in stress-induced ulcerogenesis. The experiments performed on 32 male Wistar rats weighing 180–220 g, which divided into 4 groups. Rats in the control group (n = 8) and in group II (n = 8) was administrated within 28 days intranasally 0.1 ml of 0.9-% NaCl solution. Animals of groups I (n = 8) and III (n = 8) received intranasally 0.1 ml of Dalargin (0.2 mkg/kg) within 28 days. On day 28 the animals of groups II (n = 8) and III (n = 8) were subjected to stress ulcerogenesis for 60 min [Porsolt, 1993]. Index distribution ($ID_{280/254}$) of AWM in the serum all groups of rats using the method, based on the precipitation of protein components of 20% trichloroacetic acid [Gabrielian et al., 1987] was determined. It is shown that in the blood serum of laboratory rats after exposure Dalargin $ID_{280/254}$ was significantly reduced by 54% (n = 8; $p \leq 0.01$) compared to control, and under the influence of stress ulcerogenesis (n = 8; $p \leq 0.01$) – by 38.73%. With the combined effects of stress ulcerogenesis and Dalargin the $ID_{280/254}$ almost back to the values of the control group and slightly higher, amounting to 112% (n = 8; $p \leq 0.01$). Thus, we can talk about a stress-protecting dalargin effect, which helps to maintain a natural relation of physiologically important AWM fractions in an organism under stress, showing the activity of stress-realizing (254 nm) and stress-limiting systems of the organism (280 nm).

P.09-015-Wed
Role of PGE2 in hypoxia-induced death in human proximal tubular cells

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Prostaglandin E2 (PGE2) is produced by the inducible enzyme cyclooxygenase-2 (COX-2), and acts through EP receptors (EP1, EP2, EP3 and EP4), which are located at the cell membrane. Recently, we have found that intracellular EP receptors (iEPR) also mediate several PGE2 effects, so that they are prevented by the inhibitor of PGE2 uptake transporter bromosulfophthalein (BS). Since tissue hypoxia is an important factor in renal pathophysiology, here we studied the role of PGE2 in hypoxia (1% O2)-induced apoptosis in human proximal tubular cells (PTC). Hypoxia-induced increase in the expression of both caspase-3 and annexin V (which were respectively assessed by Western blot analysis and flow cytometry) was prevented by COX-2 inhibition, antagonism of EP receptors or BS treatment. Of note, COX-2 expression as well intracellular PGE2 levels were up-regulated by hypoxia. Taken together, these results indicate that iEPR mediate hypoxia-induced apoptosis in PTC. Due to their high metabolic activity, PTC have a large oxygen demand so that they become readily hypoxic in several types of both acute and chronic kidney disease. Our work suggests that the apoptotic death of hypoxic PTC might be prevented by targeting of the PGE2 uptake transporter.

P.09-016-Mon
Unusual inorganic pyrophosphatase from *Mycobacterium tuberculosis*: from structural and functional features to effective inhibitors

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Soluble inorganic pyrophosphatases (PPases) are enzymes essential for all organisms. They hydrolyze inorganic pyrophosphate, thus providing the driving force for key biosynthetic reactions. Most of well-characterized Family I PPases share highly conserved structure of their active site; however, some PPases have unique functional properties. One of them, PPase from *Mycobacterium tuberculosis* (Mt-PPase), is the possible target for the rational design of anti-tuberculosis agents. Our previous studies have shown that Mt-PPase is evolutionary isolated from the majority of Family I PPases and has several unique features. In the present work, these functional peculiarities were studied in detail. Enzyme kinetics was used to describe interactions of Mt-PPase with known effectors of other Family I PPases. Sedimentation analysis was used to explore different oligomeric forms of Mt-PPase. Site-directed mutagenesis was used to understand the role of specific structural features of Mt-PPase. Molecular docking and molecular dynamics was used to simulate particular steps of catalytic mechanism, binding of effectors, and to predict the location of effector binding sites. Virtual screening and *in vitro* high-throughput screening was used to find novel effectors. We found that in contrast to most of other Family I PPases, calcium and fluoride ions do not inhibit Mt-PPase, while substrate analogs (bisphosphonates) activate Mt-PPase under various conditions. Oligomeric forms of Mt-PPase with unusual properties were obtained after treatment of hexameric form with water-organic solutions. Three symmetry-related cavities for binding positively charged compounds were predicted in Mt-PPase hexamer. Enzyme-substrate complex was modeled and the possible interconnection between the structural and functional peculiarities of Mt-PPase was suggested. Two novel classes of effectors were predicted *in silico*. Three new inhibitors and one new activator of Mt-PPase were discovered *in vitro*.

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P.09-017-Tue
A novel HBV chimeric antigen produced in plants and mammalian cells induces stronger immune response than the vaccine-constituent antigen

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More than 250 million people are infected with Hepatitis B Virus (HBV) and about 600,000 people/year dye due to associated liver complications. The infection is not curable and prevention through vaccination remains an effective approach. However, despite the safety and efficacy of the commercial vaccine, the high costs restrict the immunization programs in developing countries. Moreover, about 10% of the vaccinated population fails to develop a protective immune response. In this context, our research aims to produce a more immunogenic HBV antigen in mammalian and plant cells, as a low-cost production alternative. The current vaccine is based on the small (S) viral envelope protein which has the ability to self-assemble into highly immunogenic subviral particles (SVPs). Our strategy uses S protein as a carrier for an immunogenic epitope derived from the large (L) surface protein. The resulting chimera and S protein were transiently expressed in HEK293T cells, *N. benthamiana* and *L. sativa* leaves. The properties of the newly-obtained protein (N-glycosylation pattern, SVPs formation) are similar to S in either expression system, as shown by biochemical analysis. The antigens purified from HEK293T medium and tobacco leaves were further used in immunization studies in mice by injection. Transformed lettuce leaves were administrated orally. Analysis of the immune responses showed higher IgG/M titers and cytokine secretion induced by the chimeric antigen compared to S antigen, regardless the production system. Furthermore, specific anti-S and anti-preS1 antibodies were detected in the sera of chimera-immunized mice. The antibodies induced by the chimera had higher neutralization capacity of HBV infection in vitro. In conclusion, our data promote the novel chimeric HBV antigen as a promising alternative to the conventional HBV vaccine. The research leading to these results has received funding from EEA Financial Mechanism 2009–2014 under the project contract no 5SEE/2014.

P.09-018-Wed
Calcitriol as a supplement for Friedreich ataxia

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Friedreich ataxia (FA) is a rare neurodegenerative disease, which has currently no cure, due to an expansion of a GAA-repeats in the first intron of frataxin (FXN) gene leading the deficiency of FXN, a mitochondrial protein. Since dorsal root ganglia (DRG) neurons are mainly affected in FA, our lab has developed frataxin-deficient DRG neurons from neonatal rats, in which protein level is reduced by shRNAs targeting FXN mRNA, showing altered calcium homeostasis, neurite degeneration and apoptotic cell death. Using our model, we tested the effect of 1α,25-dihydroxyvitamin D_3 (or calcitriol) on decreasing apoptotic markers since calcitriol supplement has shown neuroprotective effects, low levels of Vitamin D_3 increase the risk of neurodegenerative diseases and the synthesis of Vitamin D active form depends on the mitochondrial enzyme CYP27B1, localized also in neurons. In this work, treatment with Vitamin D_3 increased cell survival, decreased neurite degeneration and reduced α-fodrin cleavage, an indicator of apoptotic cell death. Additionally, CYP27B1 levels showed a five-fold increase in frataxin-deficient cultures, indicative of deficient levels of calcitriol that were reverted to normal values by the calcitriol treatment and the reduction of neurite degeneration is more noticeable using calcitriol than his precursor (calcidiol). These results strongly support an impaired CYP27B1 function and low levels of calcitriol in FXN-deficient DRG neurons and provides clues for the use of Vitamin D_3 as a straightforward therapeutic approach to consider for patients with Friedreich Ataxia. Project funded by Ataxia UK., Ataxia Ireland and ACAH (Associació Catalana d'Ataxies Hereditaries) and by SAF2017-83883-R from MINECO (Spain).

P.09-019-Mon
Inhibition of serine acetyltransferase for the development of new antibiotics

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The rising trend of bacterial antibiotic resistance urges for new strategies for antibiotics development. Inactivation of enzymes involved in cysteine biosynthesis has shown promise, causing a decreased antibiotic resistance, increased susceptibility to oxidative stress and defects in biofilm formation. Serine acetyltransferase (SAT) catalyzes the reaction between L-serine and acetyl coenzyme A to produce *O*-acetylserine (OAS), a substrate of the last enzyme in cysteine biosynthetic pathway. OAS is unstable and spontaneously converts to *N*-acetylserine, an inducer of cysteine operon, therefore being essential for cysteine biosynthesis in plants and bacteria. Despite its key role in cysteine biosynthesis, only three molecules have been reported so far as inhibitors of SAT, leaving its inhibition quite unexploited. In order to identify new SAT inhibitors, we have prepared a construct for expression of recombinant SAT of *Salmonella enterica* serovar Typhimurium (STSAT), fused with histidine tag and thioredoxin A, and optimized its expression and purification. We developed a continuous indirect activity assay, based on the reactivity between DTNB and the thiol group of coenzyme A – a product of SAT reaction. Several compounds, derived from *in silico* screening of in house library, were tested against STSAT and inhibitors with IC50 in low micromolar range were identified. We proved them to be competitive against acetyl coenzyme A and investigated their preliminary structure-activity relationship. The most potent inhibitors were screened in microbiological assays against *Escherichia coli* and were shown to be efficient in the presence of enhancer of membrane permeability. This work was supported by Marie Sklodowska Curie ITN INTEGRATE project, grant number 642620. Nina Franko and Joana Magalhães are INTEGRATE project fellows.

P.09-020-Tue
Antioxidant mechanisms in ovary adenocarcinoma cells resistant to cisplatin do not rescue from photonecrosis: implications for photodynamic therapy

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Oxidative stress is a major mechanism of tumor cell photodamage applicable in photodynamic therapy (PDT). The effectiveness of PDT is directly associated with accumulation of a photosensitizer in cancer cells and triggering oxidative stress upon light activation. However, in cells resistant to antitumor drugs (in particular, cisplatin), antioxidant systems can seriously limit oxidation thereby attenuating the therapeutic efficacy. We investigated the redox balance in cisplatin-sensitive and -resistant cells as a factor determining the survival or death upon PDT. In particular, we studied the expression of genes that encode the enzymes that mediate generation of active forms of oxygen (NADH-oxidase) and the antioxidant enzymes (superoxide dismutase - SOD, catalase, glutathione peroxidase - GPx). To induce photodamage the human ovarian adenocarcinoma SKOV-3 cell line and its subline resistant to cisplatin (SKOV-3/CDDP) were loaded with the new fluorinated carboranylchlorin (FC). Maximum intracellular accumulation of FC was achieved within 36–48 h. This compound was virtually non-toxic in the dark (IC_{50}>50 μM). Real time PCR analysis revealed a 4-fold decrease in the abundance of the NADH oxidase (*NOX5*) mRNA, a 3-fold increased expression of the *GPx*1 gene, and a moderate elevation of SOD1 and SOD2 mRNA level. Regardless of disbalanced expression of redox genes in SKOV-3/CDDP cells, light exposure (30 J/cm^2; 5 μM FC) triggered rapid (within first minutes) necrotic death of these cells. This effect was similar in parental SKOV-3 cells. Consequently, up-regulation of antioxidant defense in cisplatin-resistant cells does not prevent their lethal photodamage in the presence of the new photosensitizer FC. Thus, antioxidant mechanisms relevant to emergence of resistance of ovarian adenocarcinoma cells to cisplatin can be circumvented by inducing photonecrosis. The publication was prepared with the support of the RUDN University Program «5-100».

P.09-021-Wed
ERK1/2 directly phosphorylate HIF-2α and regulate its activity by controlling its CRM1-dependent nuclear shuttling

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Hypoxia Inducible Factor 2 (HIF-2) is a transcriptional activator implicated in the hypoxic cellular response. The inducible subunit, HIF-2α, is regulated by post-translational modifications. To study HIF-2α phosphorylation, human full-length recombinant HIF-2α or smaller fragments were used as substrates for in vitro phosphorylation assays with ERK. A small part of the C-terminal domain of HIF-2α (aa 640–679) was shown to be phosphorylated by ERK and contained a single putative phosphorylation site (Ser672). Site-directed mutagenesis of this residue significantly reduced phosphorylation of HIF-2α and, when expressed in human hepatoma Huh7 or HepG2 cells, a HIF-2α phospho-deficient mutant (S672A) exhibited lower transcriptional activity and increased nuclear exclusion, compared to the wild-type form. Moreover, inhibition of the ERK activation by U0126 impaired phosphorylation, nuclear accumulation and activity of wild-type endogenous HIF-2α, all of which was reversed when nuclear export was concomitantly inhibited by the exportin CRM1 inhibitor, leptomycin B (LMB). These findings suggest that phosphorylation of Ser672 by ERK1/2 enhances nuclear accumulation and activity of HIF-2α by blocking its CRM1-mediated nuclear export. Indeed, immunoprecipitation experiments demonstrated selective interaction between HIF-2α (S672A) and CRM1. To gain further mechanistic insight into the regulation of HIF-2α subcellular trafficking, we used site-direct mutagenesis to target conserved hydrophobic residues that may constitute a functional NES neighboring the ERK1/2 site. The effects of these mutations on localization and transcriptional activity of HIF-2α are currently under investigation. In summary, our findings reveal a novel regulatory step in the activation of HIF-2α which may play an important role in the adaptation of cancer cells to hypoxia. IM Gkotinakou is supported by State Scholarship Foundation (IKY).

P.09-022-Mon
PPARα knock-out mice show a compensatory expression of the PPARγ isoform primarily under ethanol stimulatory conditions

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Nuclear Peroxisome Proliferator Activated Receptor-alpha (PPARa) has been proposed as a potential target for the treatment of neuroinflammation-associated neuropsychiatric disorders, including alcohol abuse, but direct experimental evidence is still weak. Ethanol binge induces neuroinflammation in frontal cortex that is prevented by several acylethanolamides, such as oleoylethanolamide and palmitoylethanolamide, by regulation of Toll Like Receptors 4 (TLR4)-associated inflammatory pathway in frontal cortex. These acylethanolamides acts mainly as endogenous PPARa agonists, although they may bind other receptors, and direct evidence on the role of PPARa in neuroinflammation is unknown. We aim to study the role of PPARa in frontal cortex under physiological or neuroinflammation-associated ethanol binge challenge. Results indicated that PPARa KO mice showed an increase in TLR4, Nuclear Factor–kB p65 subunit, cyclooxygenase 2 (Cox2), interleukin-1B (IL-1B) and Tumor Necrosis Factor-alpha mRNA levels in frontal cortex, reflect of higher inflammatory conditions. After *Drinking in the Dark* ethanol administration we observed an increase in main inflammatory markers in the WT ethanol group as expected. However, ethanol animals lacking PPARa receptors showed reduced levels of TLR4, p65, Cox2 and IL-1B mRNA levels compared with ethanol WT group. Additionally, PPARa KO animals showed a compensatory upregulation of the PPARg isoform, which is also anti-inflammatory, primarily under ethanol stimulating conditions. Indeed, PPARg/a ratio in KO animals was bigger under ethanol challenge. Additionally, the PPARg endogenous ligand 15d-Prostaglandin J_2 plasma levels were similarly upregulated in KO animals, especially in ethanol-treated animals. Results highlight an anti-inflammatory homeostatic role of PPARa in physiological conditions and indicate that the lack of PPARa may induce a compensatory PPARg isoform up-regulation mainly after inflammatory stimulus such as ethanol binge exposure.

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P.09-023-Tue
High-fat diet compromises male fertility that is not surpassed by reversion to a controlled diet

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Lifestyle is a major cause for the onset of metabolic diseases (MD), notably obesity and type II diabetes (T2DM). Men suffering from those diseases also display poorer fertility outcomes, although the cause-effect relation between lifestyle and impaired fertility is still arguable. Besides, there is no evidence that lifestyle changes can restore the fertility potential in individuals who suffered MD. Herein we studied MD onset caused by diet (fat-rich diet), its effect on male reproductive potential and the effect of a diet reversion (DR) on those issues, using a rodent model. To achieve this, 3 groups of 12 mice were fed with 3 different diets (CTRL – standard Mucedola; HFD – high-fat diet and HFDt – high-fat diet for 60 days, then replaced by standard Mucedola). At 200 days, mice were subjected to Intraperitoneal Glucose Tolerance Test (IGPTT) and to Intraperitoneal Insulin Tolerance Test (IPITT), to assess the onset of MD. Mice weight was monitored throughout the experiment. After sacrifice, several tissues were collected and weighted. Testicular tissue was used for metabolomics analysis by H^1-NMR. Sperm was obtained from epididymis, and fertility parameters were determined. Our results show that high-fat diet significantly increases mice weight, and its withdrawal quickly normalizes it. CTRL and HFDt mice showed significant lower glycaemia during IGPTT than HFD suggesting that DR improves the metabolic state of the mice. Nevertheless, DR was unable to restore all fertility parameters since HFD and HFDt mice had significantly lower sperm viability and motility than CTRL mice. Metabolomics analysis revealed an altered lipid and energy metabolism in HFD mice, coupled to increased oxidative stress. HFDt normalized lipid metabolism illustrating a limited improvement after DR. Overall our data suggest that high-fat diet compromises male fertility and DR has limited impact on the affected parameters. Thus, weight gain cause permanent damage on male reproductive tract.

P.09-024-Wed
Mitochondria-targeted antioxidant SkQ1 improves corneal healing after UV-induced damage in rabbits

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Cornea absorbs most of daily ultraviolet (UV) light. An excess of UV damages results in not only keratopathy and cataract but also maculopathy. Previously, it was demonstrated UV exposure are associated with mitochondrial dysfunction and oxidative stress in cultured corneal epithelial cells in vitro. In this work, the feasibility of mitochondria-targeted antioxidant SkQ1 based therapy for enhancement of corneal regeneration after UV irradiation was demonstrated in vivo. A model of corneal damage caused by UV radiation was developed. It was demonstrated that 312 nm light irradiation of the cornea triggers the development of corneal lesions in rabbits. In particular, loss of epithelium, stromal edema and apoptosis of keratocytes and endothelium cells were observed. The stepwise mechanism of corneal regeneration was characterized. It involves epithelium and endothelium regrowth and activation of healthy keratocytes. Regeneration takes place simultaneously in all corneal layers and it becomes completed by day 7. It was emphasized that multiple apoptosis of stromal keratocytes can be associated with UV-induced oxidative stress, which manifested in the cornea as elevation of MDA content. The feasibility of SkQ1 based antioxidant therapy for enhancement of corneal regeneration after UV irradiation was demonstrated. The therapy prevents the loss of stromal keratocytes and other corneal cells, facilitates re-epithelialization of damaged tissue area and enhances stromal remodeling. In general, clinical and histological data indicate that the treatment accelerates corneal healing by 3 days. According to biochemical studies, the instillations of SkQ1 also suppresses oxidative stress in the cornea. In conclusion, our data suggest that the mitochondria-targeted antioxidant SkQ1 is effective in improving regenerative phases of corneal healing after UV damage. This study was supported by the Russian Science Foundation (Project no. 16-15-00255).

P.09-025-Mon
Study of CD sensitivity of iron(II) clathrochelates on various globular proteins

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Iron (II) clathrochelates are cage complexes with a wide range of bioactivities. They are able to bind proteins; upon binding to serum albumins, they induce strong specific circular dichroism

(CD) signal in 350–600 nm range. The most pronounced CD responses gain compounds with carboxyphenyl ribbed substituents. Here we explored the clathrochelates' ability to induce CD response upon binding to series of proteins of various structure and functionality: beta-lactoglobulin, lysozyme, trypsin, lipase and insulin. Dependence of the binding to proteins on number (one/two/six) and isomery of carboxyphenylsulfide substituents in clathrochelate molecule was analyzed. In case of beta-lactoglobulin, clathrochelates acquired the strongest CD responses of different shapes and intensity; the most pronounced CD bands (90 mdeg) gained by di-*ortho* clathrochelate. Binding to insulin resulted in inducing the intensive CD response only by compounds with six substituents (up to 76 mdeg for hexa-*para* isomer); CD signals varied by the intensity and shape for different isomers. CD bands induced by lysozyme were less strong (up to 13 mdeg for di-*meta* isomer), had similar shape for all clathrochelates. CD bands of clathrochelate series in presence of trypsin and lipase were indistinct (1–4 mdeg). Thus, it is shown that the number and geometry of ribbed substituents are important for clathrochelate binding to proteins; these affect the fitting of the compound to a binding site and determine the arrangement of the guest-host assembly. Clathrochelates could serve as prospective scaffolds for development chiroptical probes; to reflect the protein structural peculiarities by distinctions in CD spectra, clathrochelates could be adjusted by various number and isomery of substituents. The project leading to these results has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 778245.

P.09-026-Tue
Effect of gender on blood lipids parameters of the *Ovis aries* and the *Ovis ammon* interspecific hybrids

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Hybridization of domestic and wild species is the promising way to introduce the new capacities in farm animals. Here, we estimated the gender influence on major lipids of lambs blood serum of interspecies hybrids of Argali and domestic sheep with different genotypes. Blood samples were collected before feeding from female (n = 15) and male (n = 12) lambs at the age of 5 months. The animals (of each gender) were divided among the three groups according to their genotype: 1) hybrids of 25% Argali and 75% of Romanov sheep; 2) hybrids of 18.75% Argali and 81.25% of Romanov sheep; 3) purebred Romanov sheep. The cholesterol and triglycerides concentrations by automatic analyzer ChemWell (Awareness technology, USA) with Spinreact assay kits (Spain) were measured. Kruskal-Wallis test (comparison of analog groups) and U-test (effect of gender) were used for statistical analyses. The female lambs of 2nd group had a higher concentration of triglycerides ($P < 0.01$) in comparison with 1st group (+15.9%) and 3rd group (+10.3%). Male lambs of 3rd and 1st groups had a higher cholesterol concentration ($P < 0.05$) comparing to 2nd group at 4.8% and 6.5%, respectively. We found gender-related differences in 1st group for cholesterol concentration ($P < 0.05$), in 2nd group – for triglyceride concentration ($P < 0.05$) and in purebred Romanov sheep (3rd group) - for both lipids ($P < 0.05$). Female lambs had higher concentrations of lipid metabolites in comparison with male lambs: +20.3% for cholesterol in the 1st group, +19.7% for triglycerides in the 2nd group, +14.6% and 20.6% for triglycerides and cholesterol, respectively, in the 3rd group. The patterns, established in this study, indicated the essential differences in lipid parameters between hybrid and purebred lambs as well as between male and female lambs of the same genotype. This study was supported by Russian science foundation (project No. 14-36-00039).

P.09-027-Wed
Digestive complex of Tenebrionidae insects effectively degrades resistant to proteolysis gliadins

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Gliadins are widespread dietary proteins, which contain 10–30% Pro and 30–50% Gln residues. Several toxic gliadins peptides, resistant to proteolysis by human digestive enzymes cause autoimmune Celiac Disease in 1% of the susceptible human population. Search for natural enzymatic systems, capable to hydrolyze gliadins is an urgent task. Tenebrionidae insects are stored product pests and predicted hosts of such proteolytic system. Gliadins are their main food proteins; therefore, tenebrionids should possess enzymes capable to digest them. We have carried out a bioinformatic search for proline-specific peptidases (PSP) in Tenebrio molitor larval gut transcriptome and found 11 sequences homologous to human PSP. Combination of gene expression studies of the larval gut transcriptome with biochemical localization experiments allowed us to propose the digestive function for the highly expressed secreted dipeptidyl peptidase 4 (DPP 4) and prolylcarboxypeptidase (PRCP), and also for tissue localized prolidase (XPD). Previously we have shown that cysteine cathepsins (CC) are the major post-glutamine cleaving endopeptidases (PGP) in Tenebrionidae insects. We studied the effect of individual T. molitor digestive PGP–CC and PSP–DPP 4 and PRCP, and combined action of these peptidases on gliadins and suggested the hypothetical scheme of complete hydrolysis of γ- and ω-gliadins fragments with PQQPFPQ repeats. At the first stage, CC can hydrolyze the bond between two Gln residues. Resulting fragments are substrates for DPP 4, which cleaves dipeptides from their N-terminus. Remaining tripeptides can be hydrolyzed by PRCP. XPD may complete gliadins degradation, cleaving final dipeptides. Therefore, this natural complex provides complete gliadins hydrolysis and has a high potential as a possible preparation for Celiac Disease enzyme therapy. This work was supported by RFBR grants 17-54-61008 Egypt_a, 18-04-01221_a; RFBR-National Intellectual Development grant 17-34-80158 mol_ev_a.

P.09-028-Mon
DNA-binding ability, topoisomerase I/II and anticancer activity of novel biscoumarin derivatives with different length linkers

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Coumarin derivatives exhibit antitumor activities at different stages of cancer formation through various mechanisms, such as blocking of the cell cycle, the induction of cell apoptosis or the inhibition of DNA-associated enzymes. In recent years, the *O*-

substituted analogs of 7-hydroxycoumarins and biscoumarins have attracted intense interest due to their anticancer effects. In this work, we examine the spectral and binding properties of a new biscoumarin derivatives based on 7-hydroxycoumarin (a-e), linked together by linkers of different lengths. The interaction of a-e with calf thymus DNA (ctDNA) were investigated using different spectral methods. The fluorescence test at different temperatures revealed that the quenching mechanism was a static type. The binding constant K values of biscoumarins a-e with ctDNA were in range of 5.82×10^2 - 2.57×10^3 M^{-1}. Thermodynamic parameters ΔH and ΔS measurements were taken at different temperatures and indicated that van der Waal's forces and hydrogen bonding formation played major roles in the binding process in the case of compounds b-e. However, the ΔH and ΔS values for derivative a were both positive, a finding which is indicative of hydrophobic interaction. The values of ΔG for each compound were negative, and this confirms that the binding process is enthalpy driven and spontaneous. The results of other experiments such as iodide inducted quenching, competitive binding assay with ethidium bromide and CD spectral analysis suggest that compound a-e may bind to ctDNA the groove binding mode. Gel electrophoresis analyses indicated that biscoumarin derivatives (a-e) did not display DNA nuclease activity. Topoisomerase I/II inhibition assay were performed. The studies derivatives were also analysed against A549 adherent lung adenocarcinoma cells. This study was supported by Internal Grant Programme of University of P.J. Šafarik in Košice VVGS-PF-2018-754.

P.09-029-Tue
Amicoumacin A: the molecular mechanism of translation inhibition and antibiotic resistance

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Amicoumacin A (AMI) is an insufficiently studied antibiotic, which possesses not only strong antimicrobial, but anti-inflammatory and antitumor activities. Despite the potential using AMI as medical agent, molecular mechanism of its action is still unknown. The crystal structure of bacterial ribosome in complex with AMI revealed that antibiotic binds in the E site of 30S subunit, forming contacts with 16S rRNA and mRNA, but does not interacting with tRNA. Moreover, point mutations (ins544K, G542V and G581A) in the IV domain of EF-G compensated the AMI inhibitory activity. In our studies, we show that on the initiation step, AMI inhibits binding of initiator $tRNA^{fMet}$ to 30S subunit and prevents ribosomal subunit association. AMI does not change the rate of aminoacyl-tRNA binding in the A site and does not inhibit the peptide bond formation, but changes conformation of bound tRNA. On the translocation step, it significantly reduces the rate of peptidyl-tRNA movement from the A to P site of the ribosome and changes the position of its acceptor end in the peptidyl transferase center. AMI also stimulates binding of deacylated tRNA to the E site. Substitutions in domain IV of elongation factor EF-G lead to strong deceleration of the motion of peptidyl-tRNA D-loop region and but did not change the motion of tRNA acceptor end during translocation. In the presence of AMI, mutant EF-G promotes retention of the protein synthesis efficiency, while existence only intact EF-G form considerably decreases it. Thus, AMI inhibits protein translation by the disruption of initiation complex formation and the alteration of mRNA-tRNA complex movement upon translocation. This work is supported by Russian Science Foundation grant 17-14-01416 and RFBR 17-00-00368.

P.09-030-Wed
Fine tuning of bacterial translation initiation by small compounds

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The translation of the genetic information from the messenger RNA (mRNA) into proteins starts with the formation of 30S initiation complex (30SIC). During the initiation specialized transfer RNA ($fMet\text{-}tRNA^{fMet}$) binds to the initiator mRNA codon in the P site of the 30S ribosome subunit. The initiation factors IF1, IF2, IF3 are responsible for the accuracy and the efficiency of the process. As a result of amino acid starvation, lack of iron, phosphorus, fatty acids and other stress conditions in the cell, a process called stringent control is started. As a result, the processes of protein biosynthesis and cell duplication are slowed down. Stringent control carries out a global regulation of all metabolic processes of the cell through an alarmone (p)ppGpp. At this work we study the (p)ppGpp-mediated regulation at the onset of protein synthesis in the *in vitro* bacterial system. We apply advanced techniques of fluorescent spectroscopy to study the mechanism of (p)ppGpp interaction with IF2 along the pathway of 30S IC formation. Microscale Thermophoresis (MST) confirms the cooperative character of 30S initiation complex formation, strongly dependent on all ligands, and highlights guanosine nucleotides GTP and (p)ppGpp as key components to regulate $fMet\text{-}tRNA^{fMet}$ and mRNA recruitment. Pre-steady state kinetic assays show that the alarmone prevents rapid 50S joining to the 30S subunit, indicating that (p)ppGpp promotes 30S pre-IC. Nucleotide competition experiments show that (p)ppGpp competes better than GDP with GTP. Furthermore, the competitive character of (p)ppGpp appears to be mRNA dependent. Altogether, we provide a novel model for translation initiation regulation by (p)ppGpp and suggest that upon stringent response the alarmone regulates translation initiation in an mRNA-dependent manner by regulating the transition of 30S pre-IC to 30S IC. This work is supported by Russian Science Foundation grant 17-14-01416 and RFBR 17-00-00368.

P.09-031-Mon
Novel 3,6-diamino-9-substituted acridine derivatives as potential anticancer agents and gene expression regulators via their interactions with poly(rA)

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Acridine and its derivatives are heterocyclic, aromatic, organic compounds, many of which exhibit several biological effects, including promising anticancer and cytotoxic properties. They represent an important class of drugs, capable of intercalation into DNA and inhibition of topoisomerase or telomerase enzymes. They are also used as antibacterial, antiprotozoal and antimalarial agents, biological fluorescent probes or in the treatment of HIV and Alzheimer′s disease. Since we know, that various serious human diseases (HIV, hepatitis C, AIDS, etc.) are caused by RNA viruses, designing of new RNA binding compounds seems to be necessary. All eukaryotic mRNAs possess the unique structural region, represented by 200–250 bases long polyriboadenylic acid [poly(rA)] at the 3′-end. Polyadenylation of primary transcripts is an important determining factor in maturation, initiation of translation and stability, and is catalysed by the enzyme poly(A) polymerase (PAP). Poly(rA) therefore plays an essential role in gene expression. It has been found, that human PAP is significantly overexpressed in human cancer cells and represents a tumour-specific target. For the purpose of finding new, potent, anti-tumour agents capable of selectively interact with poly(rA), new 3,6-diamino-9-substituted acridine derivatives were synthetized. Their biochemical and biophysical properties were tested using spectroscopic techniques (UV-Vis absorption and fluorescence spectroscopy, circular dichroism and thermodynamic studies). Interactions were investigated using single and double stranded poly(rA) at pH 7.1 respectively 4.5. The obtained results can serve as an inspiration for the design and development of new acridine based molecules specifically targeted to poly(rA) structures. This study was supported by Internal Grant Programme of University of P. J. Šafárik in Košice VVGS-PF-2018-754 and VEGA 1/0016/18.

P.09-032-Tue
Mechanisms of import of non-canonical mitochondrial proteins

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Mitochondria are organelles that provide cells with the vast majority of energy they need. These structures, however, are not self-sufficient and instead rely on proteins and chemicals that are imported from elsewhere in the cell. Nearly 99% of mitochondrial proteins are synthesized in the cytosol and imported into mitochondria. Two layers of membrane enclose the mitochondria, and transporting proteins across these membranes requires large molecular machines embedded within the membranes, called translocases. Translocases recognize distinct signals encoded in the protein sequence to specifically import mitochondrial proteins. However, a number of proteins destined to other cellular compartments also localize to mitochondria without any canonical targeting sequences. Hence we are interested in exploring new translocation pathways or mechanisms involved in the import of these non-canonical mitochondria proteins. In order to identify potential partners, we imported non-canonical substrates, like p53, APE1, DJ1, PKC beta II, into mitochondria and subsequently subjected them to affinity purification and mass spectrometry. The mass spectrometry analysis revealed several potential candidates that might act as a translocases or proteins involved in the import of non-mitochondrial proteins. We present a preliminary analysis of sub-mitochondrial localization and interactions of the most promising candidate proteins.

P.09-033-Wed
MicroRNA hydrolysis by autoantibodies in blood of patients with schizophrenia

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MicroRNAs are known to regulate the expression of genes involved in the brain development and synaptic plasticity. Abnormal microRNA expression levels were detected in the serum and various brain regions of patients with schizophrenia (SCZ). Recently, in our laboratory catalytic antibodies or abzymes with nuclease activity were found in the serum of patients with SCZ. Here, we present the first evidence that IgG of SCZ patients possess RNAase activity and hydrolyze microRNA. Using different criteria we confirmed experimentally that this activity belongs to antibodies itself. IgG preparations were obtained by affinity chromatography and FPLC and analyzed by MALDI MS. MicroRNA-hydrolyzing activity of IgG were revealed by the degree of hydrolysis of fluorescent labeled microRNAs as a substrate using PAGE. There was revealed site-specific hydrolysis of microRNAs associated with SCZ (miR-137, miR-9-5p, miR-219a-2-3p, and miR-219a-5p). The secondary and tertiary structures of the microRNAs were predicted. Three major of cleavage sites are located in the microRNA loops or duplex parts directly articulated with the loops. It was detected that both light and heavy chains of IgG possessed RNase activity. Both chains are involved in the formation of the active center. The kinetic parameters of RNase reaction catalyzed by IgG were significantly lower than that for native enzymes. Interestingly, the level of RNase activity correlated with the clinical parameters of SCZ. Besides, we showed that IgG from schizophrenia patients with RNase activity reduced the survival rate of neuroblastoma IMR-32 cell culture by 10–28%. Thus, the formation of catalytic antibodies is proof of immunological disturbances in SCZ. Autoantibodies hydrolyzing microRNA can contribute to the abnormal microRNAs expression levels in the blood of patients with SCZ. *This work was supported by grant from RFBR 16-04-00603 and complex SB RAS scientific program II.2П/VI/57-5 (0309-2015-0022).*

P.09-034-Mon
Mechanisms of global translation modulation upon dysfunctional mitochondria

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Mitochondria are cellular structures in the cytosol of almost all eukaryotic cells. The primary role of these organelles is the production of energy in form of adenosine triphosphate (ATP) by the oxidative phosphorylation (OXPHOS). This metabolic pathway is also responsible for generation of reactive oxygen species (ROS). ROS are known to regulate cell signaling at low levels but prolonged exposure results in irreversible damage in cellular

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components, such as DNA and proteins. Changes in mitochondrial biogenesis and OXPHOS activity correlate with many age-related diseases. Aged mitochondria show decline in ATP synthesis concomitantly with increase in production of oxidants. Adjustment of protein synthesis is an essential adaptation of cells to oxidative stress. Dysfunctional mitochondria influence global protein synthesis. We showed that upon defective protein import into mitochondria cytosolic translation is inhibited in yeast (Wrobel *et al*., 2015). Downregulation of protein synthesis is also a feature of aging. However, the signals leading to its modulation remain unclear. Cells to maintain protein homeostasis must tightly control the process of protein synthesis. Translation can be regulated at any step of the process; nevertheless most common in eukaryotic cells is the regulation at the level of translation initiation. At the beginning of the protein synthesis, the key role in its regulation plays the eukaryotic initiation factor 2 (eIF2) complex. In response to stress the *alpha* subunit of eIF2 (eIF2α; Sui2 in *S. cerevisiae*) is phosphorylated, which causes translation attenuation. Our aim is to unravel signals and mechanisms, which regulate cellular protein synthesis during stress conditions caused by dysfunctional mitochondria.

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P.09-035-Tue
Increased mean platelet volume is associated with acute myocardial infarction in patients with diabetes mellitus type 2

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Overdose of more chronic illnesses is increasingly a common health problem in the general population. The association of diabetes mellitus and acute myocardial infarction is greater every day. The mean platelet volume (MPV), which is the determinant of platelet function, is an independent risk factor for cardiovascular disease. Available research data show that increased MPV is linked to the presence of risk factors for cardiovascular disease including others: diabetes mellitus, hyperlipidemia, hypothyroidism and other autoimmune disease. The aim of this study was to investigate the effect of each disease (hypothyroidism, hypertension, myocardial infarction) individually on MPV in diabetic patients. The cross-sectional study enrolled 102 diabetes mellitus type 2 (DMT2) patients, both sexes (46 females, 56 males), with a mean value of age 58.91 (SD = 12.93) treated at the Primary Health Centre in Zenica from May to July 2017. All respondents had diabetes mellitus, the disease lasted for almost 10 years in both sexes. Mean platelet volume was significantly higher in patients with myocardial infarction than in those without myocardial infarction (11.04 ± 1.43 vs. 9.409 ± 1.53; $P = 0.001$). Regression analysis showed that the prevalence of myocardial infarction had the highest predictive significance for MPV values, (predictor importance 0.49; coefficient 1.275, $P < 0.001$). Mean platelet volume was significantly higher in patients with diabetes mellitus and myocardial infarction than in DM patients, without myocardial infarction. We propose that MPV might be an important predictive factor for damage after cardiac injury. Regression analysis showed the only association with myocardial infarction in patients with DMT2, but not with other chronic illnesses. MPV values increased with the increasing number of comorbid conditions with the highest platelet volume indices were observed in patients with myocardial infarction.

P.09-036-Wed
Novel peptide analogues of antibiotic chloramphenicol

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In view of the growing bacterial resistance, the development of new antibiotics is an urgent task. The modification of widely used antibacterials with various chemical residues is one of the main approaches for creating new compounds with improved properties. The combination in the antibiotic structure several moieties that act on different active sites will allow to trick the mechanisms of bacterial resistance. Chloramphenicol is a widespread antibiotic that acts by inhibiting bacterial protein biosynthesis. Its binding site is located in the A-site of the peptidyl transferase center of the ribosome. The replacement of the dichloroacetyl residue in the chloramphenicol molecule by a peptide will expand its binding site to the ribosomal tunnel. These chimeric molecules can also be used for studying the interaction of ribosome nucleotides with the peptide attached to the antibiotic. To find the optimal amino acid sequences of the peptides, molecular docking based virtual screening of all possible tripeptide chloramphenicol analogues for their binding to the ribosome was carried out. Compounds containing peptides FWH, VFR, RAW, AAA, both with the free N-terminal amino group and the protected by acetyl group, were selected and synthesized. The affinity of the compounds to the ribosome was measured using a competitive displacement assay. It turned out that some of the analogues bind to the ribosome 30 times better than the original antibiotic, which is consistent with the virtual screening results. The use of 23S RNA chemical probing made it possible to determine the nucleotides involved in the interactions with the peptide moiety of the molecules and suggest their binding mechanism. The ability of some compounds to inhibit protein biosynthesis *in vitro* was comparable to that of chloramphenicol, making the obtained analogues promising for further antibacterial activity studies. This work was supported by the grants RFBR (16-04-00709) and RSF (14-24-00061-P).

P.09-037-Mon
Organization of respiratory chain in human fibroblasts with cytochrome c oxidase deficiency caused by loss of COX8A subunit

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Biogenesis of cytochrome c oxidase (COX) protein complex is executed under dual genetic control. While COX deficiencies are some of the most frequent causes of respiratory chain disorders in humans, they are largely associated with aberrations in translation of mitochondrial-encoded COX subunits and disruptions of the tightly regulated process of COX assembly. Mutations in the nuclear genes of structural subunits of COX are very rare

and few clinical cases have been reported to date. One of them, manifested in the patient with the Leigh-like syndrome with leukodystrophy and severe epilepsy, was ascribed to the mutation in *COX8A* leading to aberrant splicing and decreased transcript amount. It resulted in the loss of wild type COX8A protein – the smallest nuclear-encoded structural subunit of COX, which is indispensable for stability and activity of the COX complex. To further characterize organization of the respiratory chain in the patient's fibroblasts, we studied the supramolecular complexes containing COX and its pattern of structures at different stages of assembly by blue native polyacrylamide gel electrophoresis (PAGE). We identified that a decreased amount of COX was largely recruited to the supercomplexes together with complexes I and III. Furthermore, to assess adaptive changes in the observed phenotype, we analyzed steady state levels of selected respiratory chain components with SDS PAGE and Western blot. Additionally, amount of transcripts encoding mtDNA maintenance and translation machinery, alongside with the variation in the gene expression for selected structural subunits of respiratory chain complexes, were assessed by quantitative reverse-transcription polymerase chain reaction. From our findings, supercomplex assembly emerges as a crucial step for stabilizing residual COX activity. This project was supported by the Marie Sklodowska-Curie COFUND grant No. 665735 and Polish National Science Center grant No. 2015/18/E/NZ1/00737.

P.09-038-Tue
The evaluation of endocrine disruptor effects of environmental pollutant zinc and copper pyrithione via apoptosis pathways by histopathological and biochemical analyses

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Growing living organisms can accumulate in large numbers on surfaces like pipes, tanks, and ship hulls, resulting in corrosion, clogging and contamination, known as biocides. The antifouling paint biocides copper pyrithione (CuPT) and zinc pyrithione (ZnPT) are environmental pollutants especially effect the non-target organisms. Zebrafish (Danio rerio) is a model organism of biomedical researches for disease modelling and functional neuroscience. The aim of the present study is to determine the effects of these environmental pollutants (Cu-ZnPT) on aquatic toxicology and the possible effects on humans by evaluating apoptotic pathways and endocrine disrupting biomarker, vitellogenin. To generate tissue damage four groups of zebrafish (length 3–4.5 cm) were exposed to 0.02 μg/L CuPT, 0.1 μg/L CuPT, 0.1+1 μg/L CuPT+ZnPT and 1 μg/L ZnPT for the planned time intervals as 24 and 96 h. Several biochemical assays including hematoxylin-eosin staining, immunohistochemistry, ELISA for whole body vitellogenin and Western blot were performed to elucidate the mechanism of action of Cu-ZnPT. The gill tissues were damaged in both Zn and Cu pyrithione exposed groups. Hyperemia, epithelial lifting and hyperplasia were observed in all Cu-ZnPT concentrations. The lesions were more severe in CuPT. Western blot and immunohistochemical analysis revealed significant alterations of proapoptotic Bax protein expression and Bcl-2 protein expression ($P < 0.05$) among the groups. Besides vitellogenin levels were significantly altered compared to time control groups ($P < 0.05$). As marine pollution and deterioration of ecosystems are directly affect the humans, the results presents awareness among environmental pollution, marine pollution and health problems. The toxic effects of environmental pollutants on apoptotic pathways at different stages of organisms on the food web provide basic data to understand and estimate the effects on the human beings.

P.09-039-Wed
Carbohydrate source effects on the transcriptome of *Streptococcus mutans*

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Dental caries is one of the most common chronic multifactorial disease. The plaque ecological balance is considered to be the key factor in aetiology of caries. For this balance is crucial the role of dietary carbohydrates. The primary etiologic agent of human dental is an acidophilic bacteria *Streptococcus mutans*. By employing deep sequencing of RNA (RNA-Seq) technology we have established the effects of various sugars on the regulation of gene expression in bacterium *S. mutans* growth on tryptic soy. Bacterium *S. mutans* was grown in a 5% CO_2-aerobic atmosphere at 37°C on solid and liquid tryptic soy broth with cariogenic sugars represented by sucrose, fructose, glucose, galactose, lactose and non-cariogenic sugar xylitol. Gene expression was monitored by Illumina sequencing technology after a 12-hour growth of *S. mutans* on cariogenic or non-cariogenic sugars and compared to control culture grown without carbohydrates when results were considered as significant at $p_{adj} < 0.01$. Bacteria grown on xylitol, galactose or lactose showed only minor changes in gene expression when there was mainly downregulation of expression compared with control bacteria grown on tryptic soy broth. Another group of sugars was represented by fructose and glucose where genes involved in glycolysis and butanoate metabolism were strongly upregulated. In agreement with previous studies, the largest changes in gene expression were observed in bacteria grown on medium with sucrose. The obtained data helps to understand the impact of individual sugars on *S. mutans* metabolism involved in one of the most common chronic diseases affecting the human population.

P.09-040-Mon
Novel N-alkynyl and N-indolylethyl amino steroids as inhibitors of CYP17A1 activity

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Cytochrome P450 17A1 is a core enzyme in the androgen biosynthesis and the molecular target of abiraterone, a heterocyclic steroid used for post-surgical treatment of prostate cancer. A major class of mechanism-based inactivators and probes for cytochrome P450 is based around a terminal alkyne group. In this work, the synthesis and evaluation of alkyne- and indole-containing amino steroids as inhibitors of CYP17A1 is reported. The target compounds were obtained by reductive amination of 17- or 20-ketosteroids with propyneamine/butyneamine and tryptamine in 60–80% and 20% yield, respectively. All structures were confirmed by mass-spectrometry, IR and NMR spectroscopy. Docking simulations predicted the steroids to localize in the binding site in a manner that resembled that of native substrate pregnenolone or favoured the coordination of indole side chain with the heme. The substances were tested for their capability to inhibit progesterone 17-hydroxylation by engineered Yarrowia lipolytica yeast.

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Among the alkynes, the conjugate based on pregnenolone containing an N-propynyl moiety was found to interfere with enzymatic activity most effectively (e. g. 66% relative inhibition at a concentration of 100 μM) whereas the other compounds had only a minor effect. At a concentration up to 100 μM, the compounds did not affect yeast viability by more than 10% as evidenced by tetrazolium reduction assay and cell growth. Although the inhibitory efficiency of alkyne and indole steroids was lower than that of abiraterone, it represents an interesting starting point for development of alternative lead compounds targeting the above effector of androgen-dependent prostate cancer.

P.09-041-Tue
Glyceraldehyde-3-phosphate dehydrogenase is partially inhibited during amyloid aggregation of alpha-synuclein

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The hallmark of Parkinson's disease is the formation of protein aggregates with α-synuclein being its major component. It is also characterized by oxidative stress, disruption of energy metabolism and formation of advanced glycation end products, colocalized with aggregates. In our work, we studied the influence of the aforementioned processes on glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and its implications in synucleinopathies. We have shown that α-synuclein partially inactivates GAPDH *in vitro*, while in cell lysates of SH-SY5Y lower activity of GAPDH was observed in cells expressing prone to aggregation α-synuclein A53T, rather than in cells expressing wild-type α-synuclein. GAPDH activity was decreased by day 6 of α-synuclein A53T expression, when it formed amyloid aggregates, as it was confirmed by thioflavin S staining. Partial inactivation of GAPDH also correlated with decreased lactate generation rate measured in cell lysates and directly in cell culture medium. When oxidative stress by 10 equivalents of hydrogen peroxide was applied to α-synuclein and GAPDH, GAPDH recovered 85% of its initial activity after addition of reducing agents. However, GAPDH alone remained 50% inactivated. Glycation of GAPDH by its own substrate glyceraldehyde-3-phosphate and methylglyoxal also lead to partial inactivation *in vitro* and in cell lysates. Taken together, many factors influence GAPDH activity and therefore glycolytic rate in the pathogenesis of Parkinson's disease. On one side, an abundance of α-synuclein forming amyloid aggregates disrupts glycolysis, on the other side, it is possible that α-synuclein partially protects GAPDH from oxidative stress. Additional inhibition of GAPDH in these conditions may be caused by glycation, leading to accumulation of highly reactive carbonyls and hindering energy supply to the cell. This work was supported by Russian Science Foundation project No 16-14-10027.

P.09-042-Wed
Natural polyphenols increase systemic as well as hepatic concentrations of bilirubin and decrease lipoperoxidation in mouse liver

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Bilirubin (UCB), the end product of heme catabolism, is a potent antioxidant under physiological conditions. Even though UCB has been considered a toxic waste product and a sign of liver dysfunction for decades, its role as a powerful protective molecule is been increasingly recognized. Serum concentrations of UCB are in particular affected by bilirubin UDP-glucuronosyl transferase (UGT1A1) catalyzing its biotransformation in the liver. The aim of our study was to analyze potential intracellular and systemic bilirubin-modulating effects of polyphenols contained in the milk thistle (*Silybum marianum*) via modulation of key enzymes of UCB metabolic pathway. Human hepatoblastoma HepG2 cell line was treated with individual major polyphenolic compounds isolated from milk thistle. Based on *in vitro* studies, dehydrosilybin A and B were selected and used for *in vivo* studies. Individual compounds and/or its equimolar mixture were applied *i.p.* or *p.o.* for seven days to C57BL/6 mice. Markers of liver damage, serum concentration of UCB, expression of *UGT1A1* mRNA, lipid peroxidation and intracellular concentration of UCB in the liver were analyzed. *I.p.* application of dehydrosilybin mixture and *p.o.* application of dehydrosilybin A to mice led to a significant down-regulation of *UGT1A1* mRNA expression (46% resp. 55% of control, $P < 0.05$) in the liver and also to a significant increase of intracellular UCB concentrations (0.98 vs. 1.21 resp. 1.30 nmol/mg, $P < 0.05$) and significant decrease of lipoperoxidation (61% resp. 70% of control, $P < 0.05$) in the liver tissue. Importantly, both application routes resulted in a significant elevation of UCB in serum (125% resp. 160% of control, $P < 0.05$). Polyphenolic compounds contained in milk thistle affect the heme catabolic pathway and intracellular UCB levels *in vitro* and *in vivo*, as well as its serum concentration. This phenomenon might contribute to hepatoprotective effects of milk thistle.

P.09-043-Mon
Exploring the bioenergetics of the bloodstream *Trypanosoma brucei* mitochondrion

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The infective bloodstream form (BF) of the human pathogen *Trypanosoma brucei* possesses a single reduced mitochondrion that lacks a cytochrome-mediated respiratory chain. Therefore, the essential mitochondrial (mt) membrane potential ($\Delta\psi_m$) is maintained by the reverse activity of F_oF_1-ATPase, which translocates

H+ at the expense of ATP. It is believed that the abundant glucose in the mammalian bloodstream allows the parasite to generate enough ATP for cellular functions during glycolysis. The dogma is that cytosolic ATP is transported into the mt matrix via the ATP/ADP carrier (AAC), while ADP is transported out. However, BF trypanosomes can tolerate high doses of carboxyatractyloside (CATR), a specific AAC inhibitor, suggesting that AAC might not be essential for the BF parasites and that mt ATP might be supplied by other means. Indeed, we managed to generate viable AAC double knockout (DKO) BF parasites that are able to establish a lethal infection in mice. Furthermore, in contrast to parental BF cells, AAC DKO parasites were not able to establish a $\Delta\psi_m$ during a Safranine O assay when ATP was supplied, thus excluding the possibility of another mt ADP/ATP carrier. However, metabolomics data for AAC DKO cells indicate that acetate production is increased, suggesting that mt substrate phosphorylation (SubPhos) could provide ATP, even though this activity was long thought to not exist in this life stage. Interestingly, the elimination of succinyl coenzyme A synthetase (SCoAS), the enzyme directly responsible for ATP generated by mt SubPhos, resulted in a viable *in vitro* BF parasite that none-the-less displayed decreased fitness in the animal model. Furthermore, these cells are drastically more sensitive to CATR, suggesting that AAC can compensate for the loss of SubPhos in cultured cells only. To further verify that ATP is generated from mt SubPhos, we are currently developing tools to independently measure the amount of cytosolic and mt ATP *in vivo*.

P.09-044-Tue
Antiinflammatory effect of bilirubin in lipopolysaccharide-induced sepsis

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The aim of the study is to assess the pathophysiological role of bilirubin in inflammation. Bilirubin, a product of heme catabolic pathway, is associated with antioxidant and anti-inflammatory properties in patients with mild hyperbilirubinemia. However, the exact mechanism of its protective actions needs to be elucidated. Systemic inflammation was induced in hyperbilirubinemic Gunn rats as well as their normobilirubinemic littermates by lipopolysaccharide (LPS, 6 mg/kg IP). Animals were anesthetized and sacrificed 12 h after LPS administration. Blood and organs were collected for analysis of inflammatory and liver injury markers. The proportion of neutrophils and monocytes was significantly lower (73% and 49%, respectively, $P < 0.05$), while that of lymphocytes was higher (152%, $P < 0.05$) in the LPS-treated Gunn rats as compared to LPS-treated control. ALT activity, marker of hepatotoxicity, was lower in the LPS-treated Gunn rats compared to LPS-treated controls (4.90 ± 2.78 vs. 1.65 ± 0.72 μkat/L, $P < 0.01$). The different expression of liver cytokines was observed comparing LPS-treated Gunn rats and respective controls. We observed decreased expression of *TNF*-α (62%, $P < 0.01$), *IL1*-β (65%, $P < 0.01$) and *IL6* was slightly decreased. However, the expression of *IL-10* was downregulated in the LPS-treated Gunn rats (61%, $P < 0.01$). Furthermore, mRNA expression of lipopolysaccharide binding protein (LBP) in the liver of experimental animals was upregulated in the liver of Gunn rats before and after LPS treatment (up to 48%, $P < 0.05$) compared to their normobilirubinemic littermates. We conclude that hyperbilirubinemia in Gunn rats is associated with decreased systemic inflammatory response in LPS-induced sepsis. This work is supported by grant GAUK 168216 and SVV 260370/2017 given by Charles University, Prague, Czech Republic.

P.09-045-Wed
Evaluating 25 hydroxy D3 vitamin levels among cerebrovascular stroke patients

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Vitamin D is widely known as a hormone which primarily has effects on bone health and regulates phosphate and calcium metabolism. Besides, recent researches show that there is inverse correlation between 25 OH D3 levels and cardiovascular and cerebrovascular diseases. The aim of this study is to determine the association between vitamin D3 levels and cerebrovascular stroke. Statistical data was obtained retrospectively from January 2010 to May 2017 from Kocaeli University Medical Faculty Education and Research Hospital Central Laboratory data system. Statistical evaluation was performed with IBM SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Number of D3 measured patients who have cerebrovascular stroke is 86. With obtaining exclusion criteria, non-stroke comparison group data decreased to 26.707 from over 80.000. Age, gender, season and sunshine duration time, (monthly average hours per day) is used in the assessment. In cerebrovascular stroke group, D vitamin levels found as statistically lower ($P = 0.024$). Statistical significant difference is found between age groups and D3 levels (Kruskal-Wallis test, $P = 0.017$). Elder age group has the lowest D3 vitamin levels. We evaluate seasonal and sunshine duration time's impact grade on D3 vitamin levels. In cerebrovascular stroke group, D3 vitamin levels do not signify any statistical difference among season ($P = 0.534$). On the other hand, in the non-stroke group D3 levels differs by season ($P < 0.001$). Highest D3 vitamin levels are measured in autumn. Gender is found as an alternative factor on D3 vitamin levels in stroke group. Male group has higher levels than women ($P = 0.002$). In stroke group, sunshine duration time is not found a factor that makes statistical difference for D3 levels (Spearman test, $r = 0.74$, $P = 0.467$). Our research results endorse the literature which indicates association vascular health with vitamin D3.

P.09-046-Mon
Novel cell based assay using high throughput technology for the identification of chemical structures against Hepatitis C Virus NS2 cysteine protease

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Hepatitis C Virus (HCV) affects more than 170 million people worldwide and carries a huge cost burden: economic as well as personal. Infection with HCV can led to cirrhosis and hepatocellular carcinoma, debilitating diseases. Direct acting antivirals (DAA) have provided a major improvement over the old standard of care with alpha-interferon and ribavirin. However because of the intrinsic nature of the NS5B HCV polymerase, genetic variability and drug resistance are still pressing issues. The viral proteins that have been successfully targeted so far in the clinic with a DAA cocktail are the NS3 (non-structural protein 3), NS5A (non-structural protein 5A) and the NS5B (non-structural protein 5B) polymerase. The HCV NS2 cysteine protease, achieves a very important step in the viral life cycle, the first

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cleavage that releases the second HCV protease NS3, without this cleavage the virus cannot replicate any further. The lack of an automatable screening assay has been a major problem in identifying chemicals against HCV NS2. Herein, a cell-based assay for HCV NS2 cis-protease activity was validated, miniaturized and automatized for 384 well plate for high throughput screening (HTS). A targeted library of 2000 cysteine protease inhibitors were tested against the HCV NS2 protease activity. A counterscreen was developed and used to filter out the false positive hits. The remaining hits were validated in a secondary screen using the HCV cell culture system with full length and subgenomic replicons. Using bioinformatics techniques, a series of structure – activity relationship (SAR) were tested further. We identified chemical scaffolds with potential different modes of action due to their different effect on the NS2-NS3 site processing. In conclusion, it is presented the first cell based HTS assay for HCV NS2 cysteine protease activity which identified new chemicals confirming HCV NS2 as a druggable target.

P.09-047-Tue
Moderate physical activity alters cardiac lipid metabolism of male rats on high fructose diet

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Metabolic syndrome is a growing health problem worldwide associated with modern day diet, rich in saturated fat and sugar. Increase in fructose consumption contributes to ectopic lipid storage which in turn is a risk factor for development of type 2 diabetes and cardiovascular disease. Defective substrate utilization in the heart caused by systemic derangements in metabolic syndrome leads to cardiac dysfunction. Exercise is shown to alleviate most of the symptoms related to this metabolic disorder. The aim of this study was to analyze the impact of low intensity exercise on molecular mechanism of cardiac free fatty acid (FFA) transport and metabolism and serum lipid profile of fructose fed male rats. Male Wistar rats were divided into control group and two groups that received 10% fructose for 9 weeks, one of which was additionally exposed to low intensity exercise. Concentration of circulating FFA, triacylglycerol (TAG), total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol were measured as well as TAG content in heart tissue. Protein content of fatty acid translocase CD36, carnitine palmitoyl transferase 1 (CPT1) and Lipin1 in cardiac lysate and CD36 content in plasma membrane were evaluated. High fructose diet elevated serum TAG and showed a trend of increase of total cholesterol ($P = 0.066$). Exercise didn't change TAG and total cholesterol, but FFA were decreased compared to control group. Although there was no change in protein expression of CD36 transporter, exercise raised the level of CD36 in plasma membrane compared to fructose group. Exercise increased protein expression of both CPT1, mitochondrial transporter of FFA, and Lipin1, enzyme involved in TAG synthesis, compared to control group. High fructose consumption changed serum lipid levels, whereas low intensity exercise had greater influence on increasing the transport and metabolism of FFA in the heart.

P.09-048-Wed
Oxadiazon-induced toxicity in HCT116 cells: involvement of oxidative stress and apoptosis

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Pesticides are chemical compounds used to control organisms considered to be harmful in agriculture and also in other sectors. They are ubiquitous in the environment and exposure to these compounds is suspected to increase the incidence of certain pathologies especially, cancer. At present, exposure to pesticides through food is attracting increasing interest. In this study, we were interested in an Oxadiazole family herbicide, Oxadiazon. Although many studies are available regarding its environmental and ecological impact, very little is known about its toxicity in the mammalian system. The aim of this study was to investigate the toxic effect of Oxadiazon on intestine using an in vitro model (HCT116). Therefore, we evaluated the cell viability, elucidated the generation of free radicals, measured the mitochondrial membrane potential, and valued DNA fragmentation. Our results showed that Oxadiazon is cytotoxic to HCT116. It causes damage through the generation of free radicals and induces lipid peroxidation and DNA damage. We also demonstrated that such effects can be responsible for Oxadiazon-induced apoptosis.

P.09-049-Mon
Investigation of the nucleotide metabolism and growth of *Mycobacterium smegmatis* under different genotoxic stress conditions

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The fidelity of replication is essential for every organism to maintain their genome integrity. The fine-tuned pool of deoxyribonucleoside 5'-triphosphates (deoxynucleotides, or dNTPs) is a critical factor that contributes to the accurate DNA synthesis. It has been shown that perturbation of the well conserved balance of dNTPs results in elevated mutation rate. Interestingly, the pathogenic *Mycobacterium tuberculosis* (*M. tuberculosis*) has an especially low *in vitro* mutation rate although it lacks the mismatch repair enzymes. However, *in vivo* samples from patients often exhibit resistance to more than one drugs, caused exclusively by various single-nucleotide mutations. Based on these observations, our aim is to establish the relationship between different types of genotoxic stresses and the deoxynucleotide pool size and balance in *Mycobacterium smegmatis (M. smegmatis)*. As a valid model of *M. tuberculosis*, we used the non-pathogenic *M. smegmatis*, since they share the same DNA metabolic and repair routes. Several stresses are important in the lifecycle of *M. tuberculosis*: these are oxidative stress, ionizing radiation, nutrient starvation, alkylating agents, hypoxia and currently used antimycobacterial drugs. We defined appropriate treatment period based on the growth curve of *M. smegmatis*. The concentration of chemical agents used for the treatment were specified to inhibit cell growth. dNTP concentration following genotoxic treatments were measured applying a fluorescent-based DNA-polymerization method. Our results showed that different environmental stresses cause changes in the dNTP pool size: for antimycobacterial agents there are several orders of magnitude increase in the

deoxynucleotide pool size, while exposing cells to nutrient starvation caused reduced dNTP amounts. We are now in the process of further investigating this phenomenon by determining the mutation rates and mutational pattern arising from the applied genotoxic stress.

P.09-050-Tue
Low intensity exercise prevents disturbances in insulin regulation of α subunits of Na+/K+-ATPase in the heart of fructose-fed female rats

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Na^+/K^+-ATPase is an enzyme essential for regular functioning of the heart, regulated by insulin. Since estrogen deficiency combined with fructose rich diet provokes cardiac insulin resistance we hypothesized that sodium/potassium transport would be deteriorated accordingly and exercise training can prevent this disturbance. To test our hypothesis, we used fructose rich diet as animal model of insulin resistance in ovariectomized (OVX) female rats. OVX female Wistar rats were divided into three groups: sedentary control and sedentary and exercise groups submitted to fructose diet (10% fructose for 9 weeks). We analyzed biochemical parameters relevant for insulin action. Expression, phosphorylation and/or subcellular localization of cardiac insulin receptor (IR), insulin receptor substrate 1 (IRS1), protein kinase B (Akt) and Na^+/K^+-ATPase in basal and insulin-stimulated conditions were evaluated. Fructose diet did not change blood glucose level, but it increased plasma insulin level as well as homeostasis model assessment index, indicating insulin resistance. Exercise reversed these parameters to the control level. Fructose diet didn't have effects on cardiac IR, Akt (Thr308) and IRS1 (Tyr632) phosphorylation but it reduced Akt (Ser473), increased inhibitory IRS1 (Ser307) phosphorylation and increased expression and plasma membrane level of α1 and α2 subunits of Na^+/K^+-ATPase. Exercise returned Akt phosphorylation at Ser473 and increased at Thr308, without effect on IRS1 phosphorylation (Ser307), but returned total and plasma membrane level of α1 and α2 subunits of Na^+/K^+-ATPase. In conclusion, exercise prevents disturbances in cardiac sodium/potassium transport in fructose-fed OVX rats suggesting that low intensity physical activity might be important nonpharmacological treatment for cardiac insulin resistance.

P.09-051-Wed
Resolvin E1 regulates inflammatory mediators and oxidative stress enzymes of the cementoblasts

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Resolvin E1 (RvE1) has been effective in treatment of periodontitis and regenerated lost periodontal apparatus including new cementum formation in animals. The aim of this study was to investigate the action of RvE1 on the gene expression of inflammatory mediators and oxidative stress enzymes in cementoblasts. Murine immortalized cementoblasts (OCCM.30) were treated with different concentrations of RvE1 (0–1,000 ng/mL). The impact of RvE1 on OCCM.30 proliferation was determined for 360 h. RvE1 had a dose-dependent and bimodal impact on the expression of cytokines and enzymes. There was a statistically significant downregulation of IL-1β and IL-6 and a an upregulation in IL-10 in response to lower concentrations (0.1–1 ng/mL) of RvE1 on days 1 and 6 compared to control/untreated cells ($P < 0.01$). The elevated IL-1β/IL-10 and IL-6/IL-10 ratios indicated increased activity of a resolution of the inflammatory process in the early stage of the experiment. Higher concentrations (10–1,000 ng/mL) of RvE1 failed to stimulate the proliferation and expression of tested molecular targets. In contrast, expression of IL-1β, IL-6, IL-8 was significantly increased in response to higher doses of RvE1 while IL-10 expression was reduced ($P < 0.01$). We determined that the increase of IL-1β/IL-10, IL-6/IL-10, IL-8/IL-10 ratios with higher doses of RvE1 on days 1 and 6. RvE1 dose-dependently induced the expression of SOD and GPX in OCCM.30 ($P < 0.01$). These findings suggested that cementoblast function was regulated by the RvE1 where lower doses stimulate a resolution of inflammatory process in parallel with an increased enzymatic activity, which would favor a regenerative process and higher doses favor an activation of inflammation, which would be key for the resorptive role for the cementoblast-induced tissue turnover during the periodontal regeneration (Selcuk University Scientific Research Projects BAP-17401164).

P.09-052-Mon
The anti-neuroinflammation of erinacine A on LPS-induced Parkinson's disease in vivo and in vitro

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Hericium erinaceus (*H.E.*) is a kind of medicinal mushrooms in Asia, and has anti-oxidative, anti-diabetics and anti-hypertensive effects. Erinacine A (EA), the main active compound of *H.E.*, is demonstrated that it could inhibit the inflammatory cytokine expression in the model of stroke rats. However, few of researches discussed the preventive potencies of EA in neurotoxicity which is induced by inflammatory response. The previous studies found that neuroinflammatory response is induced by activated microglia which cause neurotoxicity and promote deteriorating of Parkinson's syndrome. This study aimed to investigate the neuroprotective effects of EA on LPS-induced neuroinflammation in the microglia cell, astrocyte, differentiated neuro-2a and animal model of semi-Parkinson's disease. Results demonstrated EA and *H.E.* could improve the physical coordination in the rotation test and dramatically decrease the expression of inflammatory cytokines, TNF-a, iNOS and IL-1b, *in vivo*. In addition, pretreated EA significantly suppressed iNOS expression and NO production in LPS-induced activated BV-2 cells. Moreover, pretreated EA could decrease TNF-a mRNA expression in astrocyte which was stimulated by LPS. Additionally, EA and *H.E.* could increase TH but decrease p-NF-kB, p-JNK expression on BV-2 conditioned medium in differentiated neuro-2a. Those results show that, *H.E.* and EA could ameliorate LPS-induced the semi-Parkinson's syndrome, inhibit the iNOS expression in activated BV-2 cells and also prevented the cell death from BV-2 conditioned medium in differentiated neuro-2a. Therefore, *H.E.* and EA may possess potent anti-inflammatory activity and improvement of Parkinson's disease.

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P.09-053-Tue
The effects of high ferritin level on bone turnover biomarkers in beta thalassemia major patients living in the Turkish Republic of Northern Cyprus

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Beta thalassemia Major (BTM) is a severe life threatening genetic blood disorder. BTM patients need regular and adequate blood transfusion to survive, resulting iron overload, toxicity and other complications. Osteoporosis and osteopenia cause serious health problems such as fractures, cracks and deformations in the bones. In the study, 50 patients (25 male, 25 female) with BTM and 30 normal volunteers as a control group (15 male, 15 female) between the ages of 20 and 50 were evaluated. Blood samples were taken in the morning on an empty stomach. Calcium, phosphorus, ALP, PTH, Osteocalcin, 25OH D3 and Ferritin were studied for all participants. There was no significant difference between the BTM patient group and the control group in terms of total calcium, phosphorus, ALP and Parathormon ($P > 0.05$). The values in these parameters were determined within normal limits in both groups. Ferritin levels of patients with BTM were significantly higher than those of the control group ($P < 0.001$). Especially Ferritin levels of BTM women were found to be significantly higher than BTM men ($P < 0.05$). When we look at the results of 25OH D3, the mean values of the BTM patient group was low <20 ng/mL and for the control group was found to be inadequate <30 ng/mL. There was a significant difference between the two groups ($P < 0.001$). Osteocalcin levels were significantly lower in the BTM group than in the control group ($P < 0.001$). A low negative correlation (-.196) was found between Ferritin and 25OH D3 levels in patients with BTM. It has been shown that, the 25OH D3 levels in the thalassemia patients were fairly low and insufficient even in the control group. It is possible to link extremely low levels of 25OH D3 in patients with BTM to extremely high levels of ferritin. It is necessary to add the 25OH D3 test to the routine check-ups of healthy persons as well as the routine tests applied to BTM patients.

P.09-054-Wed
Risk of a spontaneous bacterial peritonitis in cirrhotic patients with diabetes, with high serum endocan levels

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The presence of diabetes mellitus (DM) in patients with cirrhosis is associated with an increased risk of spontaneous bacterial peritonitis (SBP), which may represent an increased susceptibility to infections. We aimed to identify the endocan as risk factor for development of decompensation event (i.e. SBP) to optimize stratification for primary prophylaxis and therapeutic strategies to improve survival. 84 diabetic subjects with compensated and decompensated liver cirrhosis were recruited. At the time of enrolment, the first SBP incident was diagnosed in 20 decompensated cirrhotic patients with diabetes. The 64 compensated cirrhotic patients with DM were followed up for the occurrence of SBP. Median follow-up was 7.7 (interquartile range, 3.6–9.2) years. Endocan levels were determined by ELISA analyses (JDIEK H1) (Lunginnov SAS, Lille, France). Diabetic patients who developed the first SBP incident during follow-up showed significantly higher levels of endocan than SBP-free patients. The best cut-off of endocan levels was 2.07 ng/mL. At this cut-off, χ^2 analysis revealed that endocan level was significantly different between the SBP-free compensated cirrhotic patients with DM and cirrhotic patients with DM who developed SBP in follow-up. While we could confirm traditional risk factors for SBP development, such as the presence of hyponatremia or MELD, we were also able to identify a moderately elevated endocan level as predictor of SBP incident development in compensated cirrhotic patients with diabetes.

P.09-055-Mon
Strain-specific effect of AMP-activated protein kinase on glucose oxidation in mice

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Differences in susceptibility to obesity and glucose intolerance in various mouse models offer a possibility to characterize novel targets for the treatment of obesity and related diseases, such as type-2 diabetes. AMP-activated protein kinase (AMPK) is a heterotrimeric protein complex that plays a key role as an energy sensor regulating energy homeostasis in cells. Once activated, it stimulates ATP-producing pathways like glucose uptake and β-oxidation and inhibits lipolysis and lipogenesis. We aimed to detect possible differences in glucose tolerance and metabolic flexibility between A/J and C57BL/6J (B6) murine strains, resistant or prone to a high-fat induced obesity, respectively, and mice with a whole-body deletion of AMPK a2 subunit (AMPKa2-KO) mice on A/J or B6 background. Mice were characterized at 4 weeks of age, e.g. at a time of weaning onto a high-carbohydrate diet. Glucose tolerance test was performed using a dose of 1 mg of glucose per g of body weight (mg/g), administered either intraperitoneally or orally. For assessment of a whole-body metabolic flexibility, we introduced an indirect calorimetry (INCA) protocol at thermoneutral temperature (34°C) including glucose delivery at 7.5 mg/g via gastric gavage. A/J mice showed lower mean glucose levels and lower glucose tolerance as well as faster increase in respiratory quotient (RQ) in response to glucose than B6 mice. AMPKa2-KO A/J mice displayed higher RQ after a glucose gavage suggesting higher *de-novo* lipogenesis on whole-body level as compared to their wild type littermates, while this effect was not detected in AMPKa2-KO B6 mice. We have detected higher glucose tolerance and metabolic flexibility in obesity-resistant A/J mice as compared with obesity-prone B6 animals. Deletion of AMPK activity had an expected effect on *de-novo* lipogenesis on A/J background only, suggesting the key role of AMPK in the lean phenotype. Funding: Czech Science Foundation (14-36804G).

P.09-056-Tue
The characteristics of Von Willebrand domain-containing protein 8

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Von Willebrand Domain-containing Protein 8 (Vwa8) has been recently described to be a mitochondrial protein. The protein possesses a mitochondrial targeting sequence, AAA+ ATPase dynein related domain, P-loop containing nucleotide triphosphate

hydrolase domain and Von Willebrand factor type A domain. Vwa8 has experimentally proven ATPase hydrolytic activity and its gene was found methylated in acute myeloid leukemia. Despite the recent progress in the Vwa8 annotation its function and physiological relevance remains unknown. To determine the role of Vwa8 in mitochondrial physiology, we developed several approaches to manipulate Vwa8 protein levels. We introduced Vwa8 siRNA to reduce its protein levels and vectors for Vwa8 overexpression with and without flag. Using the control mouse embryonic fibroblasts (MEF) and MEFs containing overexpressed form of Vwa8, we performed cell and mitochondrial fractionation to reveal exact localization of the protein inside mitochondria. In all of the mentioned models (controls, Vwa8 overexpression and knock-down) we checked mitochondrial morphology and tested the levels of selected mitochondrial proteins to reveal the role in mitochondrial dynamics and potential Vwa8 substrates and interactors. Taken together, we are going to present precise localization of Vwa8 within mitochondria and relation between selected mitochondrial proteins and Vwa8. We will also discuss the potential function of Vwa8, which according to the containing domains might be a protease, chaperon or a member of mitochondrial dynamin family.

P.09-057-Wed
Natural bispecific antibodies in norm and pathology

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Natural antibodies presented in human blood are considered as stable monospecific molecules with two identical antigen-binding sites. Exchange of Fab arms (HL-fragments) resulting in the generation of bispecific antibodies was first described only for IgG4 subclass. The mechanism of Fab arms exchange was described in details. We have shown that human milk antibodies contain up to 54% of chimeric bispecific κλ-IgG and up to 17% of κλ-sIgA. Interestingly, bispecific κλ-IgG are presented by 74% of IgG1, but not the IgG4. We have shown that natural placenta IgG undergoes extensive Fab arms exchange and contain up to 15% of κλ-IgG. Chimeric placenta bispecific IgGs were presented mostly by 43.5% IgG1 and 41.0% IgG2. Also, we have shown that the blood of healthy donors contains in average 8% of κλ-IgG, most of which are IgG1 (25%) and IgG2 (51%). The relative content of chimeric IgGs in placenta and blood of healthy donors is significantly lower than that of the milk. One can explain this due to a lower content of protein factor(s) stimulating the HL-fragments exchange comparing with milk. Here we show that the blood of autoimmune patients contains significantly higher concentrations of bispecific κλ-IgG molecules than in healthy donors. Since that we propose that the presence of bispecific antibodies in the serum of autoimmune patients is a new biochemical marker of these disorders. The study was funded by RFBR, according to the research projects 16-34-60066 mol_a_dk, 16-04-00603-a, 16-04-00604-a and with the grant from Ministry of Education and Science MK-410.2017.4.

P.09-058-Mon
The tACE/Angiotensin (1-7)/Mas axis protects against testicular ischemia reperfusion injury

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To investigate whether exogenous angiotensin (Ang)-(1-7) administration can protect against the damaging consequences of testicular ischemia reperfusion (tIR) injury. Eighteen male Sprague-Dawley rats were divided equally among the following three groups: sham, unilateral tIR injury (1 hour of ischemic treatment and 4 h of reperfusion), and tIR + Ang-(1-7) (0.3 mg/kg). Testicular tissues obtained from the rats were evaluated for the expression of testicular angiotensin-converting enzyme (tACE), Ang-(1-7), and the Ang-(1-7)-specific receptor Mas by immunohistochemistry and enzyme-linked immunosorbent assay. Reduced spermatogenesis, induction of the caspase-8 pathway, and nitric oxide (NO) generation were assessed. The effects of tIR and Ang-(1-7) treatment on the PI3K/Akt antiapoptosis pathway were also investigated. Testicular morphological changes and reduced spermatogenesis associated with decreased expression of the tACE/Ang-(1-7)/Mas axis were observed during tIR. These effects were also accompanied by increased activity of caspase-3 and -8, down-regulation of the survivin and BAD transcripts, and decreased NO formation. During tIR, PTEN expression was increased, leading to inactivation of the PI3K/Akt pathway. Acute treatment with Ang-(1-7) prior to reperfusion attenuated the tIR-induced damage described above. Expression of the tACE/Ang-(1-7)/Mas axis was down-regulated during tIR. Administration of exogenous Ang-(1-7) prior to reperfusion rescued tACE and Mas expression and protected against germ cell apoptosis and oxidative stress. Increased NO generation and activation of the PI3K/Akt signaling pathway may have partially contributed to these effects. The tACE/Ang-(1-7)/Mas axis likely plays a role in the maintenance of normal testis physiology and spermatogenesis. This study was supported by grants MB 01/15 and SRU02/13 from Kuwait University.

P.09-059-Tue
Changes in elemental status and biochemical parameters under the influence of nutrition stress

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The aim of the work was to study the effect of fast food and instant products on the metabolic parameters and elemental composition of the liver of laboratory animals. The study was conducted on male Wistar rats from two months of age. During the experimental period (8 weeks), the animals were divided into 2 groups. The first experimental group consumed a semi-synthetic diet consisting of a mixture of the main food (50%), fast food and instant food (50%) and water, the second (control) group consumed main diet and water. The elemental composition of the liver was determined in the laboratory of the ANO "Center for Biotic Medicine" (Moscow) by atomic emission methods and mass spectrometry with inductively coupled argon plasma. It was found that a diet containing fast food and instant food products significantly affects the content of essential and toxic liver elements, biochemical parameters of blood, hormonal status, and the weight of laboratory animals. Significant decrease in

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chromium (1.66 times), manganese (3.6 times), selenium (1.21 times), silicon (3.6 times) and zinc (1.25 times) content was determined. Iodine content was reduced by 1.4 times, also concentration of triiodothyronine in the blood of the animals in the experimental group was significantly lower by 3 times. Imbalance of essential elements was accompanied by the accumulation of toxic elements. So, the aluminum content significantly increased by 3.9 times, cadmium - by 2 times. Significantly lower total protein values and greater activity of aspartate transaminase and alanine transaminase in serum of rats were found. In addition, the weight of the animals of the experimental group statistically significantly lagged behind the control group at the end of the experimental period by 35.5%. The research was supported by the Ministry of Science and Education of the Russian Federation, project No. 17.4638.2017 / 8.9.

P.09-060-Wed
Correlation between prostate tissue galactose-specific lectins and the gland pathology

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The changes in the biochemical characteristics of galactose-specific lectins from subcellular fractions of the post-operative specimens from the different clinical grades of prostate gland pathology has been studied. The galactose-specific lectin extracted from mitochondria is a 35 kDa protein (SDS-PAGE). Using the Galectin antibodies, mitochondrial fractions of tissues with the diagnosis of BPH, HGPIN and AAH revealed presence of Gal-3. Galactose-specific lectins of different molecular weights were detected in cytoplasmic fractions from different cases, among them Gal-3 too. It was shown that mitochondrial and cytoplasmic lectins have different effects on viability of peripheral blood lymphocytes from healthy person and leukemia MEC1 cell line. No changes were observed in case of mitochondrial lectins on healthy lymphocytes. Cytoplasmic lectin from HGPIN and PC tissue significantly decreased the viability of healthy lymphocytes, while in MEC1 line the survival level was decreased by lectins from all cases including norm and except AAH derived one. In addition, it was studied the influence of PC and HGPIN (precursor for development of tumors) cytoplasmic lectins on the apoptosis. The cell cycles were studied by flow cytometry at incubation time 0/24/48/72 h and the number of pre-apoptotic and apoptotic cells were counted. The apoptotic cells were observed within 24 hour timeframe, while pre-apoptotic ones started to be formed after 48 h of incubation with PC-derived lectin. In contrast, HGPIN-derived lectin prevailed the formation of apoptotic cells under the incubation for 48 h. Incubation of MEC1 lineage lymphocytes with either of the above mentioned lectins results in amplification of production of pre-apoptotic cells. To sum up, it could be concluded that galactose-specific lectins from cytoplasm and mitochondria of prostate gland are somehow involved in provoking apoptosis and this action is dependent on the grade of the disorder.

P.09-061-Mon
Effects of α-lipoic acid on tissue oxidative stress parameters in experimental hypothyroidism

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The purpose of the study was to investigate the effects of α-lipoic acid (ALA) on prooxidant-antioxidant balance in liver, kidney and brain tissues, as well as liver and kidney function tests in experimental hypothyroidism. For the evaluation of prooxidant-antioxidant balance, reactive oxygen species (ROS), malondialdehyde (MDA), protein carbonyl (PC), ferric reducing antioxidant power (FRAP), glutathione (GSH) levels, and superoxide dismutase, catalase and glutathione peroxidase activities were measured. Histopathological examinations were also fulfilled. Hypothyroidism was induced by the administration of propylthiouracil (PTU, 500 mg/L) in drinking water for 10 weeks. The ALA [100 mg/kg/day; % 0.2 (w/w) in diet] was administered in last 5 weeks of experimental period. Increased oxidative stress in brain tissue of hypothyroid rats was observed. Significant increases ROS, MDA, PC levels were found in brain of hypothyroid rats. Also SOD activity decreased. ALA treatment lowered ROS, MDA, PC, GSH levels, however the decrease in ROS levels was not statistically significant in brain tissue. Effects of oxidative stress on Liver and kidney tissues were not found. ALA treatment significantly reduced ROS levels in the liver, increased FRAP and GSH levels in the kidney. Serum liver function test did not alter otherwise urea and creatinine levels increased in hypothyroid rats. ALA administration resulted in a significant increase in ALT activity. Our results indicate that ALA treatment is effective in the improvement of the changes in prooxidant-antioxidant balance and may be useful as supportive agent for the treatment of hypothyroidism.

P.09-062-Tue
Oxidative pathway - one of the mechanisms of biological action of L-lysine α-oxidase

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L-amino acid oxidases were shown to have antiviral, antifungal, antibacterial, and anticancer activity. L-lysine α-oxidase (LO) differs from other enzymes of this group due to its unique property to oxidize preferentially the essential amino acid L-lysine. Initially various biological effects of LO were explained only by the degradation of L-lysine. But later the experimental evidences were obtained that some other mechanisms of LO action may exist. As H2O2 is formed in the reaction, which is catalyzed by LO, a possible role of reactive oxygen species (ROS) in realization of LO effects was the subject of investigation in this work. LO with specific activity 98 U/mL was purified from *Trichoderma* cf. *aureoviride* Rifai VKMF-4268D. Rat pheochromocytoma PC-

12 was used as a model cell culture. Dead cells in the population were stained by propidium iodide and the percentage of dead cells was measured by flow cytometry. Incubation of cells in the presence of 5 mM H2O2 for 2 h showed their sensitivity to H2O2; the maximum level of ROS in cells was observed after 15 min, while the maximum cell death - only after 2 h. The appropriate concentrations of LO for investigation of ROS role were determined. Incubation of cells in the presence of LO (4.2×10^{-4} and 8.4×10^{-4} μM) for 15 min increased the ROS level 3 and 8 times compared to the control and then continued to grow, after 1 h incubation maximum amounts of ROS were observed, exceeding the control 6.5 and 15.3 times; later ROS concentrations gradually decreased. The dependence of cell death on LO concentration was demonstrated. The increase in intracellular reactive oxygen species detected by the 2,7-dichlorodihydrofluorescein coincides in time with the death of cells. This result suggests that the oxidative pathway is one of mechanisms underlying the cytotoxic LO action. The publication was prepared with the support of the "RUDN University Program 5-100".

P.09-063-Wed
Stabilization of papain by immobilization on chitosan

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Enzymes of medical appointment become growingly important in the last decades in view of increasing resistance of pathogenic microorganisms to antibiotics of various classes. Enzymes can be used not only as antimicrobial agents, but also as anti-inflammatory and antiedematous means. Water-soluble enzymes have a number of essential disadvantages. Free enzymes are subject to microbial and fungal degradation, they are quickly inactivated at high temperatures and extremely acidic or alkaline environments, and can be hydrolyzed by proteases of the patient organism. Similar disadvantages are removed by a biocatalyst immobilization on the insoluble carrier. Chitosan perfectly proved itself in the field of medicine and veterinary science due to antimicrobial, antitumor and immunomodulatory action. The aim of this study is to produce a series of papain preparations immobilized on a various type of chitosan for enzyme stabilization during storage and application. A virtual screening of high-affinity carriers for immobilization has been performed using computer modelling. Based on the comparative analysis of the total energy and the localization of the ligand binding sites, some assumptions about the enzyme interactions with suggested carriers were made. A number of heterogeneous enzyme preparations have been offered and the structural features of these complexes were predicted. The papain adsorption on medium-molecular (200 kDa) and high-molecular (350 kDa) chitosans allowed preserving up to 70% of the catalytic activity. The most suitable buffer system for immobilizing the enzyme on acid-soluble chitosans is a glycine buffer in the pH range of 8.6–10.0. Being immobilized on chitosan, the enzyme stability was increased by 10 times as compared with the soluble protein. The papain, sorbed on the chitosan matrix, was more thermally stable at 70 °C than its soluble form. We acknowledge the support from Russian Ministry of Education and Science (Grant No. 3.1761.2017/4.6).

P.09-064-Mon
Fatty acid synthesis activation protects brain tissue from ischemic stroke

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Brain hypoxia is one of the major factors for loss of neuronal functions and viability in many disorders, including ischemic stroke and heart disease - the leading causes of death and long-term disability. Our goal is to understand the mechanism underlying neuron adaptation to hypoxia to outline potentially new therapeutic targets and approaches for treatment of brain hypoxic conditions. To this end, we have identified in cultured cell lines and primary cells a novel, neuron-specific protective response to hypoxia through a dramatic activation of fatty acid (FA) synthesis from glutamate (Glu) and glutamine (Gln). This suggests that activation of this mechanism under hypoxia supports neuronal anaerobic metabolism by attenuating the level of Glu and its downstream excitotoxicity, while also balancing cellular reduction potential. To validate these findings in vivo, we utilized an animal model for ischemic stroke. Fatty acid synthase inhibitors significantly increased basal brain glutamate levels. Upon fatty acid inhibitor treatment, infarct volume, edema, neurological deficiency, and reductive potential were significantly increased upon middle cerebral artery occlusion, but had no effect in the sham control animals. These results are consistent with our previously published in vitro data and indicate a novel protective role for fatty acid synthesis under hypoxia through attenuation glutamate excitotoxicity, normalization reductive potential, thus supporting anaerobic energy metabolism.

P.09-065-Tue
Inhibition of histone deacetylase attenuates development of type I diabetes through reduction of oxidant stress

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Pancreas is vulnerable tissue to oxidative stress because of its lower expression level of antioxidant enzymes than other tissues. Thus, pancreas is weak to Streptozotocin (STZ) which increases reactive oxygen species (ROS) through several pathways such as damage to DNA and mitochondria, excess nitric oxide generation and xanthine oxidase. We hypothesized that HDAC11 inhibition induces antioxidant enzymes such as catalase, superoxide dismutase (SOD), and glutathione reductase (GR) which results in resistance to oxidative stress in the pancreas. Change of cell morphology and viability was investigated by IncuCyte and MTT assay respectively. Expression of antioxidant enzymes was analyzed by qRT-PCR. To establish type I diabetes animal model, STZ (40 mg/kg) was injected intraperitoneally. MGCD0103 (2 mg/week) was infused with subcutaneous osmotic mini-pump for one week. STZ treatment decreased cell viability in a dose-dependent manner in several cell lines. INS-1E was the most sensitive to STZ compared with THP-1, HepG2, and HEK293. MGCD0103 treatment protected from STZ-induced INS-1E cell death, disruption of mitochondrial membrane potential, and intracellular ROS generation. MGCD0103 increased the expression of antioxidant enzymes such as catalase (Cat), glutathione peroxidase (Gpx), glutathione reductase (GlutR), and superoxide dismutase (SOD) in INS-1E cells. STZ injection increased the blood glucose level which was inhibited by MGCD0103. STZ destroyed the structure of pancreatic islets, which was partially restored by MGCD0103 infusion. MGCD0103 infusion induced

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expression of SOD1, 2, and 3 in the pancreas. The present study demonstrated that HDAC11 inhibition induces antioxidant enzymes which results in resistance to oxidative stress in the pancreas.

P.09-066-Wed
Influence of passive smoking on some biochemical characteristics of amniotic fluid

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The aim of the research was to study the effect of passive smoking on biochemical characteristics of amniotic fluid of laboratory animals. The study was carried out on pregnant female Wistar white rats (n = 40), weighed 130 ± 10 g at the beginning of the experiment. Animals were divided into two groups: experimental and control. The animals of the experimental group underwent passive smoking in the seed chamber for 30 min 2 times a day throughout the pregnancy. Each animal received 0.048 mg of nicotine per day. The control group consisted of intact animals. The obtained data indicated that exposure to tobacco smoke led to changes in the biochemical composition of amniotic fluid of rats. Alkaline phosphatase significantly increased by 33% in experimental group. Such increase could be observed during placental insufficiency, fetal hypoxia, gestosis. In addition, in the experimental group, the level of creatinine was significantly, by 28.1% higher than in the control group. A high content of creatinine in the amniotic fluid could be accompanied by intrauterine growth restriction, acute and chronic fetal hypoxia. Also, a significant increase in urea by 41.5% and glucose by 38.9% was detected in the group of animals who underwent passive smoking. A high urea concentration could be observed in chronic fetal hypoxia and intrauterine growth restriction. Concentration of iron in the amniotic fluid of experimental animals was significantly lower by 74.2%. This microelement is necessary for the construction of hemoglobin and myoglobin, is an integral part of cytochromes and oxidation-reduction enzymes. Thus, our analysis of the biochemical characteristics of the amniotic fluid testifies the toxic effect of the studied factor, which can lead to placental insufficiency and fetal distress, accompanied by the development of hypotrophy and hypoxia.

P.09-067-Mon
Alteration of drug pharmacokinetics with medicinal plant *Viscum album* L. by rendering major cytochrome P450 activities

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Medicinal plant mistletoe *(Viscum album L.)* have been traditionally used as anti-hypertension, coagulant, analgesic, cardiotonic, and anti-cancer agent in Anatolia, Turkey. In addition, extracts of *Viscum album* have been used as prescription drugs in Europe while considered as a dietary supplement in the US. Thus, potential drug interactions have turned into a noteworthy concern in mistletoe treatment. The present study was undertaken to study the *in vivo* effects of *Viscum album L.* on crucial drug-metabolizing P450 enzyme activities in rat liver. The water extract of *Viscum album* was injected intraperitoneally (i.p.) into 15 Wistar albino rats as 10 mg per kg of body weight for nine consecutive days, while 15 rats were used as a control. Then, following the decapitation of the rats, the livers were removed, and microsomal fractions were prepared. Cytochrome P450 activities were determined using aminopyrene (CYP2C9), benzyloxyresorufin (CYP2B), caffeine (CYP1A2), dibenzylfluorescein (CYP19), erythromycin (CYP3A4), ethoxyresorufin (CYP1A1), methoxyresorufin (CYP1A2), pentoxyresorufin (CYP2B) as substrates. The results showed that the extract of *Viscum album* decreased all the tested CYP activities significantly at varying degrees between 20–40% except the CYP2C9 (aminopyrine) activity, which was increased about 2-fold with respect to control. These are the major contributing CYP isoforms for the biotransformation of the drugs in the liver; hence, the therapeutic index and the pharmacokinetics of many prescribed medicine would be changed when the mistletoe is combined with other anti-cancer drugs. In conclusion, clinically critical herb-drug interactions are expected with *Viscum album.*
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P.09-068-Tue
Effects of radiation on activity of metabolism enzymes of purine nucleotides in the experiment

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The change of metabolism of purine nucleotides in immunocompetent cells determines its functional activity state. Researches of purine exchange ferments provide new opportunities for the biochemical approach to treatment of patients with deficiency of enzymes, which will be facilitated by the studying of its functioning on a molecular level after an organism has had radiation poisoning. To understand mechanisms of adaptation reaction, researching of enzymes of purine exchange is of particular interest. The aim of our research was to study the effects of radiation on enzyme activity of the purine nucleotides metabolism – 5'-nucleotidase (5'-NT), adenosinedeaminase (ADA), adenylatedeaminase (AMP-DA) in different organs and tissues within the experiment. To attain the goals established 2 series of tests were conducted on laboratory rats: inactive (gr.I) and exposed to radiation (gr.II). Animals of group II were exposed to ionizing radiation using Teragam radiotherapy unit in a dose of 6 Gy. During the research it was identified that the activity of 5′-NT in irradiated animals' spleen was lowered by 86.3%, ADA was lowered by 50.9%, AMP-DA tended to decrease. 5'-NT activity in lymph nodes of small intestine among animals of group II was lowered by 39.1%, ADA was lowered by 34.3%, AMP-DA – by 20.1%. 5'-NT activity in thymus of irradiated animals was lowered by 40.41%, a ADA - by 34.2% and AMP-DA – by 20.1%. The results of research showed that 5′-NT and ADA enzymes activity in liver did not change in comparison with the inactive group, and the activity of AMP-DA was increasing by 80.1%. Exposure to radiation causes significant dysfunctions of purine exchange enzymes, which characterizes the exertion of an organism's compensatory/adaptive mechanisms. 5′-NT is a marker enzyme of plasma membrane, when its activity lowers adenosine is taken up in insufficient amount, which leads to cell's dwindling energy resources.

P.09-069-Wed
Characterization of AJ-compound as a novel peripheral cannabinoid 1 receptor antagonist in mouse models of obesity

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Obesity-induced adipose tissue inflammation, in which the NLRP3 inflammasome is a pivotal mediator, is implicated in the development of insulin resistance. Cannabinoid 1 receptor (CB1R) antagonists have been shown to improve insulin resistance and the associated metabolic abnormalities, but their therapeutic development was discontinued due to neuropsychiatric side effects. Although growing evidence suggests the role of CB1Rs in proinflammatory signaling, their direct effects on adipose tissue inflammation have not yet been evaluated. Here we report that the peripherally restricted CB1R antagonist AJ-compound, which exhibits beneficial metabolic effects comparable with its brain-penetrant parent compound rimonabant, suppresses macrophage infiltration into white adipose tissue, activation of the NLRP3 inflammasome, and production of proinflammatory cytokines in diet-induced obese mice. Moreover, we identified the downstream signaling pathways by which CB1R regulates proinflammatory gene expression. These results suggest that peripheral CB1R blockade improves obesity-induced insulin resistance by suppressing adipose tissue inflammation via the NLRP3 inflammasome.

P.09-071-Tue
Apoptosis in *Acanthamoeba* induced by amoebicidal agents

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Several chemotherapeutic drugs have been described as amoebicidal agents acting against *Acanthamoeba* trophozoites and cysts. However, the underlying mechanism of action is poorly characterized. Here, we describe apoptosis in *A. castellanii* induced by polyhexamethylene biguanide (PHMB) and chloroquine. We used four types of amoebicidal agents including 0.02% PHMB, 0.02% chlorhexidine digluconate, 100 μM chloroquine, and 100 μM 2,6-dichlorobenzonitrile to kill *Acanthamoeba* trophozoites and cysts. Exposure to PHMB and chloroquine induced cell shrinkage and membrane blebbing in *Acanthamoeba,* observed microscopically. Externalization of phosphatidyl serine on the membranes of *Acanthamoeba* was detected by annexin V staining. Apoptotic cell death of *Acanthamoeba* by PHMB and chloroquine was confirmed by FACS analysis. Nuclear fragmentation of *Acanthamoeba* was demonstrated by DAPI staining. PHMB induced apoptosis in trophozoites and cysts, and chloroquine induced apoptosis in cysts. These findings are discussed to establish the most effective treatment for *Acanthamoeba*-induced keratitis.

P.09-072-Wed
Chemical chaperone – TUDCA restores ER homeostasis and alleviates inflammation in human articular chondrocyte cell line NHAC-kn treated with IL-1β and tunicamycin

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Chondrocytes, the only resident cells in cartilage, are prone to develop various stresses during osteoarthritis (OA) progression. Endoplasmic reticulum (ER) stress is a hallmark of many diseases, and is also identified as a factor perturbing chondrocyte functioning. Additionally, ER stress has been known to be connected with inflammatory response - crucial event in OA pathology. Recent findings prove good efficiency of chemical chaperones in alleviation of ER stress in different cell types. Tauroursodeoxycholate (TUDCA) and 4-phenylbutyrate (PBA) are one of the best studied chemical substances displaying chaperoning activity and demonstrating good efficacy in restoring ER homeostasis. Herein, we present the effects of TUDCA and PBA on NHAC-kn cells stressed with two different stimuli, proinflammatory cytokine IL-1β and classical ER stressor–tunicamycin. Studies were conducted simultaneously in normoxic and hypoxic conditions. We demonstrated that IL-1β and tunicamycin decreased proliferation of KHAC-kn cells, as shown by MTT test. Moreover, in normoxic conditions both treatments evoked ER stress, as confirmed by increased expression of ER stress markers GRP78 and GRP94, as well as up-regulated expression of *Chop*. Surprisingly, both treatments had little or no effect on apoptosis as evidenced by flow cytometry analysis. RT-qPCR analysis revealed markedly elevated levels of *Il-1*β, *Cox2* and *NfκB* in stressed chondrocytes. Additionally, collagen II expressions were decreased under ER stress in normoxia. TUDCA supplementation improved cell proliferation, markedly reduced the levels of ER stress and inflammation markers and restored collagen II expression, while PBA exerted no beneficial effects. Altogether, these results suggest that TUDCA might be considered as a pharmacological agent presenting potentially therapeutic values for mild OA. This study is supported by Polish National Science Center grant no. UMO-2015/17/N/NZ7/01094 (to MK).

P.09-073-Mon
Lactonase gene aiiA in *Bacillus cereus* ATCC 14893

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The presence of *aiiA* gene, encoding an N-acyl homoserine lactonase (EC: 3.1.1.81) that cleaves the lactone ring from AHL is identified for some species of *Bacillus*. This quorum quenching mechanisms leads to bacteria's virulence decrease. The aim of the study was to determine lactonase activity in probiotic strain *B. cereus* ATCC 14893 ("Bactisubtil", France). *Chromobacterium violaceum* NCTC 13274 and *E. coli* JLD271 pAL103 responding to N-hexanoyl-L-homoserine lactone by pigment production or bioluminescence, respectively, was used in the work. A high lactonase cell activity of *B. cereus* ATCC 14893 was identified; it manifests in an 8-fold increase in the concentration of AHL to achieve EC50 pigment production. Using the luminescent biosensor, suppression of autoinducer activity in range 0.001–1 μM was revealed. Sequencing of the preamplified gene fragments let to identify 704 nucleotides exhibiting 99% similarity to *aiiA* gene encoding lactonase in *B. toyonensis* BCT-7112 strain ("Toyocerin", Spain). This strain was previously positioned as *B. cereus* and only recently was assigned to another species, but it belongs to the same group. The isolated *aiiA* gene was cloned into the plasmid pGEM-Easy, using which competent *E. coli* XL-1 Blue cells were transformed. The resulting *E. coli* pAiiA strain inactivates N-hexanoyl-L-homoserine lactone, it is expressed in pigment product decrease of *C. violaceum* NCTC 13274 for 5 times. The work resulted in the analysis of nucleotide sequence of lactonase gene *B. cereus* ATCC 14893 and its cloning in

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E. coli cells, retaining more than half of the initial enzyme activity. The proposed approach of searching for lactonase genes in the used probiotic strains will make it possible to create pharmacological preparations with a new principle of action on their basis, they will block intercellular communication. The research was supported by a grant from the Russian Science Foundation (project No. 16-16-10048).

P.09-074-Tue
HDAC8 regulates neural differentiation through embryoid body formation in P19 cells

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Histone acetylation and deacetylation are epigenetic processes that regulate gene expression, and in turn, cellular function. Histone acetyl transferases transfer acetyl groups to lysine residues on histones causing relaxation of the chromatin structure because of reduced interaction between DNA and histones. Histone deacetylases (HDACs) are a group of enzymes responsible for deacetylation of lysine residues on histones and non-histone proteins. Deacetylation of histones alters chromatin structure, thereby regulating downstream gene expression and subsequently many cellular processes. However, as a member of the HDAC family, HDAC8 function during neurodevelopment is currently unknown. Therefore, we investigated HDAC8 function during neurodevelopment by examining embryoid body (EB) formation in P19 cells. HDAC8-selective inhibitor (NCC-149) (HDAC8i)-treated cells showed smaller EBs than non-treated cells, as well as reduced expression levels of the neuronal marker, NeuN. Additionally, HDAC8i treatment led to inhibition of cellular proliferation by G2/M phase accumulation and downregulated cyclin A2 and cyclin B1 gene expression. Furthermore, several cadherin gene expression changed by HDAC8i treatment. Lastly, two independent *HDAC8* knockout cell lines were established by CRISPR-Cas9, which resulted in smaller EBs, similar to HDAC8i-treated cells. These results suggest that HDAC8 regulates neural differentiation by exerting control of EB formation.

P.09-075-Wed
Role and regulation of microRNAs in aldosterone metabolism

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Naturally occurring, small non-coding, single-stranded RNA molecules; microRNAs (miRNAs) downregulates gene expressions via binding to the 3′-untranslated region(3′-UTR) of target mRNA and regulate expression of many genes including cancer and cardiovascular diseases. Hypertension is one of the most common disorders (40%) and possesses the highest death incidence(51%) in the world. The aim of this study is to the identification of the interaction between aldosterone synthesis and miRNA expression and effects of miRNAs on the certain components of signaling pathways of aldosterone and its receptors. Aldosterone-induced (ALDO) rat models were generated via injecting of 0.75 μg/kg/hr of aldosterone with osmotic minipump. Expression of miRNAs and mRNAs were measured via miRNA array and microarray, respectively. Validation of the role of miRNAs in gene expression was accomplished via using miRNA mimic and inhibitors in human adrenal gland, NCI-H295r cells. Aldosterone injection was increased the blood pressure from 118 ± 9 mmHg to 164 ± 2 mmHg ($P < 0.0001$). 68 miRNAs' expression and 2705 mRNAs' expression were altered. Aldosterone biosynthesis related mRNA and protein expressions of CYP11B1, CYP17A1, CYP11A1, CYP21A2, AGTR1, AGTR2, NR5A2, NR3C2, MC2R HSD3B2, THOP1, ANG1, MAPK3, HSD11B1, ERK, ELK, and LNPEP were measured by qRT-PCR, western blot/immunohistochemically. Especially, CYP11A1 (3-fold), CYP11B2 (3.3-fold) and CYP21A2 (1.6-fold) mRNA expressions were upregulated, CYP11B1 (36%) and CYP17A1 (47%) mRNA expressions were downregulated in NCI-H295r cells. Inhibition of hsa-miR-187-3p expression with miRNA mimic was increased expression of CYP11A1 (18-fold), and CYP17A1 (4.5-fold). CYP21A2 mRNA and protein expressions were increased 12-fold after transfected with hsa-miR-128-1-5p inhibitor ($P < 0.0001$). This study clarifies the molecular mechanism of aldosterone synthesis and potential marker in the regulation of hypertension. TUBITAK supported (114Z734).

P.09-076-Mon
Demonstration of formaldehyde toxicity on renal tissue with oxidative markers

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Formaldehyde (FA) is a harmful compound, medical professionals and students are often exposed to it. We aimed to investigate Thioredoxin-interacting protein (TXNIP), Nuclear factor- κB (NF-κB p105), Reduced Glutathione (GSH), Malondialdehyde (MDA) levels due to the possible toxic effects in renal tissue caused by formaldehyde. This study is approved by the Kocaeli University Animal Experiments Local Ethics Committee (Project number: 6/1-2016). Six weeks old, 16 female Wistar albino rats were divided into two groups: the control group and exposed group (10 ppm FA) for a total of 60 days, 4 h/day, five days a week. TXNIP and NF-κB p105 levels were examined by ELISA kit. The GSH and MDA levels were measured with the spectrophotometric method. Statistical analysis was done with IBM SPSS 20.0 version. Groups were compared with non-parametric Mann Whitney-U test. Serum MDA level shows a significant increase in the exposed group compared to the control group ($P = 0.002$). Renal GSH level shows a significant decrease in the exposed group compared to the control group ($P = 0.015$). There was a positive and strong correlation between tissue MDA and NF-κB p105 levels ($P < 0.001$). In this research, it is shown that administration of formaldehyde via inhalation to rats doesn't affect particular parameters on the apoptotic pathway whereas it alters the oxidant/antioxidant system.

P.09-077-Tue
Potentiometry in the analysis of organic acids in medicinal plants and food products

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Organic acids are involved in the regulation of the metabolism of plants and animals. Bioavailability and biological activity of organic acids depend on the chemical form (ionized, molecular).

In this regard, it is necessary to know not only the acid content, but also the ratio of the molecular and ionic form of the acid. We have developed a methodic of potentiometric titration to determine the content of organic acids. This method has a number of advantages, since it does not require dilution of intensely colored extracts and excludes random errors associated with visual fixation of the equivalence point, as when using the indicator. The methodic was tested and validated for a number of herbal medicines: rose hips, guelder rose, mountain ash, black currant, syrups, infusions and tinctures based on them et el. It has been found that the potentiometric titration methodic show well reproducible and accurate data. Direct potentiometric titration is possible when the pH value of the sample does not exceed 5.5. In opposite cases, reverse titration can be used. To derive an equation describing the curve of potentiometric titration of a solution containing a mixture of several single- and dibasic weak acids, the following regularities were used: A)Equations of chemical equilibria existing in solution; B)Equations of material balance according to the forms of acid that make up the system; C) Equation expressing the electroneutrality of the solution. As a result, an expression was obtained in which the parameters determined as a result of titration (the concentration of acids and cations in the initial solution) linearly enter. The statistical estimation of these parameters from the experimental data can be obtained by the method of least squares, which greatly simplifies the calculations. A computer program was written based on the mathematical model. This publication was supported by the Ministry of Education and Science of the Russian federation (the Agreement № 02.A03.21.0008).

P.09-078-Wed
Modulatory role of mucin in activation of blood neutrophils by *Escherichia coli* in vitro

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Previously we showed that E. coli isolates obtained from the intestinal mucosa of Crohn's disease patients and feces of healthy volunteers as well as the laboratory E. coli DH5alpha strain adsorbed mucin from dilute solution (0.1 mg/mL). Role of mucin in the interaction between E. coli and immune cells is still unknown. Our aim was to investigate whether bacterium-bound mucin influences bacteria-induced activation of normal human blood leukocytes in vitro. One of the patients' isolates was treated with FITC-labeled mucin, and mucin adsorption onto bacterial surface was evidenced by confocal microscopy. Mucin-treated E. coli or non-treated E. coli (control) were added to normal human blood and incubated for 5 and 20 min at room temperature. Leukocyte activation was assessed by luminol chemiluminescence (LCL) of whole blood, morphologic analysis of blood smears, and the amount of myeloperoxidase (MPO) and lactoferrin (LF) was measured in blood plasma by ELISA. Blood LCL after 5-min incubation with mucin-treated E. coli was 80% in respect to probes with control bacteria. $58 \pm 11\%$ of neutrophils appeared morphologically activated in blood incubated with mucin-treated E. coli unlike $86 \pm 7\%$ in control probes. Plasma MPO and LF concentrations after incubation with mucin-treated E. coli were less by 15% and 25% respectively vs probes with control E. coli. Mucin solution per se did not activate leukocytes, at least under experimental conditions used. After 20-min incubation, there was no difference in the effects on leukocytes between mucin-treated and non-treated E. coli. As shown by LCL, both Crohn's patients' (n = 5) and healthy persons' (n = 5) E. coli isolates significantly varied in the extent of mucin binding-mediated effects on leukocytes activation. Probably, in inflammatory conditions mucin adsorbed onto bacteria could play role of endogenous immunomodulator. The research was supported by Russian Science Foundation (16-15-00258).

P.09-079-Mon
Glucagon-like peptide-1 (GLP-1) signaling plays a neuroprotective role against diabetes-related Aβ-induced neurotoxicity via gut-brain axis

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Growing evidence suggests a pathophysiological connection between type 2 diabetes (T2D) and Alzheimer's disease (AD). In particular, insulin signaling blockade represents a common molecular link between these two diseases, suggesting AD may be a brain-specific form of T2D. Interestingly, our previous studies have demonstrated that glucagon-like peptide-1 (GLP-1) can exert a neuroprotective role by against Aβ-induced toxicity. Because of the key role of Aβ in AD pathogenesis, it can be accepted that reducing Aβ-mediated insulin resistance may have promising therapeutic potential. However, the mechanism how insulin signaling influences the onset and progression of AD remains incompletely understood. As GLP-1 is targeted on both central and peripheral tissues, we postulate that GLP-1 may exert its neuroprotective effects by maintaining insulin signaling and metabolic homeostasis in gut-brain axis. To examine how Aβ modulates neuronal insulin signaling and evaluate the potential benefits of GLP-1, we investigated the correlation between insulin signaling and Aβ-induced neurotoxicity by both *in vitro* and *in vivo* models. Our results demonstrated that GLP-1 significantly attenuated Aβ-induced brain insulin resistance and oxidative stress, and the GLP-1-activated gut-brain axis insulin signaling may be involved in against Aβ-mediated neurotoxicity in the brain. Moreover, this protective effect is partly mediated by activation of AMP-activated protein kinase (AMPK), which restores neuronal insulin signaling and leads to upregulation of nuclear factor erythroid 2-related factor 2 (NRF2)/heme oxygenase 1 (HO-1) antioxidant pathway. In conclusion, restoration of brain insulin signaling by targeting GLP-1 may demonstrate important implications to develop novel diagnostic or therapeutic strategies for slowing disease progression of diabetes-related AD.

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P.09-080-Tue
Protective role of polycystin-1 in cardiomyocyte cell death and scar formation during myocardial ischemia/reperfusion injury

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Polycystin-1 (PC1) is a plasma membrane mechanosensor, expressed in the heart. Loss-of-function mutations in the gene encoding PC1 leads to cardiovascular diseases, suggesting its essential role for heart function. This work aims to assess the role of PC1 in cardiomyocyte death and reparative fibrosis-related effects induced by myocardial ischemia and reperfusion (I/R). For this purpose, PC1 knockout (KO) mice were subjected to *in vivo* or *ex vivo* myocardial I/R, and *in vitro* PC1 knockdown (siPC1) in neonatal rat ventricular myocytes (NRVM) subjected to simulated I/R. Infarct size was assessed by triphenyltetrazolium chloride staining, and *in vitro* cell death was evaluated by lactate dehydrogenase (LDH) release and flow cytometry experiments with Annexin V-FITC apoptosis marker. In addition, cell death-related proteins were determined: caspases 3, 8, 9, Bax/Bcl2 levels and FasL expression. NRVM production of profibrotic factors such as TGF-β and CTGF were immunodetected and paracrine effects on cardiac fibroblast α-SMA expression were evaluated using NRVM conditioned media. Results show that PC1 KO mice subjected to I/R exhibit a larger infarct size and higher necrosis than the control group. Also, simulated I/R experiments show that LDH release and protein levels of cleaved caspase-3, 8, and FasL are all increased in siPC1 NRVM. Caspase-9 and Bax/Bcl2 levels were unaffected. Furthermore, the levels of CTGF and TGF-β are reduced in siPC1 NRVM during I/R and the aforementioned conditioned media prevents α-SMA expression, impairing fibroblast to myofibroblast differentiation. Here we show that PC1 deficiency increases I/R-induced cardiomyocytes death, triggering signals that regulate necrosis and the extrinsic apoptotic pathway. In addition, PC1 emerges as a new key regulator of cardiac fibroblast and remodeling response to I/R injury. The work was supported by FONDECYT (3160549; 1150887), FONDAP ACCDiS 15130011.

P.09-081-Wed
Interactions of polymorphisms in TNF-α and antioxidative enzyme genes in bronchial asthma

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Great numbers of single nucleotide polymorphisms (SNPs) are identified in order to explain the genetic background of the complex diseases. However, even if the particular SNPs are important indicators of the genetic background of complex diseases, they explain only a part of the genetic risk. The aim of this study was to examine SNP-SNP interactions in genes for TNF-α and antioxidative enzymes in patients with bronchial asthma (BA) in Serbian population. A total of 79 patients with BA and 85 healthy controls were screened for TNF-α G-308A (rs1800629), CAT A-21T (rs7943316), CAT C-262T (rs001179) and MnSOD Ala16Val (rs4880) SNPs using PCR-RFLP method. SNP-SNP interactions were analyzed using multifactor dimensionality reduction (MDR) method. Among the predicted models, one-locus (MnSOD) and four-locus (TNF-α/CAT -21/CAT -262/MnSOD) model were selected as the best models (testing accuracy 0.63 and 0.55, respectively; cross validation consistency - 10/10). Obtained results showed that MnSOD Val containing genotypes are the risk factors for BA. Moreover, the interaction graph based on entropy measures between the individual variables showed mild positive correlation (1.25%) between CAT A-21T and CAT C-262T SNPs in BA patients which was not influenced by the LD ($r^2 = 0.005$; D' = 0.071). No interaction was observed between TNF-α and catalase, as well as MnSOD and catalase SNPs. Mutant MnSOD Val containing genotypes are high risk genotypes for BA. Catalase A-21T and C-262T SNPs have positive interaction in patients with BA.

P.09-082-Mon
The nano-silver gauze dressing can be a good choice on antibacterial ability and healing acceleration for infectious wound treatment

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Wound infected by bacteria and followed by influencing healing process is a common problem in the field of medicine. The local use of antibacterial dressing is a mainly treatment of infectious wounds, however, the use of antibiotics needs to face the growing problem of drug-resistant bacterial strains. The silver has multiple actions against bacterial strains. But there are still some side effects of silver dressing such as delay epithelialization or pigmentation of wound. Furthermore, the retention of silver on the wound site is not good for wound healing process. In this research, we used the new nano-silver gauze on the treatment for infected wound. Both of the *in vitro* and *in vivo* studies have been performed. In the *in vitro* bacterial inhibition activity test of new nano-silver gauze, the results revealed that the E. *coli* were reduced from 4×10^6 CFU to 39×10^0 CFU, and the S. *aureus* reduced from 4×10^6 CFU to 2×10^3 CFU in 24 h. After *in vitro* experiment, we used rat model to confirm the antibacterial ability and wound healing effect of the new nano-silver gauze. An 1×2 cm incision wound was made on the back of the rat, total amount of 1×10^8 CFU E. *coli* suspension was then seeded on the wound and treated by traditional gauze and new nano-silver gauze. The result showed that the new nano-silver gauze group had lower bacteria count (6.5×10^5 CFU) than the traditional gauze group (4.7×10^6 CFU) at day 2. In the wound healing experiment, the new nano-silver gauze group enhanced the healing process in the first three days. The wound area was smaller in the new nano-silver gauze group (85.5 mm^3) than the traditional gauze group (175.5 mm^3) and the traditional silver gauze group (105 mm^3). In conclusion, the new nano-silver gauze would be an ideal choice for infectious wound treatment, not only provided with antibacterial effect, but also healing facilitation on infectious wound.

P.09-083-Tue
Regulation of apoptosis of human immunocompetent cells under the effect of polyamines

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Apoptosis is the fundamental process of regulating immune system. The aim of the work was to evaluate the ability of various concentrations of polyamines (PA) spermine and spermidine to influence the apoptosis process of human peripheral blood lymphocytes in vitro. The washed blood mononuclear cells of healthy donors (n = 30) were resuspended in a complete nutrient medium (RPMI 1640 with 10% fetal calf serum, L-glutamine, HEPES and gentamicin) and were incubated for 24 h at 37°C in a humidified atmosphere with 5% CO2. The viability of the cells was at least 90%. Solutions of PAs were added into the culture at the beginning of the incubation at concentrations of 10^{-6}, 10^{-5}, 10^{-3} M. Control cells were cultured without PA. Apoptosis was assessed with fluorescein-labeled annexin V and propidium iodide with fluorescence microscopy. It was revealed that the level of spontaneous apoptosis of lymphocytes was 1–4% of apoptotic cells. When the lymphocytes were incubated with PA, the dose-dependence of apoptosis induction was observed: the number of apoptotic cells in the presence of PA in a dose of 10^{-3} M was significantly higher (8–14%) than at a dose of 10^{-5} M PA (4.6–7%), with the most pronounced effect of spermine. The presence of PA in a concentration of 10^{-6} M, close to physiological, does not induce induced apoptosis of lymphocytes, but inhibits it to 2%. The results of the study confirmed the data on the high activity of PA at the cellular level, confirming the specific effects of PA: the similarity of the effects of spermine and spermidine, with more pronounced apoptotic activity of the spermine; multidirectional effect of large and physiological doses of PA. The physiological concentrations of PA produce an antiapoptotic effect, and large doses stimulate apoptosis of lymphocytes. The study was prepared with the support of the "RUDN University Program 5-100" and the Ministry of Education and Science of the Russian Federation (the Agreement No. 02.A03.21.0008)

P.09-084-Wed
Investigation of the immunogenic properties of antitumor enzyme L-lysine-α-oxidase

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This study is devoted to study the immunogenic properties of the antitumor enzyme L-lysine-α-oxidase from trichoderma. Biological experiments were carried out on CBA mice (CBAX C57BI) weighing 18–20 g. The native L-lysine-α-oxidase was administered to C57 BI mice intravenously five times at a dose of 35 U/kg. Weekly, for four weeks from the start of immunization in animals (n = 7), blood was collected and received mouse sera were analyzed by enzyme immunoassay according to a previously developed technique. The sera were cultured in steps of 2. We found that the dynamics of the humoral immune response to the enzyme did not differ from the characteristics of antibody formation in response to the introduction of protein antibodies. The maximum concentration of antibodies was observed in animals on days 7–14 of the experiment. In subsequent terms, a decrease in antibody titers was observed in mice. It should be noted that titers in the enzyme immunoassay are very low, indicating a low immunogenicity of the drug at the tested dose of 35 U/kg or 0.8 mg protein/kg. It is possible to compare the obtained results of the study with the immunogenicity of the antitumor drug L-asparaginase *E. coli*, which was previously studied by us. Five-fold administration of L-asparaginase at a dose of 300 U/kg, which is equivalent to 2.0 mg/kg, leads to the increase in the antibody titer up to 1/256, which significantly exceeds the titers to L-lysine-α-oxidase (1/64). Thus, the intensity of the immune response to L-lysine-α-oxidase does not exceed the L-asparaginase drug, which is permitted for use. The publication was prepared with the support of the "RUDN University Program 5-100".

P.09-085-Mon
Evaluation of apoptogenic effect of a cytostatic agent on male gametes

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Therapy of human cancer almost in 100% of cases leads to infertility, since almost all the "classical" cytotoxic drugs belong to the factors triggering apoptosis of cells. The aim of the study was to investigate, in vitro, the effect on male gametes of betulinic acid (BA) used in experimental regimens for the treatment of melanoma and brain tumors. The relevance of the study is emphasized by the fact that the data on the effect of BA on the process of spermatogenesis in men, is very little. Washed sperm cells of healthy donors (n = 20) were incubated with 60 μg/mL BA for 4 h. Control gametes were incubated for 4 h in the medium Menezo B-2 with 5% CO_2 at 37° C without adding BA. After incubation, the viability, mobility of gametes and the presence of an apoptosis marker-the externalization of phosphatidylserine-were studied by staining gametes with annexin V-FITC (AnV) and propidium iodide followed by fluorescence microscopy. It was found that the incubation of gametes with BA led to a decrease in their mobility after 10 min of cytostatic exposure and an increase in the amount of stained AnV sperm cells compared to the control. The dynamics of the transmission of the apoptogenic signal had a character of gradual increase, starting from 2 h of incubation, and reached its maximum values after 4 h. The apoptosis index (IA) of sperm cells was calculated as a ratio of the value stained with AnV gametes after exposure of BC to the value of such cells in the control. The value of IA was more than 1, which indicates the induction of apoptosis of sperm cells caused by the action of BA. Thus, the use of cytostatic agents of BA type in treatment regimens can influence spermatogenesis and lead to impaired fertility which is manifested in a decreased mobility of gametes and the induction of apoptosis. This publication was supported by "RUDN University Program 5-100" and the Ministry of Education and Science of Russian Federation (the Agreement No. 02.A03.21.0008).

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P.09-086-Tue
New approach to ameliorating production of viral antigens in artificial systems

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Efficiency of recombinant producers of viral antigens is often poor. This is caused by toxicity of viral proteins and their abnormal folding. Mechanisms of viral capsid self-assembly make viral antigens prone to formation of complexes. Viral proteins usually have several binding centers. The appearance of such proteins in a producer cell results in formation of a web which disturbs normal intracellular organization. Once the viral proteins are dissected into separate domains harboring a single binding center, they become less toxic. Such derivatives have better folding. However, isolation of domains viral proteins optimal for expression by using rational design is still difficult. We propose a method of producing and screening of libraries of bidirectional deletions – LBD, and a system for their rapid phenotypic selection. LBD are produced by PCR with adapter/random primers. Self-annealing of the random primers precludes their direct use for PCR. The proposed scheme solves this problem by ligation of the random primers with 5'-adapter to a dsDNA template with preformed 20–30 nt single-stranded regions with subsequent purification of the ligation products on a silica sorbent. The single-stranded regions are produced by treatment of dsDNA template with DNAse and DNA-polymerase I in absence of dNTP. LBD produced by PCR with adapter primers is cloned to a special vector pQL30. This vector bears a full-length LacZ gene from *E. coli* in pQE30 vector by Qiagen with inserted polylinker leading to a translational frame-shift. Bacterial colonies appeared on indicator agar with X-gal after cloning of the amplified LBD to pQL30 with a repaired frame-shifting are ranged for intensity of the blue color. The brightest colonies contain the most efficiently expressed derivatives. The described system was successfully tested on a model of NS5A gene of hepatitis C virus. Supported by Theme No. 240 0526-2014-00013.

P.09-087-Wed
New biologically active peptides from medicinal leech *Hirudo medicinalis*

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Searching for new substances alternative to antibiotics such as antimicrobial peptides is actual to resolve the problem of microbial resistance. Medicinal leech may serve as a promising source of new highly effective antimicrobial peptides appeared during the co-evolution of blood-sucking leeches and warm-blooded animals. In addition to protection against external pathogens, medicinal leeches must produce antimicrobial agents to protect stocks of blood in their crop for a long time. In despite of long-term and detailed research of medicinal leeches, only several substances are known to date. A draft of the *Hirudo medicinalis* genome carried out in our study allows for *in silico* implementation of the searching algorithm to discover new antibacterial peptides followed by their synthesis and testing biological activity on different microorganisms. The developed method includes evaluation of properties and structural characteristics of short ORFs encoding proteins with signal peptides - namely, a length <60 a.a., 8<pI< 12, charge>0 at pH 7.0. Predictive algorithms AMPA (>0.15), ADAM (>1) and CAMPR3 (>0.5) were used to estimate antimicrobial properties of the peptides. Applying this technique to the genome draft data combined with profiling the proteome of the leech salivary secretion, we identified putative antimicrobial agents. Twelve peptides were selected, synthesized and their antimicrobial activity was experimentally validated. Among candidate peptides, five peptides exhibited antimicrobial activity against *Bacillus subtilis* (168HT), two of them were active against *Escherichia coli* (K-12 substr. MG1655). Additionally, nine peptides possessed antimicrobial activity against intracellular pathogen *Chlamydia trachomatis* D/UW-3/Cx. Finally, a method developed in our study may be applied to screen genome data of other organisms to identify potential antibacterial agents. This work was supported by the Russian Science Foundation (project No. 17-75-20099).

P.09-088-Mon
Evaluation of serum SuPAR levels in sepsis and septic shock patients in terms of diagnosis and prognosis

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Sepsis is accompanied by physiological, pathological, biochemical abnormalities induced by infection and is a public health problem. It is the leading cause of mortality. Septic shock is defined as the need for a vasopressor to keep the mean arterial pressure above 65 mmHg and serum lactate level above 2 mmol/L (>18 mg/dL) in the absence of hypovolaemia. SuPAR (soluble urokinase-type plasminogen activator receptor) plays a role in the migration of cells such as monocytes, macrophages from the circulation to the tissues. In this study, the effects of SuPAR on sepsis and septic shock diagnosis and prognosis were evaluated by comparison with the currently used CRP (C-reactive protein), PCT (procalcitonin), APACHE 2 score of sepsis and septic shock patients enrolled in intensive care unit. Statistical analysis was performed using the SPSS 21.0 program. Categorical variables were compared with Pearson Chi-square, Yates corrected Chi-square and Fisher tests and Continuous variables were compared with nonparametric tests (Mann-Whitney U test and Kruskal-Wallis test) since did not fit normal distribution. The relationship between variables was assessed using the Spearman Correlation Test. The decision-making characteristics of the variables were examined by ROC curve analysis. In our study, serum SuPAR levels were significantly higher in patients with sepsis (n = 26)

and septic shock (n = 23) compared to healthy controls (n = 25) The serum SuPAR levels were similar in the survivors and non-survivors at the end of the 7 day/28 day follow-up and in the intensive care unit. When all groups were taken into consideration, we determined significant correlations between the levels of SuPAR and CRP, PCT. There was no correlation between variables in patient groups. Our findings suggest that SuPAR may be used to diagnose sepsis and septic shock, but not enough to discriminate between sepsis septic shock and predict prognosis.

P.09-089-Tue
Preliminary characterization of SET domain containing protein 3 (SETD3) as actin-specific histidine N-methyltransferase

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Histidine methylation is one of the most elusive post-translational modifications (PTM) that biochemical and physiological importance remains unknown. In most eukaryotic species, actin is methylated at histidine 73 residue, which is thought to stabilize actin filaments. Absence of such PTM results in an increased rate of filaments depolymerization *in vitro*. No physiological effects of actin hypomethylation have yet been identified in cells, tissues or organisms. Knowledge of protein histidine *N*-methyltransferases is limited. The enzyme responsible for actin histidine methylation has been partially purified, but no gene encoding this enzyme has been identified so far. In the preliminary studies, we have identified mammalian SETD3 as actin-specific histidine *N*-methyltransferase. Since SETD3 has been previously shown to act as histone lysine *N*-methyltransferase, the aim of the current investigation was to verify the activity of this enzyme as actin-methylating histidine *N*-methyltransferase. Enzymatic activity of SETD3 was assayed employing either quasi-native human β-actin produced in *E. coli* or natively folded protein overexpressed in *S. cerevisiae* as substrates. Rat and human recombinant SETD3 were produced in COS-7 cells, purified to homogeneity and shown to catalyze methylation of β-actin at H73 residue as verified by Q-TOF MS analysis. The SETD3 orthologues were also active towards a synthetic peptide corresponding to residues 69–77 of β-actin, but not to its mutated form exhibiting His-to-Ala substitution. To conclude, our results indicate that SETD3 is indeed the actin-specific histidine *N*-methyltransferase. Those findings shed new light on the substrate specificity of SET-domain-containing enzymes and open new pathways for further studies aiming at determining the physiological importance of the protein histidine methylation. The investigation was financed by the DSM 501-D114-86-0115000-23 from the Polish Ministry of Science and Higher Education.

P.09-090-Wed
Glucocorticoids combined with solid lipid nanovesicles efficiently protect otic cells from damage

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The auditory receptor, the organ of Corti, is formed by support and mechanoreceptor hair cells that are connected to the brain by the nerve endings of the auditory neurons. Otic cells do not regenerate when they suffer apoptotic damage, which ultimately causes hearing loss. Therefore, there is great interest in understanding the molecular bases of the response to damaging stressors. And in finding effective therapies against deafness, which includes the development of both drugs and efficient vehicles. The HEI-OC1 cell line was derived from the postnatal mouse organ of Corti and it has been used to study the response to damage caused by ototoxics, as cisplatin, and also otic cell protection by different drugs, including glucocorticoids. Solid lipid nanoparticles (SLNs) are very promising drug nanocarriers that have demonstrated the ability to improve drug permeability in other cellular contexts. SLNs were obtained by a microemulsion method, mixing physiological lipids (stearic acid and phosphatidylcholine) and bile salts (taurodeoxycholate). SLNs presented adequate physicochemical characteristics (size, polydispersity and stability) to be used as drug carriers. Rhodamine-labelled SLNs (SLN-RHO) were efficiently uptaken by HEI-OC1 cells, and were not ototoxic. Time course and dose response experiments established a working window without apoptotic cell death or alterations in cell cycle profiles. SLN-RHO penetrated the inner ear in vivo labelling hair cells and auditory neurons. SLNs were next combined with dexamethasone and hydrocortisone. The comparison between the effects of the drugs alone or integrated into SLNs showed differences in terms of effectiveness in the protection from cisplatin toxicity. In conclusion, our results suggest that incorporation drugs into SLNs could improve the effectiveness of inner ear treatments. Work supported by European FP7-IAPP2013-TARGEAR and Spanish FEDER/SAF2017 grants.

P.09-091-Mon
Screening study of medicinal plant raw materials for antimicrobial and antimycotic activity

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Medicinal plants have a wide range of pharmacological activities, including antimicrobial and antimycotic. These activities are conditioned by the presence of such groups of compounds as essential oils, phenylpropanoids, flavonoids, phenol carboxylic acids. We analyzed 11 types of plant raw materials for antimicrobial and antimycotic activity: *herbae: Hyperici, Serpylli, Millefolii, folia: Urticae, Eucalypti viminalis, Granati fructus cortex, flores Tripleurospermum, fructus: Sorbi, Rosae, Ribis nigri, cortex Quercus.* From herbal raw materials, alcohol extracts were obtained on ethanol with concentrations of 50%, 70%, and 95%. Combinations of extracts that showed the best activity were also analyzed. Antimicrobial activity of extracts was determined by diffusion of impregnated disks of filter paper on solid nutrient media. The tasted

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microorganisms were: *Staphylococcus epidermidis*, *Candida albicans* and *Escherichia coli*. As a comparison, the Rotokan liquid extract was used. It was found that high antimicrobial activity is inherent in extracts of *flores Tripleurospermum*, *folia Eucalypti, Granati fructus cortex, herbae Serpylli*. Effective against *Staphylococcus epidermidis* were: extract of *flores Tripleurospermum* on 95% alcohol (zone of inhibition 22 mm); mixture of extracts *Granati fructus cortex, herbae Serpylli, folia Eucalypti* 1:1:1 with 70% ethanol for *Granati fructus cortex*, water-alcohol mixture with 95% ethanol *herbae Serpylli*, water-alcohol mixture with 50% ethanol for *folia Eucalypti* (15 mm) and a mixture of extracts of *fructus Rosae* and *Ribis nigri* 1:1 (15 mm) with an ethanol concentration of 40–96%; against *Candida albicans* - an extract of *flores Tripleurospermum* (17 mm) with concentration of ethanol of 95%; against *Escherichia coli* is an extract of *herbae Hyperici* with an ethanol concentration of 70%. This publication was prepared with the support of the RUDN university program "5-100".

P.09-092-Tue
Role of the antimicrobial peptide in the pathogenesis of experimental autoimmune encephalomyelitis

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An antimicrobial peptide SNS-1 is known to play an important role in innate immune responses by inhibiting bacterial growth and mediating intestinal defense. SNS-1 gene expression increases in meningeal cells and glial cells after exposure to bacterial components, and has been implicated in inflammatory activation of glia in culture. Previous studies also indicated dual immunomodulatory roles of SNS-1; pro- and anti-inflammatory effects. However, little is known about its *in vivo* functional role in the central nervous system (CNS). The present study was undertaken to examine the functional role of SNS-1 peptide in a mouse model of experimental autoimmune encephalomyelitis (EAE). Lipopolysaccharide (LPS)–induced neuroinflammation model was used for comparison purpose. The expression of SNS-1 gene was found to be increased in spinal cords and brain during EAE progression and LPS-induced neuroinflammation. SNS-1 protein was expressed in Ly6G-positive neutrophils and Iba-1-positive microglial cells, but not neurons or astrocytes, in the spinal cord after EAE induction. SNS-1 protein administration accelerated onset time and increased EAE severity. SNS-1 protein administration enhanced glial cell activation in spinal cord of EAE-induced mice, whereas it reduced glial activation in the inflamed brain of LPS-injected mice. In the mechanistic studies using glial cells in culture, SNS-1 protein differentially regulated glial activation depending on inflammatory triggers: SNS-1 protein synergized with cytokines to induce inflammatory activation of glia, but antagonized LPS-induced responses. Furthermore, SNS-1 treatment augmented T helper cell-related *Ifng* gene expression in cultured splenocytes. These results indicate that SNS-1 differentially regulates inflammatory glial activation and neuroinflammation in EAE and LPS-injected brain, and suggest that SNS-1 can be considered as a potential therapeutic target in multiple sclerosis and other inflammatory CNS disorders.

P.09-093-Wed
Ability of uracil–DNA glycosylase to move along DNA as a target therapy against poxviruses

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Uracil–DNA glycosylase excises uracil from DNA and triggers a base excision repair pathway. This function is observed in all cellular organisms investigated and plays crucial role in survival. Moreover, UNG is encoded by some viral genomes, including poxviruses. For vaccinia virus (which is most investigated) it was shown that besides glycosylase activity UNG (D4) protein in assembly with A20 and viral DNA polymerase forms the replicative complex. To copy large genomes, many DNA polymerases have special processivity factors. Based on structural data, we suppose D4 is a processivity factor of the vaccinia virus polymerase and investigate the own processivity of D4. We have purified D4 with high glycosylase activity and used multiply damaged DNA to estimate the probability of correlated cleavage (P_{cc}). Experiments with varied distances between damages and with small gaps or bound proteins have reflected high processivity of D4 protein. In an array of compounds potentially inhibiting UNG family proteins we have found few inhibitors of catalytic activity, some of them also reducing the processivity. These compounds were found to suppress viral replication in infected cell cultures. Our results allow us to assume that processive properties of D4 is a perspective target for antiviral therapy. The work was supported by RSF (grant 17-14-01190).

P.09-094-Mon
BECN1-induced autophagy in acute diphtheria intoxication

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Due to widespread use of immunological prophylaxis diphtheria morbidity has a sporadic character these days. Nevertheless in the event of infection the risk of severe complications is high. The cause of death is mainly associated with myocarditis (in over 50% of cases). Exotoxin of Corynebacterium diphtheria inhibits biosynthesis of cytochrome B which is accompanied by depression of tissue respiration and protein synthesis. But for today there is still no data concerning the effects of diphtheria exotoxin on Beclin 1 (BECN1) associated cardiomyocyte autophagy which is considered to be either a mechanism of cell survival or a type regulated cell death. Our experiments were performed on 16 male Chinchilla rabbits with body mass 3…3.5 kg. Animals were divided into 4 groups with 4 animals each: 1 control group (intact rabbits) and 3 experimental groups (rabbits with acute diphtheria intoxication 1, 3 and 5 days after the onset of the intoxication correspondently). Diphtheria intoxication was modeled by single intravenous injection of native diphtheria toxin at a dose of 0.3 DLM/1 kg which was previously titrated on guinea pigs. Activity of cardiomyocyte autophagy was assessed by immunochemical estimation of BECN1 content in the left ventricular myocardium with the use of primary goat polyclonal antibodies (Santa Cruz Biotechnology, Inc., USA). It was found that the content of BECN1 protein was significantly decreased on day 1 (0.12 vol.%) in comparison with controls (2.77 vol.%). Positively stained viable cardiomyocytes were very rarely detected

in all fields of vision on day 3 (0.04 vol.%) and were absolutely absent on day 5 (0 vol.%). It is concluded that in case of diffuse diphtheria toxic injury of the myocardium, BECN1-associated autophagy is inhibited due to depletion of the responsible molecular mechanisms. The publication was prepared with the support of the "RUDN University Program 5-100".

P.09-095-Tue
Transition metals catalyzed reactions – a new approach to the aryl-, azolyl- and triazolylsubstituted steroids –active compounds against human MCF7 breast and SCOV3 ovarian carcinoma cells

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Steroids possess a unique position among other natural compounds due to their high biological activity and involvement into the most important processes in living organisms. One of important directions of synthetic organic chemistry is creation of new drugs by modification of steroids. During 20-th century methods of classical organic chemistry were used to transform steroids. Presently the reactions, catalyzed by the complexes of transition metals, primarily by complexes of palladium, nickel and copper, are intensively applied for this purpose. The development of new efficient protocols for steroid functionalization is an important synthetic problem. On the base of computer docking experiments we prepared a big series of 4- and 6-arylsubstituted steroids by Pd-catalyzed cross-coupling 6-halogen steroids with arylboronic acids. Many of these compounds demonstrated high inhibition activity against MCF7 cancer cells. As well we have developed a highly efficient procedure for the synthesis of azolyl-substituted steroids utilizing a cheaper catalyst system based on Cu-catalyzed reactions. A number of nitrogen heterocycles was involved into the vinylation of halogen containing steroids affording the corresponding coupling products in good to excellent yields. In addition a series of triazolyl substituted steroids and triazole annelated non-steroidal derivatives was prepared and tested against ovarian carcinoma SCOV3 and human breast cancer MCF7 cell lines to investigate the antiproliferative activity. The cytotoxic effect was detected by MTT-test. Using the results on the cytotoxicity, survival curves were constructed and IC_{50} were calculated. The values of IC_{50} were in the range 3–22 μmol/L. These low values of IC_{50} indicate the prospectivity of the further study of prepared substances as antitumor agents. The publication was prepared with the support of the "RUDN University Program 5-100" and RFBR grant 16-03-00390a.

P.09-096-Wed
Selective ligands of membrane progesterone receptors and progesterone are predominantly proinflammatory immunomodulators in human peripheral blood mononuclear cells

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The immunomodulatory role of progesterone (P4) is highly important for successful ovulation, implantation, maintenance of pregnancy and initiation of labor. The functions of progestins are mediated both by nuclear (nPRs) and membrane (mPRs) receptors of adipoQ receptor family. Human peripheral blood mononuclear cells (PBMC) comprise of T and B lymphocytes, monocytes and other types of immune cells, expressing mPRα and mPRβ. Among synthesized progesterone derivatives we found two selective ligands of mPRs which do not interact with nPRs: 19-hydroxypregn-4-en-20-one and 19-hydroxypregn-3-ene-20-one. The aim of this study was to assess the effects of these selective ligands and P4 on gene expression and the secretion of cytokines (IL-1β, IL-6, IL-10, IL-2, TNFα, TGFβ and IFNγ) in PBMC using qRT-PCR and ELISA. PBMC were isolated from blood of 8 men and 8 women, stimulated with lipopolysaccharide and incubated with different concentration of hormones for 48 h. P4 and both selective ligands significantly increased gene expression and protein secretion of IL-1β, IL-6 and TNFα and gene expression of TGFβ and IFNγ. IL-10 secretion was reduced significantly after exposure with steroids, whereas mRNA level was unchanged. These compounds also significantly reduced IL-2 mRNA level. Sexual differences in cytokines levels were not detected. The selective ligands of mPRs in some cases acted at lower concentrations on PBMCs than P4 and can successfully replace it without having a side effect on the nPR-positive cells. Thus, P4 and selective analogues of mPRs have both pro-inflammatory and anti-inflammatory effects on PBMC in men and non-pregnant women with mainly pro-inflammatory action. Thus, the concept that progestins have predominantly immunosuppressive effects is oversimplistic. In summary a complex and diverse action of these hormones depending on the type of immune cells, tissue and hormonal context was proposed.

P.09-097-Mon
Alkaline phosphatase regulates the hair-inductive capacity of 3D-cultured human DP cells

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Recent studies showed that sphere formation enhances the ability of cultured dermal papilla (DP) cells to induce new hair follicles. Alkaline phosphatase (ALP) activity is known to be correlated with the hair-inducing capacity (trichogenicity) of DP cells and expression of ALPL (ALP, liver/bone/kidney) transcript is restored in DP spheres. We investigated whether restoration of ALPL expression by sphere formation plays a critical role in hair-inducing capacity of DP spheres and, if so, to investigate the mechanism. We employed *ALPL* siRNA-mediated gene knockdown and expression vector-mediated *ALPL* overexpression in combination with a hair reconstitution assay. Knockdown or

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overexpression of *ALPL* was verified by real-time PCR and enzyme activity staining. Reporter assay was performed with β-catenin responsive plasmid (pTopflash) and immunoblot was performed to measure nuclear β-catenin levels. Expression of target genes in the Wnt/β-catenin pathway and DP signature genes was measured by real-time PCR. Knockdown of ALPL impaired the trichogenicity of human DP spheres and overexpression of ALPL augmented the trichogenicity of DP spheres. Knockdown of ALPL in DP spheres reduced nuclear β-catenin levels, pTopflash activity, and the expression of target genes in the Wnt/β-catenin pathway. Overexpression of *ALPL* in DP spheres dramatically increased nuclear β-catenin levels, pTopflash activity, and the expression of target genes in Wnt/β-catenin pathway. In addition, the expression of known DP signature genes was decreased in *ALPL* siRNA-transfected spheres and increased by ALPL overexpression in DP spheres. These data show that ALPL plays a critical role in the hair-inductive capacity of human DP spheres by regulating Wnt/β-catenin signalling and maintaining the characteristics of the DP.

P.09-098-Tue
Two novel bi-functional GH16 1,3-β-D-glucanases from gastropoda *Lambis* sp.

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1,3-β-D-glucanases are widely spread enzymes and are found in all kingdoms of living nature from viruses to eukaryotes. According to IUPAC nomenclature, they can have the following numbers:

EC 3.2.1.58 (exo-1,3-β-D-glucanases)
EC 3.2.1.39 (endo-1,3-β-D-glucanases)
EC 3.2.1.6 (endo-1,3;1,4-β-D-glucanases)

1,3-β-D-glucanases can be found in different CAZy glycoside hydrolase (GH) families, but their distribution is extremely irregular. While most of endo-glucanases belong to GH16 and GH17 families, exo-glucanases belong predominately to GH5 and GH55 families. The only GH16 family 1,3-β-D-glucanase with exo-type activity from hot spring bacterium *Caldicellulosiruptor kronotskyensis* appeared to be multi-domain enzyme with GH16 domain responsible for endo-type of activity and GH55 responsible for exo-type. There were isolated and characterized two 1,3-β-D-glucanases from hepatopancreas of marine gastropoda *Lambis* sp. from South China Sea with molecular weights of 44 and 51 kDa. The properties of these enzymes appeared to be similar to endo-1,3-β-D-glucanases (EC 3.2.1.39) of tropical sea mollusks: temperature optimum was 60 °C, pH range was from 3.0 to 6.0, Km for hydrolysis of laminaran were 0.30–0.35 mg/mL. Both enzymes catalyzed hydrolysis of laminaran with retention of configuration of anomeric carbon atom, and formation of transglycosylation products with DP 2 and 3 (EC 3.2.1.39). They also catalyzed hydrolysis of periodate-oxidized laminaran and 4-nitrophenyl-β-D-glucopyranoside, showing exo-glucanase activity (EC 3.2.1.58). There were determined full cDNA sequences, both enzymes were classified to GH16 family with single-domain organization, GenBank numbers are AIY63535.1 and AIY63536.1. To our knowledge, this is the first report about bi-functional GH16 enzymes with endo- and exo-1,3-β-D-glucanase activities. The work was supported by RFBR grant No. 18-04-00905.

P.09-099-Wed
Inhibition of ABC transporters by mifepristone is responsible for enhanced etoposide toxicity in Hep G2 cells

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Etoposide has been used in cancer therapy for decades, primarily due to its inhibition of topoisomerase II. However, several adverse effects, including acquired resistance, were observed in clinical use. Thus, a combination therapy may help to solve these problems. Mifepristone is a candidate compound for combination treatment because of its influence on protein expression and activity. Majority of experiments were performed in Hep G2 cell model in which toxicity of the mifepristone/etoposide combination was evaluated by using the xCELLigence system. Our data show dose dependent enhancement of etoposide toxicity that was mirrored by increase in caspase-3 activity. Western blots of Bak protein displayed a trend supporting activation of programmed cell death, however, the Bak upregulation was relative to total protein and was non-significant. Then we tested whether mifepristone alone has any effect on ABC transporters. The effects were tested in cell model with different expression of ABC transporters ABCB1 and ABCG2 (K562 cells). Our results suggest that mifepristone effectively inhibits the ABCB1 transporter at a clinical relevant concentration in cells with low expression and partially in cells which overexpress the transporter. These experiments were followed by HPLC/MS analysis where intracellular etoposide concentration was measured. We compared 60 μM etoposide with combination of 60 μM etoposide and mifepristone (20 μM or 10 μM). Both mifepristone concentrations significantly increased accumulation of etoposide in Hep G2 cells. Finally, we evaluated the effect of different combinations of mifepristone and etoposide on viability of primary rat hepatocytes. There was no toxic effect observed for clinically relevant drug combinations. Therefore, such combination therapy could be considered for use as primary cells are resistant to the toxic effect. This study was supported by grants LO1304 and IGA_LF_2018_012.

P.09-100-Mon
Thienopyrimidines kill *Mycobacterium tuberculosis* by production of nitric oxide

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Wide distribution of latent *Mycobacterium tuberculosis* infection and the prevalence of drug resistance denote a growing need for new antitubercular compounds. The thienopyrimidine TP053 is one of the most promising new discovered antitubercular prodrugs highly active against both replicating (*in vitro* MIC = 0.125 μg/mL) and non-replicating *M. tuberculosis*. Mycothiol-dependent reductase Mrx2, encoded by Rv2466c, is known to be a TP053 activator (Albesa-Jové et al., 2014; Mori

et al., 2017); however, the precise mode of action of this compound remained unclear. As the chemical structure of TP053 is characterized by the presence of NO_2 group, reduction of a nitroaromatic moiety of TP053 by Mrx2 was hypothesized to result in NO release and toxicity of the activated compound. Analysis of the products of enzymatic activation of TP053 by Mrx2 by the Griess reagent clearly confirmed production of nitric oxide in a time-dependent manner. Mass-spectra of cell lysates of TP053-treated *M. tuberculosis* bacilli demonstrated transformation of TP053 to its non-active metabolite with Mw = 261 that corresponds the scenario of NO release accompanied by the destruction of the reduced thiophene ring with formation a highly reactive mercapto group. The multitarget mechanism of NO toxicity for bacteria is known to include DNA damage, and biosynthesis proteins and lipids. Thus, TP-053 drug-like scaffolds prospective for further development of novel anti-TB drug. This work was financially supported by the Ministry of Education and Science of the Russian Federation (Agreement No 14.616.21. 0065; unique identifier RFMEFI61616X0065).

P.09-101-Tue
Recombinant destabilase, a potential thrombolytic, cleaves isopeptide bonds in stabilized fibrin

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Thrombolytic drugs that are currently in clinical use consist of various modified serine proteases (metalloproteases) involved in the fibrinolytic system of the blood. Their mechanisms of action involve the proteolytic degradation of polypeptide bonds in fibrin polymers, a core component of thrombus. In thrombi, fibrin molecules are cross-linked by the isopeptide bonds. Formation of the isopeptide bonds is mediated by factor XIIIa. In this work, we describe a fundamentally new mechanism of thrombolysis caused by recombinant destabilase action and called 'thrombolysis-isopeptidolysis'. Destabilase is an enzyme secreted by the medicinal leech salivary cells possessing endo-ε-(γ-Glu)–Lys-isopeptidase activity that is responsible for isopeptide bond cleavage. We induced venous and arterial thrombosis in rats. Rats received intravenous injections of recombinant destabilase produced in *Escherichia coli* cells, a commercial streptokinase preparation or both. 24 h later, we analyzed weight of the thrombi and degree of cross-linking in thrombi. We found that destabilase alone or destabilase co-injected with streptokinase causes a 47.6% or 74.6% decrease of weight of venous and arterial thrombi respectively. The combined administration of destabilase and streptokinase has a greater effect than the injection of individual enzymes. We showed that destabilase also reduces fibrin stabilization in thrombi. Thus, we found that the medicinal leech destabilase is an efficient thrombolytic agent for dissolving thrombi. We believe that treatment with destabilase will increase the overall effectiveness of conventional thrombolytic drugs. This work was supported by the Russian Science Foundation (project No. 17-75-20099).

P.09-102-Wed
Effect of electric pulse stimulation on transcriptome and [Na+]i/[K+]i ratio in C2C12 myotubes: a comparative analysis

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Skeletal muscle typify as an endocrine organ producing peptides and other bioactive substances termed myokines. Numerous researches demonstrated that myokine transcription, expression and secretion are controlled by physical activity and provide a beneficial effect on the function of skeletal muscles and neighbouring tissues. In our study, we employed mice skeletal muscle C2C12 myotubes subjected to electric pulse stimulation (EPS, 2 hr, 40 V, 1 Hz, 10 ms) as in vitro model of muscle contraction. Using the Affymetrix GeneChip technology we found that EPS resulted in differential expression of dozen of myokines including statistically significant ($P < 0.05$) by more than 20% up-regulation of Angptl4, APLN, CCL2, CXCL1, FGFBP1, IL6 and down-regulation of Adipoq, Bmp4, LRP4, Tgfb2. Both EPS and inhibition of the Na,K-ATPase by 30 mkM ouabain led to dissipation of transmembrane Na^+ and K^+ gradient resulted in elevation of the $[Na^+]_i/[K^+]_i$ ratio by 50%. With an exception of FGFBP1, ouabain didn't affect transcription of any EPS-sensitive myokines. Inhibition of L-type voltage-gated Ca^{2+}-channels by 10 mkM nicardipine completely abolished $[Ca^{2+}]_i$ oscillations triggered by EPS in fura-2-loaded cells as well as differential expression of Angptl4, APLN, CXCL1, FGFBP1, IL6, Adipoq, LRP4 and Tgfb2 indicating $Ca^{2+}{}_i$-mediated mechanisms of excitation-transcription coupling. In contrast, altered expression of Bmp4 and CCL2 was preserved in the presence of nicardipine thus suggesting Ca^{2+}-independent signaling. In conclusion, our results demonstrate that both Ca^{2+}- mediated and –independent signaling contributes to transcriptomic changes seen in EPS-treated myotubes. Intermediates of Ca^{2+}-independent signaling as well as the role of elevation of the $[Na^+]_i/[K^+]_i$ ratio in augmented expression of FGFBP1 should be examined further. This work was supported by the Russian Science Foundation grant #16-15-10026.

P.09-103-Mon
Prohaptoglobin stimulates angiogenesis by upregulating placental growth factor expression via TGF-β/Smad1,5 signalling

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Our previous study demonstrated that prohaptoglobin (proHp), a precursor haptoglobin, enhances angiogenesis by increasing the expressions of vascular endothelial growth factor-A (VEGF-A) and VEGF receptor 2 (VEGFR2) in endothelial cells. However, the underlying mechanisms of proHp on angiogenesis are still unclear. Here, we investigated the angiogenic factors involved in upstream signals in proHp-treated HUVECs. ProHp enhanced the expressions of placental growth factor (PlGF) and VEGFR1 as well as VEGF-A and VEGFR2. The tyrosine-phosphorylations of VEGFR1 and 2 were also enhanced by proHp treatment. Interestingly, PlGF knockdown attenuated the proHp-induced angiogenic events - VEGF-A expression, VEGFR1/2 phosphorylation, cell migration and tube formation on Matrigel. Furthermore, transcription factor profiling assay indicated that Smad is involved in

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the proHp-stimulated PlGF expression. Transforming growth factor-β (TGF-β) expression and Smad1/5 phosphorylation were also induced by proHp. Blockade of TGF-β signalling by TGF-β receptor kinase inhibitor LY2109761 or Smad1/5 siRNA reduced the proHp-enhanced expressions of PlGF and VEGF-A, and *in vitro* tubular network formation. These findings suggest that the angiogenic effects of proHp were dependent to PlGF and mediated *via* TGF-β/Smad1,5/PlGF/VEGF-A/VEGFR1,2 signalling pathway.

P.09-104-Tue
A draft of the *Hirudo medicinalis* genome provides information about potential anticoagulant and thrombolytic proteins

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Hirudotherapy is used in medicine since ancient times. The salivary cell secretion (SCS) of medicinal leech contains a lot of biological active compounds that suppress blood clotting, decrease pain sensitivity and enhance local blood microcirculation. However, protein and peptide composition of SCS is not fully described, and structure and properties of many components remain unknown. In our work we have generated a draft of the *H. medicinalis* genome. We identified earlier unknown homologs for the genes encoding leech anticoagulants in a draft of medicinal leech genome. There were homologs of serine proteinase inhibitors (bdellin A, bdellin B3, antistasin, eglin C, hirustasin) among identified sequences. We also determined several homologs of destabilase, a polyfunctional protein. Its isopeptidase activity leads to breakdown cross-links in stabilized fibrin and subsequent thrombolysis. This makes destabilase to be a potential compound for treatment of thrombosis. Thus, the draft of the medicinal leech genome provides a database of sequences encoding the unique leech proteins for developing new pharmacological compounds. This work was supported by the Russian Science Foundation (project No. 17-75-20099).

P.09-105-Wed
Analysis of dolichol content in urine and tissues of patients with congenital disorder of glycosylation

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Dolichol (dol) is membrane lipid, which carries glycans for *N*-linked protein glycosylation, *O*-mannosylation and GPI anchor biosynthesis ongoing in endoplasmic reticulum. Its structure is composed by isoprenoid units. Dol is presented in all tissues and most membrane organelles of eukaryotic cells. Recently some types of congenital disorders of glycosylation (CDG) were described as consequence of dol biosynthesis defects (mutations in *DHDDS, SRD5A3, DOLK, NgBR* genes), while other defects are closely associated with its metabolism (genes *MDPU1, DPM1-3*). The aim of study was to analyze dol content in urine and tissues of patients with suspect deficiency in dol biosynthesis by mass spectrometry with purpose to extend screening methods for CDG. Biological material for this study consisted of urine samples from 76 controls in age 2 months to 82 years, 6 patients with CDG (1xNgBR-CDG, 1xSRD5A3-CDG, 2xPMM2-CDG, 1xDPAGT1-CDG, 1xPGM1-CDG) and 43 patients with suspicion of CDG syndrome; samples of frontal cortex, liver, muscle and heart tissues from 2 NgBR-CDG patients and controls. Urine samples were stored in -20°C and tissue homogenates were stored in -80°C until used. Lipid content after extraction was separated by *Agilent 1290 Infinity LC System*. Dols were analyzed by *API 4000 LC-MS/MS System Sciex*. Peaks of dols with 17, 18, 19 and 20 isoprenoid units were captured and the ratio of Dol-18/Dol-19 was calculated. In group of controls, significant correlation between Dol-18/Dol-19 ratio and age in urine was found ($P < 0.005$). Reference range of controls in urine was evaluated. There were not detected differences between genders. Ratio of Dol-18/Dol-19 was significantly increased in NgBR-CDG urine and tissues in comparison with control. Our results showed a new possibility for diagnosis of patients with rare CDG, who cannot be identified by usual screening methods. Supported by: *AZV-16-31932A, RVO-VFN64165, SVV-UK 260367/2017*.

P.09-106-Mon
SGBS preadipocyte cell line can serve for human beige type of thermogenic browning adipocyte differentiation

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In contrast to white adipocytes, brown and beige adipocytes contain high amount of mitochondria which express uncoupling protein 1 (UCP1) and its main function is thermogenesis and energy expenditure. However, there is only limited data about regulatory networks that drive human brown or beige adipocyte differentiation. Therefore, human cell line models are needed in order to explore the key molecular targets of novel pharmacological treatments that can enhance browning and help the therapy of patients suffering from metabolic syndrome. The Simpson-Golabi-Behmel syndrome (SGBS) preadipocyte cell line provides a useful tool for studies of human adipocyte biology. Our aims were to investigate whether brown or beige adipocyte differentiation can be induced in SGBS cells and to clarify the effect of Irisin (myokine which stimulates beige differentiation in response to exercise) and BMP7 (autocrine mediator which induces classical brown development) treatment during adipocyte differentiation. We aimed to test the involvement of the creatine phosphate substrate cycle in the heat production of SGBS derived beige adipocytes and investigate whether beige differentiation can be reversed to white adipocytes or they maintain their beige morphology. We applied white and PPARγ-driven browning (including long-term Rosiglitazone treatment) differentiation cocktails to induce adipocyte differentiation. The application of Rosiglitazone could be successfully used to induce browning of SGBS cells. Irisin treatment resulted in up-regulation of UCP1 and TBX1 genes. BMP7 moderately induced a classical brown phenotype. The browning protocol or Irisin induced a beige phenotype with high oxygen consumption rate for UPC1-dependent and creatine phosphate

futile cycle mediated heat production. Thus, SGBS cells can be shifted into both white and beige adipocytes. The continuous Rosiglitazone or Irisin treatment could maintain a beige phenotype under long-term (28 days) differentiation program.

P.09-107-Tue
Protective effect of alpha lipoic acid on apical periodontitis-induced cardiac injury

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Oxygen free radicals are involved in pathophysiology of apical periodontitis. This study was designed to assess the possible protective effect of alpha lipoic acid (ALA) on apical periodontitis (AP)-induced cardiac injury. 200–250 g weighed Wistar albino male rats were randomized into four groups; control group, ALA group, AP group and ALA+AP group. In control and ALA groups, rats were not treated, saline and ALA (100 mg/kg) were administrated. In AP and ALA+AP groups, the left maxillary first molar teeth of the rats were opened with a round dental bur until the pulp chamber was exposed. This application was done under 100 mg/kg ketamine and 10 mg/kg xylazine anaesthesia with using high-speed water cooling. It was then left open for 30 days to induce apical periodontitis. Saline and ALA (100 mg/kg) was administered intraperitoneally every 24 h during the experiment. At the end of the experiment, animals were euthanized by high dose of ketamine-xylazine combination. Serum ALP, LDH, CK and SOD activities were determined using an automated biochemical analyser and the structural cardiac injury was assessed pathologically. Results obtained were then statistically analysed using GraphPad Prism 7. Results were compared by means of one-way analysis of variance (ANOVA). Tukey's was used as a further analysis in binary comparisons. Serum ALP, LDH and CK activities were elevated in AP group. Besides, SOD activities were decreased in the AP group. While the changed enzyme activities was significantly normalized by ALA treatment. Since ALA administration alleviated the apical periodontitis-induced heart injury and improved the cardiac structure and function. It seems likely that ALA with its anti-inflammatory and antioxidant properties may be of potential therapeutic value in protecting the cardiac tissue against systemic oxidative injury due to apical periodontitis.

P.09-108-Wed
ATP6AP1-CDG: Biochemical and molecular-genetic characterization of two cases with severe phenotype

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Introduction: Congenital disorders of glycosylation (CDG) are a rare, clinically heterogeneous group of >100 metabolic diseases caused by deficiencies of enzymes/proteins participating in glycosylation pathways. Here we describe a case of two CDG-suspected male siblings (P1 and P2) with hyperbilirubinemia, hepatopathy, splenomegaly and wrinkled skin, who both died due to progressive liver failure at the age of 3 (P1) and 11 (P2) months. Results and discussion: The finding of hypoglycosylated TF (CDG-II pattern) and ApoC-III in sera from P1 and P2 pointed to a combined defect of N- and O-glycosylation. Altered morphology of Golgi apparatus (GA) and delayed retrograde GA transport, assessed by brefeldin A treatment, was detected by immunocytochemistry in the cultivated fibroblasts of P1. Moreover, peroxisomal disturbance and increased reactive oxygen species were observed, demonstrating a complex impact of the defect on the cellular function. Whole-exome sequencing in P1 identified a novel hemizygous mutation c. 221T>C (p.Leu74Pro) in exon 2 of *ATP6AP1* gene, and the same mutation was later confirmed in P2 by Sanger sequencing, while the mother was found to be a carrier of the heterozygous variant. ATP6AP1 is an accessory protein of the vacuolar H+-ATPase, a proton pump which participates in acidification of various intracellular compartments including GA. ATP6AP1-CDG was first reported in 2016, and compared to the so far 12 described cases presenting predominantly with immunodeficiency, hepatopathy and cognitive impairment, our patients manifested with a more severe phenotype. Supported by grants: AZV MZ CR 16-31932A, RVO-VFN64165, UNCE 204011.

P.09-109-Mon
Hippocampal Ras protein through downstream effectors - Akt and ERK plays a significant role in the nongenomic regulation of thyroid disorders

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Thyroid hormones (THs) are important regulators of growth, development and metabolism. The central nervous system is an important target for the THs and hypothyroidism in adulthood has been clearly linked to neurocognitive dysfunctions. It is also known that THs exert nongenomic effects on the mitochondrial energy metabolism. Nongenomic effects of THs can be mediated by ERK and Akt signaling pathways, and their upstream regulators - Ras proteins. Therefore, we decided to investigate quantitative changes of these signaling molecules in the different compartments of neural cells in the hippocampus of adult rats in following groups: euthyroid (control), hypothyroid (methimazole-treated), nobiletin (nobiletin-treated) and T4-treated hypothyroid states. It was observed that level of phosphorylated ERK was slightly increased in the cytoplasm of hypothyroid rats and significantly decreased in case of nobiletin supplementation. Level of phosphorylated Akt was increased in the cytoplasm of

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hypothyroid rats' and was restored to control level for T4-treated animals. Level of K-Ras was increased on the plasma membrane and significantly decreased on the endoplasmic reticulum of hypothyroid rats. These parameters were restored to the control level in both cases: with nobiletin supplementation and under T4 treatment. In addition, level of H-Ras was increased on the endoplasmic reticulum of hypothyroid rats and decreased to control level for the rats taking nobiletin supplementation. Our results suggest that hippocampal Ras protein through downstream effectors - Akt and ERK plays a significant role in the nongenomic regulation of thyroid disorders.

P.09-110-Tue
FcrR/ROS/CK2a is the key inducer of NF-κB activation in a murine model of asthma

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The transcription factor, nuclear factor (NF)-κB plays a pivotal role in the development of allergic airway inflammation, However, the mechanism of NF-κB activation in asthma remains to be elucidated. CK2a activation was assessed by CK2a phosphorylation and protein expression. Airway levels of histamine and cytokines were determined by ELISA. We have used two (active and passive) forms of allergic pulmonary inflammation. models. In active form, animals were immunized with OVA via intraperitoneally, followed by airway challenge with OVA. In passive form, animals were passively sensitized by intratracheal instillation with either anti-OVA IgE or anti-OVA IgG, followed by airway challenge with OVA. The role of NADPH oxidase (NOX) in CK2a activation was assessed using $NOX2^{-/-}$ and $NOX4^{-/-}$ mice because NOX2 and NOX4 contribute to many inflammatory diseases. Second airway challenge increased CK2a phosphorylation and protein expression in airway epithelial cells as well as the nuclear translocation of the p50 and p65 subunits of NF-κB, all of which were inhibited by the CK2a inhibitor, 4,5,6,7-tetrabromobenzotriazole and the antioxidant N-acetyl-L-cysteine. CK2a phosphorylation and protein expression were significantly impaired in $NOX2^{-/-}$, but not in $NOX4^{-/-}$, mice. Induction of passive sensitization using anti-OVA IgE, activated neither CK2a nor NF-κB. In contrast, induction of passive sensitization using anti-OVA IgG activated both CK2a and NF-κB. These data suggested that Fc gamma receptor (FcgR)/reactive oxygen species (ROS)/CK2a is a key inducer of NF-κB activation in airway epithelial cells in a murine model of asthma.

P.09-111-Wed
Tetracenomycin X novel inhibitor of translation

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Last few years people faced a problem that there are a lot new species of bacteria which could not been influenced by certain antibiotics. Also already known pathogenic bacteria gained resistance genes. Antibiotic resistance is one of the biggest threats to global health, according to WHO. By 2050, it is expected that mortality rate caused by bacterial diseases will increase 14 times. Therefore, there is great necessity of novel antibiotics in the whole world. Due to reasons mentioned above we decided to make screening for novel compounds with antibiotics activity. Screening was performed using reporter *E. coli* strain pDualrep2, which can detect translation inhibitors and DNA damaging antibiotics. In this screening we found significant antibiotic activity for cultural broth sample of bacterium *Amycolatopsis* sp. which shown inhibition of translation. In further study a compound was purified by HPLC chromatography, the structure was determined by combination of NMR and mass spectrometry and it turned out, that we re-discovered old antibiotic - Tetracenomycin X (ThX). However, a mechanism of antibiotic activity has not elucidated yet. Further study showed that in vitro translation with this compound shows opportunity to decrease level of translation. This result was proved by toe-printing assay, which demonstrates a translational inhibition as well. By now we also have found some mutant strains of *E. coli* which have ThX resistance because of mutations in rRNA, this data is also proved by probing assay. Therefore, it could be reasonably assumed that ThX probably provides inhibition of translation on elongation stage. In view of all data obtained, Tetracenomycin X is of great medical and scientific interest as a molecule which might be a basis to development of novel antibiotic drug. Thereby it requires a further scrutiny. Work was supported by the Russian Science Foundation (grant 18-44-04005) for I.A.O.

P.09-112-Mon
Lactuca capensis mitigate cognitive dysfunction on Aβ1-42-induced a rat model of Alzheimer's disease through regulating BDNF mRNA and IL1β mRNA expression and antioxidant system activity

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We investigated the neuropharmacological effects of the methanolic extract from *Lactuca capensis* Thunb. leaves (100 and 200 mg/kg) for 21 days on memory impairment in an

Alzheimer's disease (AD) rat model produced by direct intraventricular delivery of amyloid-β1-42 (Aβ1-42). Behavioral assays such as Y-maze and radial arm maze test were used for assessing memory performance. Aβ1-42 decreased cognitive performance in the behavioral tests which were ameliorated by pretreatment with the methanolic extract. Acetylcholinesterase activity and oxidant-antioxidant balance in the rat hippocampus was abnormally altered by Aβ1-42 treatment while these deficits were recovered by pretreatment with the methanolic extract. In addition, rats were given Aβ1-42 exhibited in the hippocampus decreased BDNF mRNA copy number and increased IL1β mRNA copy number which was reversed by the methanolic extract administration. These findings suggest that the methanolic extract could be a potent neuropharmacological agent against dementia via modulating cholinergic activity, increasing of BDNF levels and promoting antioxidant action in the rat hippocampus.

P.09-113-Tue
Assessment of lipid peroxidation and antioxidant protection indicators in ascariasis before and after treatment

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An important role in the development of pathogenesis, diagnosis of parasitic diseases is the definition of products lipoperoxide cascade. A total of 110 patients were examined: 30 with ascariasis, 20 healthy women aged 18 to 45 years served as a comparison group. Researched indicators lipoperoxide cascade and antioxidant protection in the blood of 60 women after 1.5 months after the standard and an integrated deworming therapy. Indicators of LPO in the blood of women with ascariasis before treatment, after standard and complex therapy indicate the activation of free radical processes. In relation to the comparison group, there was a statistically significant increase in the content of MDA, TTP and TSP. The average blood levels of TTP and TSP exceeded the values of the comparison group by 2 times, the average level of MDA–by 3.75 times. The content of MDA in women with ascariasis was 1.8 times higher. The level of MDA content in groups before and after standard treatment was significantly higher than the values in the comparison group (3.7 and 2 fold, respectively). The activity of GPO and CAT in groups before and after standard treatment significantly differed from the norm. After complex treatment, increased activity of GPO was observed. In the main group, after the use of standard and complex treatment, CAT activity decreased slightly in relation to the group with ascariasis, but the activity of CAT remained increased compared to control values. In the blood of women with ascariasis, activation of free radical oxidation processes was observed, which was not compensated by an appropriate increase in the activity of AP enzymes. The activation lipoperoxide cascade is associated with impaired metabolism of membrane lipids and change their physicochemical properties permeability of biological membranes.

P.09-114-Wed
Potential mechanisms of CD58 gene regulation by differential binding of transcription factors to allelic variants of SNP rs1335532 associated with the risk of multiple sclerosis

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B-cells are known to play a key role in multiple sclerosis (MS) progression and autoimmune response. CD58 is expressed on the surface of antigen-presenting cells (APC), including B-cells, and provides their interaction with most types of T-cells. *CD58* gene is considered as a MS susceptibility locus with the protective genotype associated with higher CD58 mRNA expression. Protective effect of CD58 is presumably associated with activation of Treg cells as a result of CD58-CD2 interaction. ASCL2 binding to DNA was assessed using pull-down assay. Wnt pathway stimulation was performed using LiCl. Activities of variants of CD58 enhancers containing different rs1335532 alleles were estimated using luciferase reporter assay. We determined that minor rs1335532 allele creates functional ASCL2-binding site within CD58 enhancer area required for the basal activity of *CD58* promoter. *CD58* enhancer containing minor rs1335532 variant, that is statistically associated with low risk of MS, demonstrated stronger *CD58* promoter induction during Wnt pathway activation in B-lymphoblastic cells. ASCL2 is highly represented in follicular T-helpers and it is able to regulate the range of B-cell specific genes. We now identified ASCL2 as the main regulator of rs1335532-dependent modulation of CD58 enhancer and promoter activities in B-lymphoblastoid cell lines. This link may be directly related to the ability of B-cells to activate Tregs and suppress inflammation in multiple sclerosis. This project is supported by grant 14-14-01140 from Russian Science Foundation.

P.09-115-Mon
Semi-high throughput, blood-based microRNA analysis identifies specific molecules with deregulated expression in patients with type-2 diabetes mellitus

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Type-2 diabetes (DM2) epidemic is ascribed to the interplay between genetic and environmental factors: the latter affects the epigenome which interacts with the genome, finally orchestrating DM2-pathophysiological mechanisms. Certain microRNAs (miRNAs) are known to influence genes involved in insulin resistance or predicted to impact DM2-susceptibility genes, in liver, adipose tissue, skeletal muscle and/or β cells. *Aim:* To unravel the DM2-specific miRNA signature, we studied the expression pattern of a panel of DM2-related miRNAs in patients and controls (CT). *Methods*: Based on that molecular profiling of blood cells reflects pathophysiological events in affected tissues, total RNA was isolated from peripheral blood of 40 DM2 and 37 CT subjects, polyadenylated and reverse transcribed. The expression patterns of 84 DM2-related miRNAs were tested in representative DM2 (n = 3) and CT (n = 4) samples, using the Human Diabetes

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miScript PCR Array (QIAGEN). Validation of the differential expression was performed in the total cohort by specifically developed qPCR protocols. Non-parametric statistical tests were applied to evaluate possible correlation with disease development. *Results:* Among the 84 miRNAs tested, let-7f-5p, miR-23b-3p, miR-451a, miR-24-3p, miR-27b-3p and miR-29b-3p were found to be down-regulated in the representative samples of patients *versus* CTs (fold-change ≥2; $P < 0.05$). Specific qPCR protocols applied in the total cohort validated the differential expression of miR-24-3p [median (range): DM2 = 2.4 (0.4–10) *vs.* CT = 4.1 (0.7–22.7); $P = 0.008$] and let-7f-5p [median (range): DM2 = 3.2 (0.7–39.2) *vs.* CT = 6.8 (1.1–61.1); $P = 0.01$]. *Conclusion:* Study of DM2 patients' peripheral blood reveals deregulated expression of miRNAs associated with insulin resistance (miR-24-3p, let-7f-5p) or targeting the *CDKN2A* DM2-susceptibility gene (miR-24-3p). Association with clinical data would add to the biological interpretation of these findings, with possible prognostic value.

P.09-116-Tue
Differentially expressed plasma microRNAs at early stages of Alzheimer's disease

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Alzheimer's disease (AD) is the most common age-related dementia. One of the major challenges in the AD field is deciphering in peripheral tissues the molecular signatures, characteristic of early stages of the disease in patients with mild cognitive impairment due to AD (MCI-AD). Using qRT-PCR we evaluated microRNA (miRNA) profiles in blood plasma collected from 15 MCI-AD patients, whose neuropsychological diagnoses were confirmed by cerebrospinal fluid (CSF) biomarkers, 20 AD patients and 15 non-demented, age-matched individuals (CTR). In the first screening 179 plasma miRNAs were compared between AD and CTR, and between MCI-AD and CTR. 23 differentially expressed miRNAs reported earlier as AD biomarker candidates in blood were confirmed in the current study and 26 novel differential miRNAs between AD and CTR were detected. The potential of these 15 miRNAs to be used as biomarkers was further verified in independent AD, MCI-AD and CTR groups. Finally, 6 miRNAs (3 novel in AD context and 3 reported) were selected as the most promising biomarker candidates differentiating early AD from controls with the highest fold changes (from 1.32 to 14.72), consistent significance, specificities from 0.78 to 1 and sensitivities from 0.75 to 1), (patent pending, PCT/IB2016/052440). The miRNA panel is promising for diagnostics of early AD. The TargetScan, MirTarBase and KEGG database analysis indicated putative protein targets of the differential miRNAs involved in key cell processes such as cell cycle, apoptosis and cancerogenesis.

P.09-117-Wed
Association of common KCNJ11 polymorphism E23K with type 2 diabetes and markers of glycaemic control, inflammation and obesity

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KCNJ11 gene encodes an inward-rectifier potassium ion channel (Kir6.2), and together with the high-affinity sulfonylurea receptor 1 (SUR1), forms the KATP channel. This channel modulates insulin production and secretion through glucose metabolism. The functional effects of *KCNJ11* polymorphism E23K (rs5219 C>T) on insulin secretion and insulin sensitivity in humans are also controversial, and has been reported to be inconsistent with Type 2 diabetes (T2D) in various ethnic groups. The aim of this study was to analyze the association of *KCNJ11* polymorphism E23K (rs5219 C>T) with clinical and biochemical parameters of T2D in Caucasian population from Bosnia and Herzegovina and the Republic of Kosovo. The study included 638 patients with T2D and prediabetes and 360 healthy controls of both sexes, aged from 40 to 65 years. Patients were recruited at the Clinical Centre University of Sarajevo, University Hospital of Clinical Centre in Banja Luka, General Hospital in Tešanj and Health Centre in Prizren. Genotyping of analyzed *KCNJ11* polymorphism E23K (rs5219 C>T) was performed by qPCR allelic discrimination at University Hospital Hradec Kralove (Hradec Kralove, Czech Republic), and by Sequenom MassArray IPLEX platform, in collaboration with the Lund University Diabetes Centre (Malmo, Sweden). Result of our study showed that allele T of *KCNJ11* polymorphism E23K (rs5219 C>T) was significantly associated with the most important causative agents in development of T2D, lower values of insulin and HOMA IR index, but on the other hand with lower values of anthropometric parameters (BMI and waist circumference and higher levels of fibrinogen. Also, important is a tendency of association of T allele with lower values of HbA1c in group of T2D patients, and with higher triglycerides and total cholesterol levels in group of T2D patients without any therapy.

P.09-118-Mon
Propanediol utilization genes as inflammatory bowel disease markers

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Increase of *Escherichia coli* content in intestinal microbiota is a dysbiosis often observed in Crohn's disease (CD) patients. Genes involved in utilization of propanediol were shown to be more

often found in genomes of CD-isolated *E. coli* than in isolates from healthy people. In this work four genes of this operon were evaluated as potential CD markers. Intestinal microbiota was extracted from feces or liquid ileum content by differential centrifugation. DNA was extracted using a CTAB-based protocol. Abundancy of four genes (*pduC*, *ccmK2_1*, *ccmL* and *pduU)* was evaluated by qPCR with primers targeting conservative regions. Tested samples were isolated from patients suffering from following inflammatory bowel diseases (IBD): CD (39 ileocolitis, 33 small intestine, 28 colon), ulcerative colitis (48 total UC, 32 left-sided or right-sided colitis, 30 distal colitis) and 10 other intestine diseases (diverticulosis, pancreatitis, irritable bowel syndrome). Non-IBD group included patients with colon cancer (17) and hepatic cirrhosis (16). Control group included 39 healthy volunteers. Samples from patients were collected in clinics of Moscow, St Petersburg and Kazan (Russian Federation). As a result, healthy group clustered together with cancer and cirrhosis. CD and UC cohorts were distinct from healthy but partly overlapped among themselves. CD group was more distant from healthy, than the UC group. Thus, the increased content of propanediol utilization genes in intestinal microbiota can be regarded as a marker of inflammatory bowel disease. Supported by Russian Science Foundation [16–15-00258].

P.09-119-Tue
DNM1L mutation and its impact on mitochondrial network and ultrastructure

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DNM1L gene encodes the dynamin 1- like protein DNM1L (Drp1) which is crucial in the mitochondrial fission process as well as fission of peroxisomes. Several mutations in DNM1L gene were described. The most cases were de novo missense mutations in middle domain that is important for the self-assembly of the protein and its oligomerization and few cases were in GTPase domain. We identified novel de novo mutation c.176C>T (p.Thr59Ile) in DNM1L gene. The mutation affects highly conserved Thr59 in the GTPase domain of the protein. Thr59 has been previously shown to be indispensable for GTPase reaction since it is involved in the positioning of the catalytic water molecule and Mg2+ coordination (Wenger et al 2013). In patient cells, regular occurrence of "mega-mitochondria" along elongated mitochondrial network was found in cultured myoblasts and fibroblasts which confirms impaired mitochondrial dynamic. Moreover, immunocytochemistry staining with catalase antibody revealed disturbances of peroxisomal fission. The dominant-negative effect of the mutation on DMN1L function was confirmed in cultured skin fibroblasts. Protein analysis in cultured skin fibroblasts (SDS-PAGE followed by Western blotting and immunodetection) revealed decreased amount of some OXPHOS subunits (COX2 and COX5a), decreased amount of Mitofilin protein plus differences in representation of OPA1 isoforms. Total amount of DNM1L protein was comparable to controls. Furthermore, in cultured skin fibroblast we compared impact of DNM1L mutations localized in GTPase domain and middle domain on mitochondrial network and mitochondrial ultrastructure. Our data provide further insight on into pathogenic mechanism of DNM1L deficiency. Supported by research projects GAČR 14-36804G, AZV 17-30965A, RVO-VFN64165/2012.

P.09-120-Wed
Effect of natural flavonoids - myricetin and dihydromyricetin on the wound healing process in vitro

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Myricetin (MYR) and dihydromyricetin (DMY) are classified as natural flavonoids. MYR is one of the key constituents of various human foods including fruits, vegetables, berries, teas and red wine, whereas DMY is found especially in two plants - *Hovenia dulcis* and *Ampelopsis grossedentata*. The structures of these two compounds are differ only in the double bond between the second and the third carbon of the flavone ring. Both substances are characterized by their anti-inflammatory and antioxidant properties. They are able to regulate the inflammatory response via NFκB and Nrf2 signalling pathways. MYR was reported to possess anti-inflammatory effect by inhibiting the activation of NFκB and with suppression of the production of free radicals. Both flavonoids inhibit the production of pro-inflammatory cytokines, especially interleukin 6 and 8 (IL-6, IL-8) and enzymes, such as cyclooxygenase (COX-2) and metalloproteinases (MMPs). However, there are only limited data in the literature about the anti-inflammatory activity of DMY. In this study, *in vitro* model of inflammation was demonstrated on monolayer of scratched fibroblasts exposed to *Pseudomonas aeruginosa* for six-hour. MYR and DMY were subsequently applied to this model for 24 h at sub toxic concentrations selected using MTT assay. Inflammatory parameters were analysed in collected cell medium and lysate after the incubation period using the Enzyme - Linked ImmunoSorbent Assay (ELISA) and the Western blot. The results of the individual methods will be discussed in the poster presentation. This work was supported by the grant IGA_LF_2018_012 and by LO1304.

P.09-121-Mon
Acute kidney injury in rats is affected by pregnancy

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Acute kidney injury(AKI) is a major factor of morbidity in the clinic. AKI occurs in 20% of patients hospitalized with acute illness, and the mortality rate can reach 40%. Pregnancy is a factor which affects AKI: AKI can occur in 10% of all pregnancies, and the mortality rate of pregnant women can reach 18%. On the other hand, studies report an increase in regeneration rate of some tissues during pregnancy. A contradiction emerges: pregnancy is a major risk factor for kidney pathologies, but basic research suggests, that it might be vice versa. We tried to address these issues using an experimental model of ischemic AKI on pregnant rats. In the study, we have shown, that during pregnancy kidneys are more tolerant to AKI. Pregnant animals showed remarkable preservation of kidney functions after ischemia/reperfusion(I/R), indicated by the drop of serum creatinine levels, compared to the non-pregnant group with kidney I/R. The

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pregnant rats also demonstrated a significant decrease in kidney injury marker NGAL and in MDA levels. Two months after I/R pregnant group has drastically decreased rate of fibrosis formation in kidney tissue. These effects are likely linked to increased cell proliferation in pregnant kidneys tissue after injury: using real-time cell proliferation monitoring we have shown, that after ischemic injury cells, isolated from pregnant animals kidneys, increase their proliferation rate in more pronounced manner, than cells from control animals. This observation was supported by increase of proliferation marker PCNA levels in pregnant kidneys. These effects at least partially were due to hormonal changes in mothers' organism since hormonal pseudopregnancy simulated pregnancy's effects. Our results demonstrate, that kidney during pregnancy are more tolerant to ischemic damage and suggest, that clinical outcome might be more affected by complications, rather than AKI itself. The study was supported by RFBR grants 17-04-01045, 18-04-01034, 18-34-00776.

P.09-122-Tue
Ellagic acid protects from myelin-associated sphingolipid loss in experimental autoimmune encephalomyelitis

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Experimental autoimmune encephalomyelitis (EAE), the most common model for multiple sclerosis, is characterized by inflammatory cell infiltration into the central nervous system and demyelination. Previous studies have demonstrated that administration of some polyphenols may reduce the neurological alterations of EAE. In this work, we show that ellagic acid, a polyphenolic compound with potent antioxidant and anti-inflammatory properties, is beneficial in EAE, most likely through stimulation of ceramide biosynthesis within the brain. EAE was induced in Lewis rats by injection of guinea-pig spinal cord tissue along with Freund's complete adjuvant containing *Mycobacterium tuberculosis*. Clinical signs first appeared at day 8 post-immunization and reached the peak within 3 days, coincident with body weight loss and reduction of the brain contents of both myelin basic protein (MBP) and sphingolipids, two main components of myelin. In contrast, rats receiving ellagic acid in the drinking water from two days before immunization showed delayed clinical signs and cumulative disease scores. This amelioration of clinical signs was accompanied by sustained levels of both MBP and sphingolipid classes in the brain cortex, without apparent changes in infiltration of inflammatory CD3+ T-cells, microglial activation or weight loss, which together suggest a neuroprotective effect of ellagic acid. Finally, we demonstrate that urolithins, the physiological ellagic metabolites found in serum, stimulate the synthesis of ceramide in a glioma cell line. These findings indicate that ellagic acid consumption has a neuroprotective effect in EAE rats. This work was supported by SAF2011-29951 (Ministerio de Economía y Competitividad e Innovación cofinanced by ERDF), S2013/ABI-2728 (Comunidad de Madrid) and CIBEROBN, ISCIII, Spain.

P.09-123-Wed
Autophagy activation induced by the first genetic variant of β2-microglobulin oligomers

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It is widely recognized that the mechanism of amyloid aggregate cytotoxicity requires the primary interaction between the aggregates and the cell membrane, resulting in functional and structural perturbation of the latter, which can induce cell death by apoptosis or autophagy. Autophagy is the process that helps to clean the cell from an exogenous or endogenous material that is useless or harmful to cells. This mechanism involves the transport of material present at the cytoplasmic level material through specific vesicles called autophagosomes in the lysosomal compartment. Generally, autophagy is induced by cell starvation, but other stresses can be caused by the activation of this process, such as the presence of misfolded protein deposits. Our study is focused on understanding the signaling pathway responsible for the autophagic process induced by the interaction of the first genetic variant of β2-microglobulin (B2M-D76N) aggregates with cell membranes. B2M-D76N is associated with a familial form of systemic amyloidosis. We have investigated the interaction process of amyloid aggregates at different stages of aggregation with specific membrane sites characterized by the presence of GM1 and the consequent activation of the signaling cascade responsible for the autophagic process. In fact, data acquired by Western Blot and confocal microscopy techniques have confirmed the activation of autophagy in SH-SY5Y cells treated with B2M-D76N oligomers. The formation of autophagosomes and the increase of LC3-II, the main marker of autophagy, were observed at 5 h of treatment. At the cytoplasmic level, a precise autophagic pathway has been identified that involves AKT and GSK3β upstream of the mTOR complex, whose inhibition promotes the activation of autophagy, and P-S6 downstream. Finally, the latest experiments seem to indicate the involvement of the IGF1R receptor in the activation of the above-described signaling pathway but this data needs further analysis.

P.09-124-Mon
Probucol downregulated the expression of angiogenesis-associated proteins in SW480 colorectal cancer cells

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Probucol is a diphenolic compound and it's been used in clinical application for a long time because of its effects anti-oxidative, anti-hyperlipidemic, anti-diabetic, and anti-inflammatory properties. Clinical studies have shown that probucol has different pharmacological effects on cardiovascular and metabolic diseases. It can also serve as a potent chemopreventive agent by suppressing tissue injury and modulate the toxicity-promoting effect. The aim of this study was to evaluate the effects of probucol on cellular proliferation in SW480 human colorectal cancer cells and investigate the anti-proliferative and anti-metastatic mechanism of probucol in these cells. We found that probucol significantly decreased cell growth of SW480 cells in a dose dependent manner ($P < 0.0001$). The inhibition of cellular proliferation by probucol was caused by a cell cycle arrest at G0/G1 phase. Treatment with

probucol had significant effect on the percentages of Annexin V-FITC positive cells and the increase in multicaspase levels when compared with the vehicle group. We found a decreased protein expression of IκBα, phospho-IκBα, p53 and bcl-2, whereas a significant increase in bax protein levels. Probucol has also down-regulated the expression of angiogenesis-associated proteins such as VEGF, MMP-9 and eNOS. Our results indicate that probucol induces anti-proliferative effects via blocking of cell cycle progression and represent an antimetastatic potential by inhibiting mRNA expression of metastatic MMP-9 protein in SW480 cells.

P.09-125-Tue
Synthesis of charged meso-arylporphyrins – potential photosensitizers for antimicrobial PDT

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Infectious diseases are the second leading cause of death worldwide and it's directly related to the increasing resistance of many pathogens to the antibiotics. Antimicrobial photodynamic therapy (APDT) resolves these problems. Tetrapyrroles is widely uses in APDT due to the ability of these dyes to generate singlet oxygen 1O2 upon irradiation. In this study, new PS for APDT based on charged cationic and anionic amphiphilic meso-arylporphyrins was created. First, approaches to the synthesis of charged amphiphilic meso-arylporphyrins (cationic and anionic) have been developed and optimized. The main strategy is based on the preparation of pre-functionalized benzaldehydes, which was introduced into the reaction of monopyrrolic condensation according to Lindsay's method. Further, cationic porphyrins (pyridinium and ammonium) were obtained by the quaternization reaction. Target anionic porphyrins were received by alkaline hydrolysis of the porphyrin precursors with terminal carbomethoxy groups. We've varied degree of lipophilicity, arrangement of the substituents, charge in porphyrins. Considering the prospects of synthetic porphyrins using for inactivation of microorganisms, the aggregation of potential PS in aqueous microheterogeneous systems imitating the intracellular environment was investigated. It was shown that the cationic derivatives of porphyrins in aqueous microheterogeneous systems forms J- and H-type homoaggregates, as well as mixed ionic associates in the presence of oppositely charged polyelectrolytes and premicellar aggregates of anionic surfactants. Also, we investigated binding of cationic porphyrins with cholesteric DNA dispersion. It was experimentally proved the formation of two types of complexes of all porphyrins with DNA: intercalated and external bedding along a small groove. Received porphyrins are promising candidates for further antimicrobial studies. This work was supported by the Russian Science Foundation, project No. 17-73-10470.

P.09-126-Wed
Transcription regulation of contents of some mitochondrial enzymes and transcription factors in human skeletal muscle

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Skeletal muscle adapts to changes in contractile activity by production of specific regulatory and structural proteins, leading to optimization of the contractile function. The aim of our work was to study the transcription mechanisms regulating the training-induced increase in content of mitochondrial enzymes and transcriptional factors involved in mitochondrial biogenesis in human skeletal muscle. For this purpose ten young untrained males performed the one-legged moderate intensity knee-extension for 1 h. Biopsies from *m. vastus lateralis* were taken prior to, and after the exercise. This test was repeated after 8 weeks of cycling aerobic training. After 8-wk training, the content of mitochondrial proteins at rest (NDUFB8, SDHB, UQCRC2, MT-CO1, ATP5A1) increased (by 35–162%, $P < 0.05$). There were no changes in the expression of genes encoding these proteins at rest and after single exercise (both before and after 8-wk training). An increase in the content of transcriptional regulators was found after 8-wk training for CRTC2 (60%, $P < 0.05$), NR4A3 (100%, $P < 0.05$) and TFAM (20%, $P < 0.06$). The expression of the *NR4A3* and *TFAM* genes increased ($P < 0.05$) after single exercise, both before and after 8-wk training. An increase in the expression of genes in response to single exercise was also observed for other transcriptional regulators (*PGC-1α, ESRRG*), but there was no increase in the content of their proteins after 8-wk training. The reason for the increase in the content of mitochondrial enzymes could not be linked to the increase in basal or exercise-induced expression of their genes. On the contrary, the training-induced increase in the content of some transcriptional regulators was associated with the activation of their mRNA expression after each single exercise. This work was supported by the Russian Science Foundation (grant no. 14-15-00768).

P.09-127-Mon
Modified GDNF has stimulated the neural differentiation of progenitor cells and it may be used in the treatment of Parkinson's disease and ischemic stroke

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GDNF therapy is effective for treating degeneration of dopaminergic neurons in Parkinson's disease. It is possible that pre-(α) pro-GDNF is needed for conventional neuron survival, and pre-(β) pro-GDNF serves as SOS system during traumatic injury of neurons or neurodegenerative diseases. To study 'pro' region function during fast transport and factor induction properties several versions of modified GDNF were made. Secretion of GDNF into medium has been shown. Then modified GDNF were introduced into HEK293 cells. Condition media after transgenic cell culturing was added into culture medium of rat embryonic spinal ganglion explant. Deletion of 'pro' region essentially increases GDNF effects as neural inductor. Study of culture of dissociated spinal ganglion and calculation of neural sprouts yielded the same results. Deletion of both pre- and pro-regions enhances trophic activity of GDNF (mGDNF). Spinal ganglia cultured in the presence of medium conditioned by cells transfected with mGDNF exhibited active growth of β-3-tubulin-positive axons by day 4. Then we also demonstrate neurotrophic effect of mGDNF for PC12 cells *in vitro*. Studies were also conducted in vivo. A model of Parkinson's disease was used, which was obtained by subcutaneous injection of MPTP into C57Bl / 6 mice. Implantation of cells producing mGDNF in caudatum-putamen smoothed out symptoms of Parkinson's disease in motor activity tests. Research is supported by Grant from RNF.

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P.09-128-Tue
Pt(II) and Pd(II) polyamine complexes in the spotlight of metastatic prostate cancer treatment

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Prostate cancer is the sixth cause of cancer-related death in men. When patients develop hormone refractory prostate cancer (HRPC), Docetaxel is administered. However, this drug is frequently related to acquired resistance which leads to a limited chemotherapeutic success and a poor prognosis. In the last few years, platinum drugs in combination regimens have been the object of a growing interest, although they induce several deleterious side-effects and acquired resistance, which often limits their clinical use. Thus, new strategies are needed for obtaining improved anticancer agents with a lower toxicity coupled to an optimised cytotoxic profile. Multinuclear polyamine complexes differing in the nature of the metal centre (either Pt(II) or Pd(II)) as well as in the characteristics of the alkylamine ligands (spermine or spermidine) were investigated in this study: $M_3(Spd)_2Cl_6$ and $M_2(Spm)Cl_4$; M = Pt(II) or Pd(II); Spd, Spermidine; Spm, Spermine. Their effect was tested towards the PC-3 human prostate cell line, derived from advanced androgen independent bone metastasis with a high metastatic potential the results having been compared with the non-tumour prostate cells PNT2. The antiproliferative activity (cell density) of the tested compounds was evaluated through the SRB spectrophotometric method, while the cytotoxicity (cell viability) was assessed by the mitochondrial dehydrogenase activity MTT colorimetric assay. Drug concentrations ranging from 5 to 100 μM were screened, for incubation periods of up to 72 h. The reversibility of the drug effect was also determined, by removing the drug-containing medium and replacing it by fresh, drug-free, medium. It was concluded that the Pd(II) complexes display a higher cytotoxic and antiproliferative profile relative to their Pt(II) analogues. The toxicity of both agents towards the non-neoplastic cells was found to be reversed upon drug removal (after *ca.* 3 days).

P.09-129-Wed
Design and in-vitro cell-based evaluation of lonidamine-loaded vitamin E TPGS-conjugated polymeric nanoparticles

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Lonidamine has generally been used as an antineoplastic agent against a wide range of solid tumors, such as metastatic breast cancer, and advanced ovarian cancer, for many years. It shows its activity through the inhibition of the aerobic glycolysis pathway via the direct inhibition of the enzyme, hexokinase 2. The main objective of this research is to design of lonidamine-loaded Vitamin E TPGS-conjugated polymeric nanoparticles (PNPs) and to evaluate their cytotoxic and apoptotic activities. In this respect, the physico-chemical characteristics of the PNPs were evaluated in terms of particle size, shape, loading capacity, surface charge, redispersibility index, storage stability, cytotoxicity, apoptosis, fluorescent imagining and cell cycle arrest in SW480 cancer cells. In this perspective, Vitamin E TPGS-conjugated PNPs of lonidamine were prepared using nanoprecipitation technique. The effects of PNPs on cell viability were determined by MTT test and in order to evaluate apoptosis, annexin V binding assay, multicaspase assay, cell cycle and fluorescent imaging studies were performed. The average diameter of PNPs were obtained between 149.3–312.3 nm. The FESEM images of the nanocarriers demonstrated that the particles were in spherical shape. The potency of the PNPs on the proliferation proficiency of SW480 cells was showed that all formulations inhibited cell proliferation efficiently in a dose-dependent manner. Cell cycle analysis demonstrated that lonidamine loaded PNPs showed a significant arrest in G0/G1 phase of cell cycle when compared to control ($P < 0.001$). The results also showed that all PNPs induced apoptosis in a dose dependent manner. Among them, the nanoparticle formulation containing the lowest concentration of Vitamin E TPGS has represented the most potential apoptotic effect, since it has induced annexin V binding at 10 μM and higher concentrations in accordance with the increase on caspase levels.

P.09-130-Mon
The combined effect of probiotic and chondroprotector on genes expression Ptgs2, Tgfb1 and Col2a1 in the cartilage of rats with monoiodoacetate-induced osteoarthritis

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Osteoarthritis (OA) is a chronic degenerative-dystrophic disease of joints caused by a broad range of factors. Chondroprotector (CP) therapy is widely used treatment of OA but still causes disputes over efficiency. Previously, we showed the anti-inflammatory effect of probiotic (PB) diet. In this study, we try to compare the effectiveness of CP course and PB diet alone and with their administration together during OA in rats. We used a single injection of monoiodoacetate through the infrapatellar ligament of Wistar male rats to start OA model. Therapeutic groups got an intramuscular injection of CP ("Drastop", World Medicine, UK) daily from 2nd to 29th days of experiment and intragastric feeding of PB ("Symbiter", O.D. Prolisok, Ukraine) from 8th to 21st days of the experiment, alone and together. Cartilage sampling was provided on the 30th day. We made a histological observation of the samples and analyzed gene expression of proinflammatory mediators (Ptgs2 codes cyclooxygenase-2, Tgfb1 – tumor growth factor β) and Col2a1, which produces the basis of joint cartilage - collagen II type. We used the single-step method with acid guanidinium thiocyanate-phenol-chloroform extraction to isolate RNA. Synthesis of cDNA with real-time quantitative RT-PCR was made with specific forward and reverse primers to Ptgs2, Tgfb1, and Col2a1 primers. In OA group, the expression of Ptgs2 and Tgfb1 raised and Col2a1 decreased comparing to control group. Separated administration of CP and PB in OA group showed positive changes in the expression of the genes, but it did not reach control values. Their combined using was more effective than separate applying and it was approaching to control. Histological analysis approved molecular findings. PB applying may be perspective to improve the standard treatment of OA and it needs further investigation.

P.09-131-Tue
Studies on the structure activity relationships of natural and semisynthetic polyene antibiotics of the Amphotericin B group

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Despite Amphotericin B (AmB) therapy is limited by considerable toxicity, it is still the drug of choice for the treatment of mycotic infections caused by a wide range of fungi especially in cases of life-threatening infections in immunocompromised patients. Better understanding of structure – activity relationships and clarified molecular description of the biological activities of polyenes are two critically important objectives for improving the poor therapeutic index of AmB and the development of other resistance-refractory antimicrobial agents on the bases of polyene antibiotics. We have performed a comprehensive comparative analysis of the structure – antifungal activity relationship for the vast series of nystatin analogues, their novel semisynthetic derivatives, as well as AmB and its semisynthetic derivatives including AmB-benzoxaborole conjugates. Some of the derivatives were tested to determine whether they form pores in lipid bilayers and to compare the characteristics of channels, also their ability to bind with ergosterol was evaluated by the quantum chemical method. The data obtained revealed the influence of the structure of the C7 – C10 polyol region and the substituent in the position C16 (CH_3, COOH or CONHR) of the macrolactone ring or in the 3'-N-position of mycosamine moiety on the antifungal activity of the polyene antibiotics. The significant correlation between the ability of the tested derivatives to increase the permeability of model membranes—causing the appearance of single channels in lipid bilayers or inducing calcein leakage from unilamellar vesicles—and the minimal inhibitory concentration indicated that the antifungal effect of the conjugates was due to pore formation in the membranes of target cells also all of retain the ability of AmB to interact with ergosterol. This work was partly supported by the Russian Foundation for Basic Research (grant No. 16-34-60110).

P.09-132-Wed
Osteoporosis related to celiac disease in children

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Untreated celiac disease can lead to certain complications. One of these complications is osteoporosis. Children with gastrointestinal symptoms, including chronic diarrhea and malabsorption, developmental delay were considered to have classical Celiac Disease (CD). CD is an inherited intestinal disorder in which the body cannot tolerate gluten. Gluten is a protein found in wheat, rye, barley, farina, and bulgur. When people with celiac disease eat foods containing gluten, their immune systems respond by attacking and damaging the lining of the small intestine. The small intestine is responsible for absorbing nutrients from food into the bloodstream for the body to use. When the lining of the small intestine is damaged, nutrients cannot be absorbed properly into the body. Bone mineral density(BMD) was measured with dual-energy X-ray absorptiometry (DEXA) in two children with developmental delay, and gastrointestinal symptoms. Z-scores and T-scores were determined. Osteoporosis was considered present when a Z-score was <−2, T- score less than -2.5. First girl was 5 years old, T- score was -5.9; second girl was 13 years old and T- score was -5.5. Complete blood analysis were performed for two of them. Definitive diagnosis was made with biopsy by Esophagus gastro duodenoscopy. Measurements of bone density in children with developmental deficits appear to be a finding of the disease. Various blood tests as well as measurements of bone density may be useful. In the case of osteoporosis, celiac should definitely be suspected.

P.09-133-Mon
Reactive oxygen species production by neutrophils activated by Crohn's disease *Escherichia coli* in vitro

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Crohn's disease (CD) is characterized by chronic and/or acute inflammation and is often accompanied by an increased presence of *Escherichia coli (E. coli)* in the gut microbiome. We studied *E. coli* isolates from CD patients in regard to their pathoadaptivity and capability of evading the attack of neutrophils, key players of innate immunity in inflammation. *E. coli* isolates (n = 27) were obtained from ileal aspirate, ileal biopsy and faeces of seven patients. *In vitro* activation of neutrophils in the whole blood in response to *E. coli* was assessed by luminol chemiluminescence (LCL) and morphological examination of blood smears. Bacterial peroxidase and catalase activities were measured without cell disruption using spectrophotometric methods. The results revealed a diversity in parameters under study not only among isolates from different patients, but also among isolates from one and the same patient. LCL values ranged from 2.9 ± 1.2 to 52 ± 13 mV, peroxidase activity differed at the most by 3 times, catalase activity – by 12 times. Despite of this diversity, a significant negative correlation ($P < 0.05$) was found between LCL and bacterial peroxidase activity. In control experiments, the addition of an exogenous peroxidase at the relevant concentrations had no effect on the *E. coli*-stimulated LCL of blood. Lucigenin-enhanced CL showed positive correlation with LCL ($p < 0.01$). Morphologic signs of neutrophil activation were observed even for isolates capable of inducing only low LCL. However, isolates that caused low LCL and those that caused high LCL differed in their ability to stimulate phagocytosis. Complex regulation of the level of reactive oxygen species produced by activated neutrophils could be considered a part of Crohn's *E. coli* pathoadaptivity. The research was supported by Russian Science Foundation (16-15-00258).

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P.09-134-Tue
Novel inhibitors of HIF prolyl hydroxylase and their preclinical characterization

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Hypoxia-inducible factor (HIF) triggers the genetic program of cell survival under conditions of limited oxygen. HIFα consists of two subunits, where the HIF subunit is subject to proteasomal degradation in the presence of oxygen and is stabilized under hypoxia. HIF prolyl hydroxylase (PHD), an a-ketoglutarate-dependent non-heme iron dioxygenase, hydroxylates the C-terminal Pro564 and N-terminal Pro402 in HIFα, providing their recognition by von Hippel–Lindau protein (VHL), ubiquitinylation and proteasomal degradation under the state of hypoxia. It has been previously shown that luciferase fusion reporter of transcription factor HIF1 can be successfully used for both research and drug discovery purposes. In this work the new development of such reporter, including a combination of interacting pairs on one and the same reporter vector, is presented. This new strategy is illustrated by the construction and characterization of reporters combining HIF ODD-luc and HIF prolyl hydroxylase isoforms (HIF PHDs). In this case the cell is used as a microreactor for an enzymatic assay. This new approach has been used to optimize the structure of a branched tail oxyquinoline pan-inhibitor of HIF PHDs (adaptaquin). The negatively charged substitutions in the "tail" of the branched oxyquinolines have been shown to increase the inhibitor preference for PHD1 and PHD3 over the PHD2 isoform. The effects of optimized versions of HIF PHD inhibitors have been compared with hypoxia and commercially available HIF PHD2 inhibitors by microarray technique. The newly developed HIF PHD inhibitors work at submicromolar concentrations and show no toxicity up 200 μM in a liver-on-a-chip device; they are metabolized by CYP3A4 and CYP2B6. Activation of the latter by the HIF PHD inhibitors of the oxyquinoline group has been observed. The work is supported by Russian Scientific Foundation grant 16-14-10226.

P.09-135-Wed
The protective effects of conivaptan and mannitol on liver and kidney in a cerebral ischemia-reperfusion animal model

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The aim of this study was to investigate the post-ischemic effects of an antidiuretic hormone antagonist conivaptan and a sugar alcohol mannitol on liver and kidney injury in an experimental cerebral ischemia-reperfusion (I/R) model. 58 eight-week-old Sprague-Dawley rats were randomly divided into 5 groups: Control (Sham), I/R+saline, I/R+20% mannitol, I/R+10 mg/mL conivaptan, and I/R+20 mg/mL conivaptan. Cerebral ischemia was carried out using bilateral common carotid artery occlusion (CCAO). After 30 min, clamps removed and brain reperfused for 6 h. Blood, liver/kidney tissue samples were taken at 6th h of reperfusion. Serum urea nitrogen, creatinine, calcium, magnesium and phosphorus concentrations and also alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase activities were measured using commercial kits. Liver/kidney tissue slices were stained by Hematoxylin-Eosin in order to evaluate histopathological changes. Statistical analyses revealed that conivaptan was significantly reduced the concentrations/activities of the measured serum parameters when compared with I/R and MAN groups. Histological examinations showed that cerebral I/R caused only a few slight changes in structure of liver, and mannitol or conivaptan eliminated these changes. There was no edema, inflammatory cell infiltration, haemorrhage or necrosis in any of the groups. In kidney, cerebral I/R has led to changes in structure of glomeruli such as compacting, shrinkage and narrowing of Bowman's capsule space, without causing any change in tubular structures. Conivaptan has removed these changes bringing renal histology to a similar state in control. This is the first study which showed the effects of conivaptan against mannitol on remote organ injury in an experimental cerebral I/R model induced by CCAO. Our findings revealed that conivaptan is very efficient against remote tissue injury than mannitol as evidenced by biochemical/histopathological examinations.

P.09-136-Mon
Periostin deficiency increases osteoclast numbers and aggravates bone erosion in murine models of rheumatoid arthritis

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Periostin (POSTN), a matricellular protein, is involved in many fundamental biological processes such as bone metabolism, cell proliferation, cell invasion, and angiogenesis. Although POSTN expression has been reported to promote migration and invasion of fibroblast-like synoviocyte (FLS) in vitro, there is no study to investigate the role of POSTN in mouse models of rheumatoid arthritis (RA). This study was performed to assess the function of POSTN in 3 mouse models of arthritis, K/BxN serum transfer arthritis (STA), collagen-induced arthritis (CIA) and collagen-antibody induced arthritis (CAIA). STA, CIA and CAIA was induced in $POSTN^{-/-}$ and $POSTN^{+/+}$ mice. Arthritis was monitored in 3 mouse models of arthritis using defined criteria (clinical and histologic). Osteoclastogenesis was assessed using bone marrow monocytes (BMM) cultures from $POSTN^{-/-}$ and $POSTN^{+/+}$ mice. POSTN level in synoviocyte tissue were increased in patient with RA compare to OA patient. In STA studies, the clinical score and hind paw thickness were significantly increased in $POSTN^{-/-}$ mice compared with $POSTN^{+/+}$ mice. Mean histologic severity scores including synovial inflammation, bone erosion and cartilage damage were increased in diseased joints from $POSTN^{-/-}$ mice compared with those from $POSTN^{+/+}$ mice. The IL-1β was increased in the serum, and TNF-α, IL-1β, and MMPs were increased in the ankle of $POSTN^{-/-}$ mice than wild type control. BMMs from $POSTN^{-/-}$ mice showed increased osteoclast formation compared with BMMs from $POSTN^{+/+}$ mice. Similarly, in CAIA and CIA model, both mean clinical severity scores and ankle joint swelling were significantly increased in $POSTN^{-/-}$ mice compared with $POSTN^{+/+}$ mice. In conclusion, this study suggests that POSTN contributes to pathogenesis of RA and might have a potential protective role in RA.

P.09-137-Tue
Biological activity of echinochrome included in polysaccharide matrix

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Sulphated polysaccharide carrageenans (CRGs) of different structural types isolated from red algae of the Pacific coasts exhibit diverse biological activity. Due to its high ability to adsorb water, CRG can improve drug dissolution and thus increase the oral bioavailability poorly water- soluble drugs. Echinochrome (Ech) is the water-insoluble active substance in the cardioprotective drug Histochrome®, produced in Russia. CRGs matrixes have been used to incorporate of Ech in order to study the effect of the polysaccharide on the Ech biological properties. CRGs modified the biological activity of Ech, they decreased the Ech ability to induce the IL-6 and TNFα synthesis and increased the IL-10 synthesis which was inhibited by Ech alone. LPS-induced synthesis of the TNFα level was insignificantly decreased by Ech, whereas the IL-10 synthesis was marked by the activating effect of Ech in CGRs matrix. Blocking TLR4 on blood cells with monoclonal antibodies revealed no effect of CRG on TNFα induction, whereas some inhibition by Ech in the CRGs matrix was observed. HT-29 cells were investigated under normal and stress conditions; induced by exposure to ethanol to study the influence of CRGs and Ech on their viability in a real-time system. This effect was evaluated in combination with LPS, a common component of the gastrointestinal tract. CRGs with low sulphate content and the presence of 3,6-anhydrogalactose alone or in combination with LPS are prerequisites for the recovery of ethanol-exposed. Ech also possessed an ability to restore cells after exposure to ethanol. This work was supported by the Russian Science Foundation (RScF grant, project 16-14-00051).

P.09-138-Wed
Disruption of protein arginine N-Methyltransferase 1 in germ cells causes infertility in male mice

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PRMT1 is a member of the Protein N-Arginine Methyltransferase (PRMT) family, which catalyzes the formation of monomethylarginine and asymmetrical dimethylarginine, and regulates important biological processes through post-translational modification of various proteins such as chromatin proteins, structural proteins and transcription factors. However, the function of PRMT1 in spermatogenesis remains largely unknown. In this study, we found that PRMT1 was abundantly expressed in mouse male germ cells. Especially, the early spermatogonial cells located immediately beneath the seminiferous tubular wall retained strong expression of PRMT1. Generation of germ cell-specific PRMT1 KO mice demonstrated that PRMT1 was essential for male fertility and spermatogenesis. The PRMT1 KO mice were normal in growth, but were completely infertile and had much smaller testes with 70% reduction in testis weight at 5 weeks. Histological analysis revealed that in PRMT1$^{-/-}$ testes, spermatogenic cells failed to differentiate, without producing haploid cells during the first round of spermatogenesis. Asymmetric dimethylation of histone 4 arginine 3, which is one of the major function of PRMT1, was found to be much reduced in germ cells of PRMT1 KO testis. It was also found that PRMT1 deletion altered expression pattern of spermatogenesis-related genes as well as some meiosis-specific genes. Taken together, the results suggest that the PRMT1 deficiency influences the expression pattern of spermatogenesis-related genes, affecting multiple biological processes including meiosis and differentiation during spermatogenesis, which finally leads to infertility in male mice.

P.09-139-Mon
Effect of Korean red ginseng on hypertriglyceridemia in high fat/cholesterol diet rat

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Korean Red Ginseng (RG) are used as a traditional treatment for improve blood circulation. This experimental study was designed to investigate the inhibitory effects of RG (from JinAn) on lipid metabolism in high fat/cholesterol diet (HFCD)-induced hypertriglyceridemia. Sprague Dawley rats were fed the HFCD diet with/without fluvastatin (Flu, positive control) 3 mg/kg/day, and RG 125 or 250 mg/kg/day, respectively. All groups received regular diet or HF diet, respectively, for 13 weeks. The last three groups treatment of Flu and RG125, and RG250 orally for a period of 9 weeks. Treatment with low or high doses of RG markedly attenuated plasma levels of triglycerides and augmented plasma levels of high-density lipoprotein (HDL) in HFCD-fed rats. RG and Flu also led to an increase in lipoprotein lipase activity in the HFCD group. On the other hand, RG and Flu led to a decrease in fatty acid synthase and free fatty acid activity in the HFCD group. Treatment with RG suppressed increased expressions of PPAR-α and AMPK in HFCD rat liver or muscle. In addition, the RG attenuated triglyceridemia by inhibition of PPAR-γ and FABP protein expression levels and LXR and SREBP-1 gene expression in liver or muscle. The RG significantly prevented the development of the metabolic disturbances such as hypertriglyceridemia and hyperlipidemia. Taken together, the administration of RG improves hypertriglyceridemia through the alteration in suppression of triglyceride synthesis and accentuated of triglyceride decomposition. These results suggested that JinAn Red Ginseng is useful in the prevention or treatment of hypertriglyceridemia-related disorders such as triglyceride metabolism.

P.09-140-Tue
DAX1 expresses in Leydig cells modulates testicular steroidogenesis

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Testosterone is male sex hormone which is essential for the development of male reproductive system and function. Biosynthesis of testosterone in animal mainly occurs in testicular Leydig cells. Many steroidogenic proteins such as StAR, 3βHSD and P450c17 are involved in testicular steroidogenesis. The orphan nuclear receptor Nur77, which is induced by LH/cAMP signaling in Leydig cells, plays an important role in the steroidogenesis by activating the expression of steroidogenic genes. DAX1, which is a member of orphan nuclear receptor superfamily of transcription

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factor, is necessary for proper testicular development and function. It was previously found that DAX1 functions as a negative coregulator of Nur77 and represses Nur77-induced P450 promoter activity in Leydig cell lines. In this study, we examined a role of DAX1 in testicular steroidogenesis *in vivo* by generating Leydig cell-specific Dax1 knockout (KO) mice. RIA assay revealed that the level of testosterone was increased in Leydig cell-specific Dax1 KO testis compared to wild-type. In addition, the expression of steroidogenic genes and proteins such as P450c17, StAR and 3β-HSD were significantly increased in Dax1-deficient Leydig cells. Taken together, these data suggest that Dax1 regulates the expression of steroidogenic genes, thus controlling and fine-tuning steroidogenesis during testicular development.

P.09-141-Wed
Myelin basic protein-induced RAGE expression in M1/M2 macrophages

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Myelin basic protein(MBP) is one of the candidate autoantigens of the human inflammatory demyelinating disease multiple sclerosis(MS), which is characterized by the active degradation of the myelin sheath. MBP has extensive posttranslational modifications, including deimination of arginine residues. The deiminated (citrullinated) MBP C8 isomer is divided into two fractions: stathmin-containing C8a and C8b. Macrophages are important effector cells involved in the pathogenesis of MS. They play a dual role in MS which is explained by the fact that upon activation with specific cytokines macrophages can assume M1-classically activated or M2-alternatively activated cellular states and produce either pro- or anti-inflammatory cytokines and thereby perform their functions. Multiple studies have elucidated that ligand– receptor for advanced glycation end products(RAGE) interaction on cells, such as macrophages mediates cellular migration and upregulation of proinflammatory molecules. Considering all this data, we have investigated the effect of MBP isomers (C8a and C8b - most citrullinated and C1-the most cationic) on the expression RAGE in different phenotypes of Raw264.7 macrophages. We have found that in control-untreated macrophages membrane RAGE expression is significantly decreased in both M1/M2 phenotypes to compare with nonpolarized cells. Addition of C8a significantly increases intracellular RAGE to compare with C8b/C1. C8a has the same effect on the M1/M2 polarized macrophages, as C1, it decreases intracellular RAGE in M1/M2. On the other hand, the expression of RAGE on the plasma membrane of MBP-treated polarized and non-polarized macrophages looks differently: in nonpolarized cells RAGE expression is increased by C8b and C1 compared with control. In M1 cells significantly increase is marked for C8b activation and in M2 significantly increase is marked for C1 activation. We suggest that RAGE internalization may play a significant role in polarized macrophages.

P.09-142-Mon
The effect of the myelin basic protein charge isomers on the intracellular and extracellular HMGB1 level in M1/M2 macrophages

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Myelin basic protein (MBP) is one of the principal constituents of the mammalian myelin sheath and plays a structural role in maintaining myelin stability. Based on the post-translational modifications, this protein contains several forms termed charge isomers. Alteration of MBP cationicity may represent a regulatory mechanism for normal myelin assembly or a degradative mechanism in multiple sclerosis. In the progression of MS also is inserted immune system, especially macrophages. Macrophages are capable of producing pro-inflammatory cytokines, including high mobility group-B1 (HMGB1) proteins, which have the potential to damage myelin. HMGB1 has recently been shown to play a critical role in several inflammatory diseases. We have studied the effects of myelin basic protein charge isomers on the release of HMGB1 in M1/M2 Raw264.7 macrophages. We determined the content of intra- and extracellular HMGB1 in polarized and non-polarized macrophages after stimulation with MBP isomers. We have found that macrophages release HMGB1 in response to myelin basic protein charge isomers. Addition of the C8- citrullinated MBP isomer to the non-polarized macrophages is characterized by high level of intracellular HMGB1, as compared with non-citrullinated isomer C1. The same effect is characteristic for the M1 macrophages (classically activated macrophages). As about the M2 cells (alternatively activated macrophages), the amount of intracellular HMGB1 is not changed by the addition of MBP charge isomers. On the other hand, the secretion of HMGB1 by action of MBP charge isomers in the cell media has a different effect. In the media of non-polarized cells is decreased as for C8 as well for C1 compared with control cells. In the media of M2 cells, the concentration of HMGB1 is increased by acting C8 and C1 compared to the control, especially high is for C1. We propose that MBP –derived HMGB1 may be one of the major players in macrophage polarization.

P.09-143-Tue
Regulation of lipid production by TRK-fused gene (TFG) in human sebocytes

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TRK-fused gene (TFG) is located in endoplasmic reticulum (ER) exit sites. This protein is known to be involved in COPII-coated vesicle formation, together with many scaffold proteins such as Sec16 and Sec13. Since it has been recognized that many lipid molecules are synthesized in ER, we investigated the putative role of TFG in lipid production of sebocytes. We cultured immortalized human sebocytes, and induced lipid production by treatment of insulin-like growth factor 1 (IGF-1). The putative role of TFG was investigated using the recombinant adenoviruses expressing TFG and/or microRNA specific for TFG. When sebocytes were treated with IGF-1, the lipid production was increased. During IGF-1-induced lipogenesis, the protein level of TFG was increased in the time- and dose-dependent manners. When TFG was overexpressed using recombinant adenovirus, the lipid production of sebocytes were increased. Consistent with this result, regulators for lipid synthesis such as PPARγ, SREBP1 and SCD

were also increased by TFG overexpression. Conversely, knockdown of TFG using microRNA resulted in the decrease of SREBP1 and SCD. These findings suggest that TFG is a novel regulator for lipid production in sebocytes, which can be a new target for acne treatment.

P.09-144-Wed
Regulation of biofilm formation in bacilli

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Biofilms are a factor of pathogenicity, have multiple resistance, cannot be treated by traditional antibiotic methods and are the basis for the development of chronic processes in the body. Until now, there are no reliable ways to combat biofilms, this problem requires further development. Bacteria of the *Bacillus* genus are convenient model microorganisms for studying the architecture of biofilms and the action of various biological agents. In our laboratory we selected a culture medium for the formation of biofilms by *B. subtilis* strains. There are differences in the molecular mechanisms of formation and regulation of biofilms in different species of bacilli. Common is the process of repression of the biofilm matrix formation by the *SinR* protein, which inhibits the transcription of the operon from 15 genes, the eps-operon, whose function is the biosynthesis of extracellular matrix polysaccharides. In our laboratory comparative analysis of sinR genes was performed in different representatives of bacilli, including pathogenic species - *B. cereus, B. thuringiensis, B. anthracis* and saprophytic - *B. subtilis* 168, using the Clustal Omega program. It was found that the sequence of sinR genes in pathogenic strains is almost 100% identical. However, the similarity of the gene sequences of sinR pathogenic species and *B. subtilis* is only 72%. This probably explains the formation of biofilms with different architectures in *B. subtilis* and pathogenic. In the longer term, we plan to construct mutant strains of *B. subtilis* 168 with the inactivated sinR gene, as well as bearing point mutations in this gene. This will allow us to evaluate the role of mutations in the gene sinR in the formation of various phenotypes of biofilms in bacilli. Understanding these mechanisms is important for finding new strategies in creating agents to combat biofilms. The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

P.09-145-Mon
Colloid-chemical regression model in analysis of dynamic surface tension correlation with triglycerides and cholesterol content in the cow blood serum

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The application of mathematical methods in biochemical sciences leads to the expansion of analytical methods for diagnostics of the physiological-biochemical status of various animals. The use of the regression method in modeling the interrelation between some biochemical parameters and dynamic surface tension (DST) of the animal blood serum was reported for the first time. The main aim of our research is to study the major lipid parameters of the cow blood serum, as well as the application of a regression model for the estimation of the triglycerides (Tg) and cholesterol (Ch) content in the cow blood serum using the measured DST parameters at the liquid/air interface. Blood from experimental animals was taken before morning feeding (on an empty stomach) from the vein. All procedures were carried out with the approval of the Animal Care Committee of the Moscow SAVMB and internationally recognized guidelines. Six DST parameters of the cow blood serum were obtained at different times of surface existence (sigma 0–3: s_0, s_1, s_2, s_3, and lambda 1–2: λ_1, λ_2). The biochemical composition was characterized by Tg and Ch content. The regression coefficients obtained as the result of the regression model provided the following regression equation for the Tg content in cow blood serum: $[Tg] = -0.11\ \sigma_1 + 0.14\ \sigma_2 - 0.1\ \sigma_3 - 0.09\ \lambda_1 + 5.79$. The regression equation for the Ch content is the following: $[Ch] = 0.25\ \sigma_1 - 0.22\ \sigma_2 - 0.1\ \lambda_0 + 0.12\ \lambda_1 + 0.71$. The proposed model has predictive value and can be used to estimate Tg and Ch content in cow blood serum by the obtained DST parameters. The regression correlation analysis can contribute to better understanding of the influence of the major individual components of biological liquids and for the diagnostics purposes in animal medicine and zootechnology. This work was supported by the Russian Foundation for Basic Research (grant 18-016-00207).

P.09-146-Tue
Enzymatic production of anticancer thiosulfinates by targeted to ovarian cancer cells methionine γ-lyase

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Pyridoxal 5'-phosphate-dependent methionine γ-lyase (EC 4.4.1.11, MGL) is involved in the metabolism of sulfur-containing amino acids of bacteria, unicellular eukaryotes and fungus. We have shown that MGL effectively catalyzes the β-elimination reaction of S-alk(en)yl-L-cysteine sulfoxides (alliin, methiin, propiin) to yield thiosulfinates which are capable of killing cancer cells. A variety of malignant cells contain specific markers. The possibility to use carboxy derivatives of isoflavones as carriers for affinity targeting of drugs to tumor cells expressing estrogen receptor of the β type such as ovarian carcinoma and others was recently reported. The isoflavone daidzein is a naturally occurring compound found in soybeans and other legumes. Acting as a weak estrogen, daidzein recognizes a putative plasma membrane estrogen receptor and a membrane-located estrogen receptor of β–related protein. The conjugates between daidzein and *Citrobacter freundii* MGL and its mutant form C115H MGL were prepared. Conjugates were specifically bound to ovarian carcinoma cells (SCOV-3) and the substrates (alliin, methiin and propiin) were added. Thiosulfinates enzymatically produced by tumor anchored MGL and C115H MGL killed tumor cells. The IC_{50} values for daidzein-enzymes conjugates with S-alk(en)yl-L-cysteine sulfoxides were determined. The usage of daidzein-mutant form C115H MGL conjugate is more promising due to its higher catalytic efficiency in the β-elimination reactions of S-alk(en)yl-L-cysteine sulfoxides. Thus the pharmacological couples of the mutant form enzyme with sulfoxides might be new anticancer agents. This work was supported by Russian Foundation for Basic Research (project # 18-04-00916-a) and by the Program of fundamental research for state academies for 2013-2020 years (# 01201363820).

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P.09-147-Wed
The association between clusterin rs11136000 C/T single nucleotide polymorphism and clusterin protein level in tear fluid in pseudoexfoliative glaucoma risk

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Pseudoexfoliation syndrome is an age-related systemic disorder of extracellular matrix characterized by the presence of fibrillar deposits in the anterior segment of the eye. Glaucoma due to pseudoexfoliation is called pseudoexfoliative glaucoma (PEG) and can cause blindness if it is not treated. Clusterin (CLU) is a multifunctional glycoprotein that is accumulated in pseudoexfoliative material. CLU expression level is unexpectedly low in aqueous humor of PEG patients. This study aimed to investigate the association between rs11136000 C/T SNP and CLU protein level in tears in PEG risk. The study population consisted of 80 PEG patients and 80 controls. All samples were obtained from Gülhane Education and Research Hospital, Ophthalmology Unit, Ankara, Turkey. Genomic DNAs were isolated from whole blood of subjects by salting out method. Genotypes were assigned by PCR-RFLP. Total protein concentrations were determined by Bradford protein assay and CLU concentrations were determined by ELISA in tear samples. CLU concentration in tear samples was found as 9.86 ± 9.75 μg/mL in PEG, 7.83 ± 6.67 μg/mL in controls ($P = 0.519$). PEG patients with TT genotype had significantly higher CLU ($P = 0.045$) and total protein concentration ($P = 0.032$) as compared to controls with the same genotype. Within controls, tear CLU concentration is significantly higher in TC genotyped subjects compared with CC genotyped subjects ($P = 0.011$). CLU concentration was correlated with total protein concentration ($r = 0.631$, $P = 0.000$ for PEG, $r = 469$, $P = 0.000$ for controls), but not with rs11136000 C/T SNP ($r = -0.090$, $P = 0.427$ for PEG, $r = -0.157$, $P = 0.164$ for controls). CLU rs11136000 C/T SNP, total protein and CLU concentrations in tear samples were analyzed for the first time in Turkish population in PEG. This work shows that total protein concentration in tear samples is significantly higher in PEG patients. This study was supported by TUBITAK (115S360).

P.09-148-Mon
The protective effect of nebivolol on renal ischemia/reperfusion injury in rats through the p38 MAPK and PI3K/Akt signaling pathways

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Renal ischemia-reperfusion (I/R) injury is a serious cause of acute renal failure. Nebivolol, a third generation β1-adrenergic receptor blocker with the capability to release NO from endothelium, has been shown to attenuate detrimental effects of prooxidant and inflammatory mechanisms. However, the effect of nebivolol on p38 MAPK and PI3K/Akt in renal I/R has not been studied yet. Hence, it was hypothesized that nebivolol may exhibits renoprotective effects through its possible effects on p38 MAPK and PI3K/Akt signaling pathways to prevent oxidative stress and inflammation. 20 Wistar albino rats were randomly divided into 3 groups; Sham, IR, IR+nebivolol. Renal I/R was performed through bilateral clamping of the pedicles 45 min ischemia followed by 24 h of reperfusion. Nebivolol (10 mg/kg, gavage) was administered 7 days before ischemia. High performance liquid chromatography (HPLC) and a colorimetric kit were used to analyze malondialdehyde (MDA) and superoxide dismutase (SOD), respectively. Also, mRNA expressions of interleukin-1 (IL-1) and tumor necrosis factor-a (TNF-a) were analysed by quantitative real-time PCR (qRT-PCR). Activation of p38 MAPK, PI3K/Akt signaling pathways and NF-κB p65 were evaluated by western blot. Besides, mRNA and protein expressions of kidney injury molecule-1 (KIM-1) were analyzed with qRT-PCR and enzyme-linked immunosorbent assay (ELISA).Nebivolol pretreatment significantly reduced interstitial inflammation during renal I/R, which was consistent with decreased TNF-α and IL-1β mRNA expressions. It also decreased KIM-1 mRNA and protein expressions. Nebivolol pretreatment also reduced MDA level and attenuated the reduction of SOD activity in the kidney during I/R. Moreover, nebivolol pretreatment could further suppress the p38 MAPK and NFκB p65, and also activate PI3K/Akt signalling pathway during renal I/R. In conclusion, our study suggests that nebivolol may represent a potential strategy to attenuate renal I/R.

P.09-149-Tue
A comparison of the effectiveness of silibinin and resveratrol in preventing alpha amanitin induced nephrotoxicity

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Effective antidotes are needed to prevent nephrotoxicity due to Amanita phalloides type mushrooms containing Alpha-Amanitin (α-AMA). In our study, the efficacy of protective effects of resveratrol in nephrotoxicity was investigated and compared with silibinin, a known antidote in a mice model of α-AMA-induced toxicity. α-AMA and %0.9 sodium chloride (α-AMA+NS) was administered simultaneously in the control group. In the treatment groups, resveratrol (30 mg/kg) was administered simultaneously with the a-AMA (α-AMA+SR), 12 hour (α-AMA+12R) and 24 hour (aAMA+24R) after α-AMA administration. Silibinin (5 mg/kg) was administered simultaneously with the α-AMA (a-AMA+Sil) and repeated every 6 h in silibinin group. Renal tissue specimens were examined histomorphologically, which were stored in paraffin-embedded blocks. Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (Gpx) and catalase levels were measured in renal tissue homogenates. Urea and creatinine levels were also measured. Histomorphological damage scores were significantly decreased in a-AMA+SR ($P < 0.01$) and a-AMA+12R ($P < 0.05$) groups compared to a-AMA+NS group. While catalase levels increased in a-AMA+SR group compared to a-AMA+NS group ($P < 0.001$), they decreased in a-AMA+12R($P < 0.01$), a-AMA+24R ($P < 0.01$) and a-AMA+Sil ($P < 0.001$) groups compared to α-AMA+SR group. MDA levels decreased in a-AMA+SR, a-AMA+12R and αAMA+24R groups ($P < 0.01$). SOD levels increased in a-AMA+SR, a-AMA+12R groups compared to a-AMA+NS group ($P < 0.05$) and they decreased in a-AMA+24R group compared to α-AMA+SR and a-AMA+12R groups ($P < 0.05$). While a-AMA increased serum urea and creatinine levels, histomorphologic injury scores and it decreased antioxidant enzymes in kidney. Early administration of resveratrol might prevent or heal the α-AMA-related nephrotoxicity partly through its antioxidant and anti-inflammatory effects.

P.09-150-Wed
Isomerization of Asp7 residue in amyloid beta alters its interaction with nicotinic acetylcholine receptor α7 and influences its receptor-mediated neurotoxicity pathways

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The amyloid-beta peptide (Ab) is a normal subnanomolar component of biological fluids; however, its deposition in the form of amyloid plaques is one of the hallmarks of Alzheimer's disease (AD). For the majority of AD cases, the initiating factor is still unknown, and such form of the disease is termed sporadic AD (sAD). A possible factor that triggers sAD is the aberrant post-translational modification of Ab. Earlier we have shown that Ab peptide, containing isomerized Asp7 residue (isoD7-Ab), shows increased toxicity for neuronal cells and, moreover, represents the only known synthetic Ab form, which promotes cerebral amyloidosis in AD model mice. We have hypothesized that the increased neurotoxicity of isoD7-Ab is attributed to its altered interaction with alpha7 nicotinic acetylcholine receptor (a7nAChR), which was identified as the important mediator for the pathogenicity of Ab oligomers. Indeed, inhibition of a7nAChR by α-bungarotoxin reduced the neurotoxicity of non-modified Ab, but not of isoD7-Ab in differentiated human neuroblastoma cells SH-SY5Y. Further, we have studied short-term and long-term effects of amyloid peptides on a7nAChR. Short-term (up to 30 min) pre-incubation with either Ab or isoD7-Ab reduced the activation of a7nAChR expressed in both neuronal cells and *Xenopus laevis* oocytes, however, the different kinetics of inhibition suggested different binding sites for these peptides on a7nAChR, which was further confirmed by molecular modelling. Long-term exposure (72 h) to Ab in accordance with previously published data reduced the surface a7nAChR in neuroblastoma cells by 40%, whereas isoD7-Ab had no effect on the receptor amount. Based on these findings, we can assume that more prominent pathogenicity of isoD7-Ab is governed by the distinct molecular mechanism, further investigation of which can help in better understanding of pathways underlying sAD development. Supported by the Russian Science Foundation grant #14-24-00100.

P.09-151-Mon
Study of 17β-HSD10 inhibitors with implications in neurodegenerative disorders

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17β-hydroxysteroid dehydrogenase type 10 (HSD10) is a mitochondrial protein involved in many physiological pathways, but was also found to be overexpressed in Alzheimer's disease (AD) and steroid-dependent cancer forms. In AD HSD10 can bind β-amyloid resulting in oxidative stress and cell toxicity. Modulation of this enzyme may be novel target for treatment of neurodegenerative disorders and/or cancer growth. For evaluation of potential inhibitors, the recombinant enzyme was produced in *E. Coli* and purified using standard chromatographic methods. Enzymatic assay was performed spectrophotometrically at 37 °C in microplate reader and kinetic parameters of enzyme were determined. Number of potential HSD10 inhibitors based on 1-(benzo[d]thiazol-2-yl)-3-phenylurea were tested. As a control was used known inhibitor of HSD10, AG18051, having IC_{50} 136 nM. Novel inhibitors were screened at concentration 10 mM and 1 mM to identify these with best inhibition ability. We identified 10 inhibitors (K845, K848, K849, K903, K1093, K1151, K1152, K1153, K1156 and K1392) with IC_{50} lower than 10 mM, the determination of basic kinetic parameters is in progress. These candidate compounds with optimized properties will be further studied using *in vivo* methods with implications to neurodegenerative disorders and/or cancer. This work was supported by the Ministry of Health of the Czech Republic (No. NV15-28967A) and Specific Research Project of Faculty of Science, University of Hradec Kralove (No. 2115-2018).

P.09-152-Tue
The ABC-type efflux pump MacAB is required for motility of *Serratia marcescens* SM6

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Serratia marcescens is a Gram-negative bacteria with increasing clinical importance. *Serratia* infections are often difficult to treat due to the prevalence of resistance to multiple antibiotics. Multidrug efflux pumps play a crucial role in the emergence of drug resistance. Macrolide-specific efflux pump MacAB was first identified in *E. coli* and later was shown to be important for virulence of *Salmonella enterica* ser. Typhimurium in mice. Motility is an important virulence factor for many bacterial pathogens. Here we show that *S. marcescens* MacAB efflux pump is not involved in protection of bacteria against macrolide antibiotic erythromycin, but it is required for protection against aminoglycoside antibiotics. We also show that inactivation of *S. marcescens* SM6 MacAB efflux pump severely affected swimming motility of the mutant strain in semisolid agar compared to the wild type. We further show that swimming motility of *ΔmacAB* mutant strain is restored when *macAB* genes are returned on the plasmid. Furthermore, transmission electron microscopy (TEM) showed that *ΔmacAB* mutant cells express fimbria-like surface appendages that are not present on the cell surface of *S. marcescens* wild type cells. Therefore, in addition to its well-established role in drug efflux, *S. marcescens* MacAB efflux pump is also required for motility of this bacterium. This work was supported by the Russian Science Foundation project 16-14-10200 and performed in accordance with the Russian Government Program of Competitive Growth of Kazan Federal University.

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P.09-153-Wed
Intermediates of orange carotenoid protein photocycle

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The 35-kDa Orange Carotenoid Protein (OCP) is responsible for photoprotection in cyanobacteria. It acts as a light intensity sensor and efficient quencher of phycobilisome excitation. Photoactivation triggers large-scale conformational rearrangements to convert OCP from the orange OCPO state to the red active signaling state, OCPR, as demonstrated by various structural methods. Such rearrangements imply a complete, yet reversible separation of structural domains and translocation of the carotenoid. In this study, we took advantage of single 7 ns laser pulses to study carotenoid absorption transients in OCP on the timescale from 100 ns to 10 s, which allowed us to detect a red intermediate state preceding the red signaling state, OCPR. In addition, time-resolved fluorescence spectroscopy and the assignment of carotenoid-induced quenching of different tryptophan residues derived thereof revealed a novel orange intermediate state, which appears during the relaxation of photoactivated OCPR to OCPO. Our results show asynchronous changes between the carotenoid- and protein-associated kinetic components in a refined mechanistic model of the OCP photocycle, but also introduce new kinetic signatures for future studies of OCP photoactivity and photoprotection. This work was supported by a grant from the Russian Science Foundation No. 17-74-30019.

P.09-154-Mon
Digestomics of cow's milk: casein-derived digestion-resistant peptides aggregate into functional complexes

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Allergy to cow's milk proteins is commonly reported in infants, with majority of them successfully outgrowing it by the end of childhood. Safer alternatives for introduction of milk into children's diet have been investigated and milk proteins hydrolysates with fragments less than 3 kDa are considered as hypoallergenic. The aim of this study was to identify digestion products of major milk allergens and to examine the IgE reactivity and allergenicity of short digestion-resistant peptides (SDRP) released by pepsin digestion of whole milk. Here, raw milk was subjected to simulated gastric digestion and analyzed by electrophoresis and Western blotting. In gastric digests α-lactalbumin and β-lactoglobulin were present mostly as intact proteins. High-resolution mass-spectrometry peptidomics analysis of SDRP fractionated from digests revealed that majority of the digestion-resistant peptides originated from caseins (97% of peptides). SDRP mostly overlapped with the known IgE epitopes of cow's milk allergens, with the average peptide length of 10.6 ± 3.5 amino-acids. The ability of SDRP to compete for IgE binding with individual milk allergens and the mixture of the milk proteins was demonstrated. Since thirty amino acids has been suggested as a minimal length of a peptide able to cross-link two IgE molecules on the surface of mast cells and provoke an allergic reaction, it was unexpected that SDRP of induced allergic *in vivo* responses (positive skin-prick tests) in 4 out of 5 milk-allergic subjects. Hence, aggregation ability of the SDRP was assessed and confirmed by size-exclusion chromatography. Our results prove that short digestion-resistant peptides mainly corresponding to the continuous epitopes of milk proteins induce an allergenic *in vivo* response due to aggregation.

P.09-155-Tue
Antineurocytoskeletal antibodies and their immune complexes in patients with neurodegenerative diseases

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Neurocytoskeletal proteins, such as neurofilaments (Nf), may be released from neurons during a neurodegenerative process and induce the synthesis of specific autoantibodies. These antibodies could form immune complexes with corresponding antigens. The autoantibodies are present both in a free form and bound in immune complexes. An analysis of free antibodies alone cannot give the information on their total production. The aim of our study was to introduce and optimise the ELISA (Enzyme-Linked Immuno-Sorbent Assay) method for the determination of specific immune complexes of IgG antibodies against the heavy subunit of neurofilament (NfH) with the corresponding neurofilament subunit. Levels of anti-NfH antibodies and immune complexes determined by our in-house ELISA method were expressed in the same arbitrary units of concentration using the commercial calibrator for immunoglobulins. We evaluated this method on the pilot groups of patients in serum and cerebrospinal fluid (CSF) samples. Simultaneous determination of free antibodies against the heavy subunit of neurofilament and corresponding immune complexes have been performed in patients with mild cognitive impairment (MCI), Alzheimer′s disease (AD) and age-matched control subjects. Levels of free antibodies and antibodies bound in immune complexes were significantly lower in patients with MCI than in the patients with AD and control subjects. Significant differences between levels of free antibodies and antibodies bound in the immune complexes were observed especially in CSF. Our ELISA method is suitable for analysis of both serum and CSF. The parallel analysis of free anti-Nf antibodies and their immune complexes could be evaluated together and provide more complex information about autoantibody response against neurocytoskeletal proteins in neurodegenerative diseases. The study is supported by PROGRES Q25/LF1 and RVO-VFN64165.

P.09-156-Wed
Investigation of iFGF23, 1,25-dihydroxyvitamin D3, phosphorus and soluble α-klotho levels in hemodialysis patients

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Fibroblast growth factor 23 (FGF23) is a bone-derived hormone that acts on kidney to increase renal phosphate clearance and plays an important role in vitamin D metabolism by inhibiting the synthesis of 1,25-dihydroxyvitamin D_3 [1,25- $(OH)_2$ D_3]. The interaction of FGF23 with its receptor to activate signaling pathways requires the membrane protein klotho as a cofactor. Extracellular domain of klotho is cleaved and released to bodily fluids and this is known as soluble α-klotho (sαKl). In our study, we aimed to investigate serum iFGF23, 1,25- $(OH)_2$ D_3, phosphorus and sαKl levels in hemodialysis patients. Sixty hemodialysis patients, who are treated at Gazi University Hospital Nephrology Clinic, and 34 healthy individuals were included in the study. Serum iFGF23 and sαKl levels were analyzed using ELISA kits, 1,25- $(OH)_2$ D_3 was determined by LC-MS/MS and phosphorus levels were measured using routine biochemistry systems. iFGF23 levels, as well as serum phosphorus levels of the hemodialysis patients were found to be significantly elevated than the control subjects ($P < 0.001$, for both). Whereas soluble Klotho and 1,25- $(OH)_2$ D_3 levels were significantly lower in hemodialysis group compared with control ($P < 0.001$, for both).

P.09-157-Mon
Physical and chemical mechanisms of selective cytotoxicity in polyacrylate polymer complex with tetrachloroaurate (Aurumacryl)

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Aurumacryl was found to cause oncostatic and oncolytic effect on transplantable murine carcinomas Akatol, Lewis and C-755 *in vivo* at 50 - 300 mg/kg of body weight upon an intraperitoneal administration. No systemic toxicity on mice was found. These data characterize Aurumacryl as a promising oncotherapeutic agent. However, mechanism of its selectivity to tumors remains unknown. Grekhova et al, 2017 reported that Aurumacryl in concentration 1 mg/mL caused double-stranded breaks in DNA visualized with *in situ* staining of histone H2AX and with DNA rocket-assay in MCF-7 human breast cancer cells. Most cells were arrested in G0 period of the cell cycle. Staining with a ROS sensor exhibited no effect of Aurumacryl on mitochondria and anti-oxidation system. A high cytotoxicity of free tetrachloroaurate on cell lines was reported formerly. But it is inapplicable in pharmacy due to insolubility in water and a high affinity to serum albumin. Here we report a low affinity of Aurumacryl to human serum albumin measured with spectrofluorimetry. Analysis of Au oxidation degree in Aurumacryl with o-phenylenediamine gave an evidence that polyacrylate polymer may serve as an inert carrier providing circulation of encapsulated tetrachloroaurate in blood. The Au(III) in Aurumacryl is protected from reducing with blood components (glucose and serum albumin). Pharmacokinetic study on mice with mass-spectroscopy demonstrated that 24 h after intraperitoneal injection of Aurumacryl, Au was uniformly distributed in all tissues. No accumulation and no avoidance of the solid tumors were registered. Our data allow hypothesis that genomic DNA should be considered as an intracellular target of Aurumacryl rather than any proteins or electron-transfer chain. The study was supported by Agreement # 14.607.21.0199 with Ministry of Education and Science of Russia (unique identifier RFMEFI61315X0042).

P.09-158-Tue
Nuclear localization of the glycolytic enzyme phosphoglucose isomerase

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In recent studies we have found that the glycolytic enzyme phosphoglucose isomerase (PGI) of *Escherichia coli* and yeast type III PGI catalyze *in vitro* the breakdown of DNA-fructosamine 6-phosphate (DNA-N-Fr6Ph) to G6Ph and DNA. DNA-N-Fr6Ph represents an Amadori product (APs) resulting from the non-enzymatic interaction of G6Ph with DNA in the early step of the Maillard reaction known as glycation. As result of glycation *in vivo* DNA accumulates both APs and advanced glycation end products with mutagenic potential. Therefore, we hypothesized that the observed DNA-N-Fr6Ph deglycation activity of PGI serves to repair G6Ph-derived lesions in DNA *in vivo*. In view of the conserved function of PGI from bacteria to humans we further reasoned that in order to carry out the proposed DNA repair activity in eukaryotes PGI should translocate to the nucleus. To explore this possibility we studied the human PGI for nuclear localization by a variety of methods including Western blotting, fluorescence microscopy and chromatin immunoprecipitation. All data unambiguously indicated that besides in the cytosol human PGI resides also in the nucleus. In support of this finding, bioinformatics analyses revealed a strong nuclear localization sequence in two of the human PGI isoforms. Experiments are now in progress to address the nuclear function of PGI in eukaryotes in terms of the observed DNA-N-Fr6Ph deglycation activity of prokaryotic and yeast PGI. This work was supported by grant DN01/5/16.12.2016 from the National Science Fund of the Republic of Bulgaria.

P.09-159-Wed
Antidiabetic screening of selected African natural products using an in vitro target-directed screening platform and cellomics

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Several synthetic drugs have been developed to date in an attempt to manage the symptoms of diabetes; however, most of these medications are accompanied by adverse side-effects and remain expensive and largely inaccessible to the vast majority of those who need it. In order to provide enough scientific evidence to support the inclusion of African antidiabetic medicinal plants and macrofungi into healthcare programs, this study sought out to develop a comprehensive *in vitro* antidiabetic target directed screening platform incorporating high content screening thus combining image cytometry with high content computational analysis. To test the success of this model, the antidiabetic potential of five plants (*Aspalathus linearis, Brachylaena discolor,*

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Carpobrotus edulis, *Sutherlandia frutescens* and *Tarchonanthus camphoratus*) and two macrofungal species (*Ganoderma lucidum* and *Hericium erinaceus*) were compared. Due to the role of post-prandial hyperglycaemia in the development of both long and short-term complications of diabetes the alpha-amylase, alpha-glucosidase, pancreatic lipase and DPPiv inhibitory activities were assessed. Furthermore, protein glycation inhibition and anti-oxidant capacity (DPPH, FRAP and nitric oxide scavenging) was measured due to the role of protein glycation and oxidative stress in the pathology of diabetes. Lastly, pancreatic beta-cell function using INS-1 cells was assessed by measuring calcium-dependent insulin secretion, glutamate dehydrogenase activation, apoptosis inhibition and protection against ROS induced oxidative stress. *C. edulis* showed the greatest potential in preventing post-prandial hyperglycaemia and while several of the tested plant species showed particularly strong anti-oxidative capacities, *C. edulis* also showed the strongest glycation inhibitory potential. With regards to beta-cell function, several species including *T. camphoratus*, *C. edulis*, *H. erinaceus* and *A. linearis* showed favourable activities.

P.09-160-Mon
The role of histidine residues in metal binding by human cystatin C

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The hypothesis connecting dyshomeostasis of selected metal ions in the brain with the development of neurodegenerative diseases is gaining increasing attention. Excessive exposure to redox-active metals like copper or iron can trigger oxidative stress and damage of native, physiologically indispensable proteins or peptides, resulting in deadly changes in the central nervous system. The mechanisms of metal toxicity are increasingly better understood what opens the prospects of novel therapeutics for treatment of neurodegeneration. One of the approaches is based on peptides as mimetics of the metal binding sites in proteins. In our studies we have focused on human cystatin C as one of the proteins involved in neurodegeneration. Native hCC is present at particularly high concentrations in cerebrospinal fluid and was shown to possess both neurodegenerative and neuroprotective propensities. To study the impact of metal ions on this protein, we have obtained synthetic peptides encompassing the potential metal binding region in the hCC sequence. Its C-terminal fragment 86–94 (sequence FHDQPHLKRK) contains two histidine residues than can be involved in binding of copper (II) ion(s). Interestingly, His residues are separated by proline what opens the possibility of formation of two metal binding sites. The complexometric properties of the hCC fragment were studied by spectroscopic, potentiometric and calorimetric methods, confirming the presence two possible metal binding sites with different affinities. To assess the role of particular histidine residue in complex formation, they were exchanged to alanine and the obtained analogues were investigated. The obtained data allowed to characterize metal binding domains of the model peptides and suggest the model of metal binding by the whole protein. Work supported by National Science Centre, Poland, grant OPUS 11 (UMO-2016/21/B/NZ1/02823) and Wroclaw Medical University grant ST.D080.16.006 (potentiometric studies).

P.09-161-Tue
Microarray-based molecular assay for the estimation of the *Neisseria gonorrhoeae* drug resistance level in Russia

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Progressive growth in the degree of antimicrobial resistance of *N. gonorrhoeae* creates a prospect for gonorrhea to move towards the category of potentially incurable infections. Genetic determinants of *N. gonorrhoeae* resistance to antimicrobial drugs used for the therapy of gonorrhea in Russia during the last decade have been analyzed in clinical isolates collected in 17 regions of the Russian Federation. Original gel-based microarrays developed in the EIMB with immobilized oligonucleotides aimed for identification of resistance determinants were used in this study. The most frequent mutations in chromosome genes were the insertion of aspartate at position 345 of *penA* gene, L421P in *ponA* gene (resistance to penicillins), V57M substitution in *rpsJ* gene (resistance to tetracyclines), S91F and D95G in *gyrA* gene and S87R in *parC* gene (resistance to fluoroquinolones). Several isolates with decreased susceptibility to azithromycin had no mutations in 23S RNA gene. The resistant strains also possessed mutations in codons 120–121 of *porB* gene and in the genes encoding efflux pumps. The presence of the plasmid *tetM* gene that resulted in the appearance of very high resistance to tetracycline was found in 7% of strains involving genes of both American and Dutch types; homological sequences from *Streptococcus* and *Enterococcus* genera were found for both *tetM* alleles whereas similar genes from mollicutes were found only for the American ones. The change in the resistance profile in the Russian population of *N. gonorrhoeae* over a 10-year time period was analyzed. This study of the *N. gonorrhoeae* population in Russia revealed high level of resistance to previously recommended drugs such as penicillin G, fluoroquinolones, and tetracycline. Thus, cephalosporins remain the drug of choice for first-line empirical antimicrobial monotherapy for gonorrhoea. The work was supported by the Russian Science Foundation (Grant No. 17-75-20039).

P.09-162-Wed
Anti-inflammatory effect of the processed *Paeonia lactiflora* extract on monosodium iodoacetate-induced osteoarthritis

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Paeonia lactiflora has been known as traditionally an important medicinal herb in Asia with the analgesic effects. In this study, we investigated the change of main component in *Paeonia Radix* extract prepared by the processing and anti-inflammatory effect of its extract on monosodium iodoacetate (MIA)-induced osteoarthritis (OA) by processing. we carried out quantification analysis of the three marker components (paeoniflorin, albiflorin, and paeonol) in the ethanol extracts of non-processed and processed *P. Radix* using a UPLC. The amount of paeonol was increased 1.5-fold after the processing when compared non-processed extracts, but not the amount of paeoniflorin and albiflorin. The anti-inflammatory effect of processed *P. Radix* was assessed in vitro in lipopolysaccharide-induced RAW 264.6 cells. The data

showed that the processed *P. Radix* extract leads an anti-inflammatory effect via reducing the production of the nitric oxide (NO), TNF-α and IL-1β in LPS-induced RAW cells. Oral treatment of processed *P. Radix* extract also reduced the blood levels of proinflammatory cytokines (TNF-α and IL-1β) and the production of inflammatory mediator (MMP-2) in the OA model. The results supports that the processed *P. Radix* extract might be an effective therapeutic drug for the treatment of the OA and OA-related symptoms.

P.09-163-Mon
Selenoprotein S1 (SEPS1) – 105G>A polymorphism and male infertility

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Abnormalities in semen parameters are often associated with reduced fertility in male and may be attributed to single nucleotide polymorphisms (SNP) in many not identified genes. Recent reports have shown the-105G>A promoter polymorphism (rs28665122) in selenoprotein S1 gene (SEPS1) to increase proinflammatory cytokine expression and thus to be correlated with autoimmune diseases, cancer and preterm birth in some populations, but there have been no reports on role of the SEPS1-105G>A polymorphism in male infertility. The aim of this study is to explore the association of this polymorphism with infertility in men with poor sperm morphology in Moscow region. The method of PCR-RFLP was used to detect SEPS1 - 105G>A polymorphism in 52 infertile males suffered from severe infertility and participated once with negative result in the assisted reproductive technologies program (ART) with abnormalities in sperm parameters as azoospermia, oligospermia, asthenoteratospermia and 51 fertile men with one or more children. Statistical analysis was carried out using "EXCEL" and "STATISTICA 6.0 program". The frequencies of genotypes were GG (30.8%), GA (65.4%), AA (3.8%) in the group of unfertile males and GG (62.5%), GA (37.5%) and AA (0%) for control group. The frequencies of SEPS1-105G>A of minor allele A (36.5% vs 18.7%) and the carriers of minor allele A (65.4% vs 37.4%) in infertile male with poor sperm morphology are significantly higher than that in the control group. The carriers of the SEPS1-105G>A polymorphism are at increased risk of developing azoospermia. Such findings suggest that SEPS1 may be a potential gene marker for idiopathic infertility in men. Larger population-based studies are needed for clarifying the relation between male infertility and SEPS1 -105G>A SNP. The publication was supported by the "RUDN University Program 5-100" and the Ministry of Education and Science of the Russian Federation (the Agreement No. 02.A03.21.0008).

P.09-164-Tue
The enhanced permeability and retention (EPR) effect of caulerpin and heparin on doxorubicin and liposomal doxorubicin: a nanotherapeutical and cancer efficiency approach

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The lack of tumor selectivity is a critical limitation for effective anticancer therapy. To take advantages from the abnormalities of tumor vasculature is the one way to accomplish selective drug targeting. Enhanced permeability and retention (EPR) effect is a phenomenon, which increase the entrance and accumulation of molecules into the tumor tissue like a "royal barrier". At this point, nanotechnology allows us to get advantages on loading capacity, conservation from degradation, controlled releasing rates and also leaking through the permeable tumor vessels due to the generation of different nano-drugs with the combination of chemotherapeutics with nano-particles. To increase the EPR effect by using nanoparticle-based therapies is a new promising approach for effective cancer management. The aim of this study is to evaluate the ERP effect of caulerpin and heparin on doxorubicin and liposomal doxorubicin. In this study, human umbilical vein endothelial cells (HUVEC) were seeded with Matrigel at the top well of ThinCerts; and human ovary adenocarcinoma (ONCO-DG-1) cells were seeded in the lower wells. Doxorubicin and liposomal doxorubicin were applied to HUVEC cells; after the exposure of caulerpin and heparin for 1 hour. Crystal violet assay was used to evaluate the viability of ONCO-DG-1 cells after the treatment of doxorubicin and liposomal for 48 h. Permeability was assessed by the measurement of viability of ONCO-DG-1 cells. According to the results, when single treatment of liposomal doxorubicin, the cytotoxic effect on ONCO-DG-1 cells was found as the highest level. Also, the results reveal that the lowest cytotoxic effect was found when the combined application of caulerpin and heparin with doxorubicin. In conclusion, caulerpin and heparin stimulate the permeability and retention of doxorubicin. Hence these chemicals may be promising agents to increase EPR phenomena for the effective cancer management.

P.09-165-Wed
The keys of cancer may be in nature

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Quercetin is a plant derived flavonoid present in diet and has been shown to possess many biological activities, including anti-inflammatory, neuroprotective, antiviral, and antifungal properties. It is known to contribute as an apoptosis inductor decreasing the growth of tumors in several tissues and inhibiting the spread of malignant cells. In the present study, we examined the effect of quercetin on cell viability in a variety human cancer cell lines. Human prostate cancer cell lines; PC-3 and LNCaP, human colon cancer cell lines; HT-29 and SW-620, human cervix cell line; HeLa, human hepatocellular cell line; HepG-2 were cultured in suspension using growth mediums with 10% FBS at 37°C.

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Cells were seeded in 96-well plates and cultivated for 24 h before the onset of treatment and treated with quercetin (1–200 μM) for 48 h. The cell viability and proliferation were assessed by using Alamar Blue assay and the absorbances read at 570 nm using the spectrophotometer. Then sigmoidal graphics were drawn by obtained proliferation data and the data converted to IC_{50} values. Quercetin inhibited cell viability of HeLa (IC_{50}: 27.1 μM), LNCaP (IC_{50}: 19.4 μM), SW-620 (IC_{50}: 13.5 μM) HEPG-2, (IC_{50}: 79.1 μM) PC-3 (IC_{50}: 153.9 μM) and HT-29 (IC_{50}: 172.4 μM) cells in a dose-dependent manner. Although quercetin inhibited the proliferation of HeLa, LNCaP and SW-620 cells at lower concentrations, it has been found less effective against proliferation PC-3 and HT-29 cells. We found that quercetin displays quite different selectivity on proliferation of human cancer cell lines. Our results demonstrate that quercetin may have a potential benefit for cancer prevention, especially colon and prostate cancer. One of the main problems is solubility and targeting of quercetin towards cancerous cells, which can be overcame via specialized water-soluble carrier compounds. This project was supported by Selcuk University (BAP: 16401083).

P.09-166-Mon
Altered neuropeptide Y and ghrelin levels BDNF heterozygous mice

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Brain-derived neurotrophic factor (BDNF) plays a crucial role in neuronal survival, synaptic plasticity and neural circuit development. BDNF heterozygous mice (BDNF (+/-)), which is deprived of one of the two alleles coding BDNF protein, is used as an animal model to study the physiological roles of BDNF. These mice are characterized by the reduced BDNF expression. Neuropeptide Y (NPY) has a role in the regulation of various basic physiological functions, such as food intake, metabolic functions, circadian rhythm, cognition and neuronal excitability. Ghrelin is the hormone that regulate appetite and energy balance in the hypothalamus. In this study, NPY and ghrelin parameters were aimed to compare between BDNF (+/-) and wild type (WT) littermates. BDNF (+/-) and WT mice were identified by conventional PCR. 11 WT and 9 BDNF (+/-) mice were used. Serum NPY and ghrelin levels were investigated by enzyme-linked immunosorbent assay method. We found that NPY levels of BDNF (+/-) mice were lower than WT mice (2567.5 ± 994.5 pg/mL; 3821 ± 1633.5 pg/mL, respectively). Ghrelin levels were higher in BDNF (+/-) mice than WT mice (9 ± 3.5 ng/mL, 8 ± 3.5 ng/mL, respectively). BDNF and its receptor tropomyosin receptor kinase B interaction lead to the induction of NPY gene expression. Our results were suggested that reduced BDNF may cause decreased NPY levels. However, reduced BDNF may cause increased ghrelin levels. But, the direct mechanism between reduced BDNF and ghrelin has not been fully identified. Further studies are needed to evaluate the effects of reduced BDNF on energy metabolism.

P.09-167-Tue
Evaluation of FNDC5/irisin levels of BDNF heterozygous mice

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Brain-derived neurotrophic factor (BDNF) is a factor that released by neurons and has role in the development and growth of neurons. BDNF heterozygous mice (BDNF (+/-)), which is deprived of one of the two alleles coding BDNF protein, is used to study the physiological roles of BDNF. These mice are characterized by the reduced BDNF expression in nearly all tissues. Irisin is formed by proteolytic cleavage of the transmembrane protein fibronectin type III domain containing 5 (FNDC5), the expression of which is most abundant in skeletal muscle. It is responsible for browning of white adipose tissue through enhancing oxygen consumption and induction of expression of thermogenin. In this study, FNDC5/Irisin parameter was aimed to be evaluated between BDNF (+/-) and wild type littermates. BDNF (+/-) and WT mice were identified by conventional PCR. 11 wild type (WT) and 9 BDNF (+/-) mice were used. Serum FNDC5/Irisin levels were determined by enzyme-linked immunosorbent assay method. We found that FNDC5/Irisin levels in BDNF (+/-) mice were higher in comparison to WT mice (718 ± 384 pg/mL; 658 ± 520 pg/mL, respectively). BDNF, which is the downstream mediator of FDNC5/Irisin, decreases *Fndc5* gene expression as part of potential feed-back loop. Our results were suggested that reduced BDNF may have increased FNDC5/Irisin levels. Further studies are needed to evaluate the effects of BDNF on metabolism.

P.09-168-Wed
Identification of functional non-coding SNPs in the autoimmunity-associated human locus 17q12-21 to be edited by CRISPR/Cas system in order to find new therapy targets

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At the moment, protein targets for drug design are encoded by only a small fraction of the human genome. Genome-wide association studies are a powerful tool for identification of loci involved in the development of complex diseases with "missing heritability", such as autoimmune disorders. However, much effort is additionally required to reveal causative SNPs in the associated loci - mainly non-coding regulatory SNPs - as well as their target genes. To this end, we systematically searched for and experimentally validated potential causative SNPs in an autoimmunity-associated locus 17q12-21 for subsequent integration of the risk alleles into the genome of relevant human leukocyte cell lines. We focused on 6 non-coding SNPs, which are most likely to be causal according to the PICS (Probabilistic Identification of Causal SNPs) algorithm of fine-mapping. We determined the coordinates of putative enhancers bearing selected SNPs using the epigenetic data generated by the ENCODE 2

project for primary human immune cells. The luciferase reporter assay was used to test the effect of SNP alleles on the activity of the putative enhancers in a set of relevant cell lines. Three out of the six non-coding polymorphisms proved to be functional in model regulatory T cells and one of them was also functional in a B-lymphocytic cell line. Moreover, we compared binding of the transcription factor MEF2C to the alternative alleles of rs12946510 using modified pull-down assay. Further introduction of the alternative alleles of causal SNPs into the genome of appropriate cell lines using CRISPR/Cas9 system will allow us to determine target genes for these regulatory SNPs. Gene editing efforts are supported by the Program of fundamental research for state academies for 2013–2020, research topic 01201363823.

P.09-169-Mon
Impact of alcohol consumption on myocardial characteristics in patients with cardiovascular disease

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Alcohol consumption is related to various clinical manifestations of cardiovascular diseases (CVD) ranging from hypertensive disease and arrhythmias to ischemic heart disease (IHD) and alcoholic cardiomyopathy. Alcohol impacts on CVD via multiple pathways and the effects of alcohol consumption on the risk of CVD are complex as there are beneficial and detrimental effects depending on volume and patterns of drinking. The aim of this study is to evaluate the damaging effect of alcohol intake on myocardium characteristics in patients with CVD. The 314 patients, 148 males and 166 females, the age 33 - 65 with major categories of CVD (hypertension, IHD and arrhythmia) were divided into three categories according to volume of alcohol consumption: lifetime abstainers (0 doses of alcohol per day), low to moderate drinkers (1- 4 doses per day) and heavy drinkers (5 and more doses per day). The changes in electrophysiological characteristics (EPC) of myocardium in patient with CVD were detected according to analysis of echocardiography (ECG) signal by dispersion mapping method. The patients with CVD use 5 and more doses of alcohol per day have significantly higher sizes of left and right atria and right ventricle than patients with low alcohol consumption or abstainers. The more pronounced changes in the EPC of the myocardium, left ventricle (LV) end systolic dimension (LVESD), left ventricular ejection fraction (%) and interventricular septum thickness were found in patients with CVD use 5 and more doses. As a conclusion, heavy drinking (≥ 5 doses per day) provides the damaging effect on EPC of myocardium. The early stages of cardiovascular disease's deterioration can be identified by detection of electrophysiological abnormalities and electric instability of the myocardium by dispersion mapping of ECG – signal method. The publication was supported by the "RUDN University Program 5-100" and the Ministry of Education and Science of Russia (the Agreement No. 02.A03.21.0008).

P.09-170-Tue
Human neuromodulator Lynx1 affects synaptic plasticity and ameliorates cognitive deficits in neurodegeneration

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Many neurodegenerative diseases (e.g. Alzheimer's disease) associated with cognitive impairment are accompanied by the loss or dysfunction of nAChRs. Lynx1 is a GPI-tethered nAChR modulator expressed in brain areas important for learning and memory. Previously, we have shown that water-soluble domain of Lynx1 (ws-Lynx1) competes with oligomeric beta-amyloid Aβ(1-42) for binding to different nAChR subunits in the brain extracts and abolishes the Aβ(1-42) cytotoxic effect in cultured cortical neurons. We hypothesized that ws-Lynx1 could be used for improvement of cognitive decline and prevent neurotoxic effects of Aβ(1-42) *in vivo*. To understand the Lynx1 role in cognitive function, we studied the effects of ws-Lynx1 preparation in wild-type mice and transgenic animals modeling Alzheimer disease (2xTg-AD). Ws-Lynx1 penetrated the blood-brain barrier upon intranasal administration with specific accumulation in the cortex, hippocampus, and cerebellum. Chronic ws-Lynx1 treatment reduced anxiety and prevented memory impairment in wild-type and 2xTg-AD mice. Positive modulation of synaptic plasticity associated with activation of presynaptic α7 type nicotinic acetylcholine receptors and neurotransmitter release facilitation was observed in ws-Lynx1 treated mice. Ws-Lynx1 enhanced mRNA level of endogenous Lynx1, normalized AChE level in the cortex, and restored synaptic density in the hippocampus in 2xTg-AD mice. Lynx1 could be considered a natural protector of the brain cholinergic system and its soluble counterpart may be a potential candidate for the cognitive impairment treatment in neurodegenerative diseases. The work was supported by the Russian Science Foundation (project #16-14-00102).

P.09-171-Wed
The first experience of tomosynthesis in diagnostic of nonpalpable breast cancer

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The urgency of the problem of studying the incidence of the breast diseases is a task of modern health, as there has been a steady increase of this problem including malignant nature. At present time, some progress has been made in diagnosing early forms of the diseases due to the introduction of modern

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technologies of radiation diagnostics. Despite the extensive use of mammography, a number of limitations remain. This, first of all, is associated with the complexity in interpreting images of volumetric formations due to superimposition of structural elements of breast tissue, located in different planes. Tomosynthesis of the breast is a new technology that helps to avoid these shortcomings. The clinical data of the study were data of clinical, X-ray and sonographic survey of 250 women in outpatient conditions. Nonpalpable lesions in the mammary glands were found in 150 women. X-ray examination was carried out on the Hologic Selenia Dimensions in machine «COMBO» mode. On standard mammograms on a background of diffuse changes nonpalpable signs of cancer were found in 55 (93%) of women in the mode of tomosynthesis in 58 patients (97%), and in 45 (75%) under US. In conclusion the use of tomosynthesis in diagnostic of non palpable breast formations enhances X-ray method, increases the percentage of detection of unsuspected cancer, 4% improves the differential diagnosis of diseases associated with architectural distortion of the breast tissue, 5% improves the diagnosis of diseases associated with the accumulation of calcifications. This publication was prepared with the support of the "RUDN University Program 5-100" and the Ministry of Education and Science of the Russian Federation (the Agreement No. 02.A03.21.0008)

P.09-172-Mon
Search for azolo[1,2,4,5]tetrazines biotargets in mycobacteria

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Tuberculosis (TB) has recently become the deadliest among the infectious diseases, with an estimated incidence of 10.4 million and mortality of 1.8 million in 2016. The emerging *M. tuberculosis* strains with multidrug and extensive drug resistance are a threat to global TB control and mark the urgent need to develop anti-TB drugs with a new mechanism of action. Five novel azolo [1,2,4,5]tetrazines were previously selected as inhibiting *M. tuberculosis* growth, and as potential serine-threonine protein kinase (STPK) inhibitors on original test-systems *M. smegmatis aphVIII+* and *S. lividans aphVIII+*. Mycobacterial genomes harbor several genes encoding STPKs. We used *M. smegmatis* mc^2 155 as a model for biotarget determination, as it has at least six close homologs of *M. tuberculosis* STPKs. The minimal inhibitory concentrations (MICs) of these compounds on *M. smegmatis* ranged from 20 to 64 mkg/mL. We were able to obtain mutants, resistant to 3.5x-4x MIC to all the compounds except for one (TSV-409) with a frequency from 4×10^{-6} to 2×10^{-9}. We selected three mutants resistant to each of the compounds for further analysis. A drug susceptibility assay revealed no significant changes in resistance levels to rifampicin, erythromycin, ofloxacin, imipenem and kanamycin, thus the compounds had a mechanism of resistance different from these antibiotics. At the same time the mutants had increased resistance to all the azolo[1,2,4,5] tetrazines, except for TSV-409, suggesting that the compounds have a common biotarget. No change in resistance level to mitoxantrone (a PknB inhibitor) was observed, thus the compounds are not likely to inhibit PknB. TSV-409 is a smaller molecule, thus it might have lower specificity and more than one biotarget. Currently the genomic DNA was isolated from the mutants and is being subjected to whole-genomic sequencing that might elucidate mutations conferring resistance. This work was supported by the Russian Science Foundation grant 17-75-20060.

P.09-173-Tue
A polymorphism associated with wheeze phenotype in asthma and regulation of IL33 gene expression

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Cytokine IL-33 is secreted by epithelial and endothelial cells during necrosis and stimulates humoral immune response. Elevated IL33 expression in pulmonary epithelium of asthmatic patients correlates with exacerbation of allergen-induced inflammation and disease progression. We hypothesized that individual variations in the IL33 gene expression may be explained by polymorphisms of non-coding regulatory regions, in particular by SNP rs4742170 located in an enhancer region in the il33 locus and associated with asthmatic wheezing phenotype. Activities of the IL33 enhancer variants containing different rs4742170 alleles were assessed upon transfection of the corresponding luciferase reporter constructs into NCIH pulmonary epithelial cell line followed by cortisol stimulation. We observed that rs4742170 risk allele no longer contained a binding site for glucocorticoid receptor (GR), a transcription factor that inhibits expression of a number of inflammatory mediators. Reduced binding of GR to IL33 enhancer correlated with elevated IL-33 promoter activity in lung carcinoma cells. Our data suggest that differential binding of the rs4742170 alleles to may underlie the emergence of asthmatic wheezing phenotype in response to increased IL33 gene expression and systemic inflammation. This study is supported by Program of fundamental research for state academies for 2013–2020 (research topic 01201363823).

P.09-174-Wed
The action of nobiletin on brain mitochondria and yeast cells

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Citrus flavonoid nobiletin has anticancer, antiviral, neuroprotective, anti-inflammatory activities and depending on the cell types exhibits both pro- or anti-apoptotic properties. The aim of our study was to investigate the possible target of nobiletin on the components of the mitochondrial respiratory chain. Our preliminary results have shown that one of possible target of nobiletin may be is complex I. Thus in this study we used isolated bovine brain mitochondria and two strains of yeast, whereas yeast foam doesn't have first complex proton pumping activity, while Candida utilis mitochondria exhibits whole complexes. Analysis of mitochondrial enzymes' activities, estimation of respiration rate in bovine brain isolated mitochondria, mitochondrial membrane potential, ROS production were performed in bovine brain mitochondria to evaluate the direct effect of nobiletin on the mitochondrial bioenergetics, yeast strains culture medium and growth conditions, oxygen consumption assay. We have found that nobiletin decreases oxygen consumption in the presence of glutamate and malate and increases in the presence of succinate. In parallel, nobiletin increases NADH oxidation, alpha-ketoglutarate

dehydrogenase activities and alpha-ketoglutarate-dependent production of ATP. Additionally, nobiletin reduces the production of peroxides in the presence of complex I substrates and does not change succinate-driven peroxide formation. Nobiletin inhibits mitochondrial respiratory chain on whole cells in a similar fashion for Yeast foam and Candida utilis. Nobiletin may act as a mild "uncoupler", which through activation of alpha-KGDH-complex and acceleration of matrix substrate-level phosphorylation maintain membrane potential at a normal level. This switch in mitochondrial metabolism could elevate succinate-driven oxygen consumption.

P.09-175-Mon
Morphological and functional characterization of macrophages derived from embryonic and monocyte sources

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Macrophages are the key participants of various morphogenetic processes, including inflammation and reparation. Any organ contains macrophages derived from several sources; however, the brain and the liver macrophage populations are represented almost entirely by cells originating from the yolk sac and the fetal liver, whereas the dermis contains macrophages predominantly derived from the bone marrow precursors. The cause of such a distinction are not clear. Heterogeneity and variability of macrophage content results in organ-specific differential tissue homeostasis and pathophysiology, which also remains understudied. The aim of the study is comparison of the immunophenotypes and the gene expression profiles of naïve and activated macrophages of differential origin, exemplified, respectively, by the connective tissue monocyte-derived macrophages (MDM) and the resident liver macrophages - Kupffer cells (KC). Comparative characterization of these populations shows that both of them are capable of being activated/polarized to both M1 and M2 phenotypes upon induction by various M1- or M2-specific inducers. KC, however, responded to induction by more pronounced upregulation of anti-inflammatory cytokines, whereas the MDM were significantly more prone to the pro-inflammatory cytokine upregulation. The induction of the genes of pro-inflammatory cytokines in KC is found to be slower than in the MDM. Gene expression profiles, immunophenotypes, and phagocytic activity of resident liver macrophages was studied. The majority of KC is found to have a $F4/80^{+}$, $CD68^{+}$, $CD163^{+}$, and $CD11b^{-}$ immunophenotype. The M2-specific induction led to an increase in the CD86 expression, and also to Ki67 expression. The inducers of both types, M1 and M2, promoted predominantly the anti-inflammatory cytokine expression in KC. The publication was supported by the "RUDN University Program 5-100" and the Ministry of Education and Science of the Russian Federation (the Agreement No. 02.A03.21.0008).

P.09-176-Tue
Exoproteome profiling of lactic acid bacteria *Lactobacillus reuteri* during co-cultivation with clinical isolates of *Klebsiella pneumoniae*

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Currently, the problem of treatment and prevention of hospital-acquired infections is an urgent task for researchers and physicians. Infectious diseases caused by hospital strains are difficult to treat, as pathogens are frequently multidrug resistant (MDR), and the determinants of resistance actively circulate among different strains. To develop rational therapy against opportunistic infections, an extensive search for new antimicrobial agents is necessary, including search of antimicrobial potential of probiotic bacteria *Lactobacillus*. In this study we analyzed exoproteome of *Lactobacillus reuteri* during its co-cultivation with *Klebsiella pneumoniae* – an MDR hospital-acquired strain. *L. reuteri* was able to suppress effectively the growth of *K. pneumoniae*. The lactic acid bacteria provided differential protein profile in response to co-cultivation with pathogen. We have shown that bacterial proteolytic enzymes, proteins of cell wall peptidoglycans breakdown, ribonucleoside hydrolase RihC, molecular chaperone GroEL and L-lactate dehydrogenase formed the exoproteome in the presence of *Klebsiella* cells. The latest two are also known to perform moonlighting functions in *Lactobacilli*. Several proteins of unknown function were also identified. These proteins may represent potential novel antimicrobial agents that could be effective against clinical isolates of *Klebsiella pneumoniae* and other nosocomial infections. This work was supported by Grant of Russian Science Foundation #16-16-00094.

P.09-177-Wed
Cytocompatibility assessment of amorphous calcium phosphates modified with tartaric or citric acid

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Bone diseases significantly decrease the quality of human's life, may be life-limiting and are recognized to be among the major health and social challenges of our time. The problems associated with application of auto- and allografts in current clinical practice stimulate the demand for improved new biomaterials for

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bone implants. The aim of our study was to evaluate the effect of newly synthesized amorphous calcium phosphates modifies with tartaric acid (ACP-TA) or (5% or 18%) citric acid (ACP-CA-5, ACP-CA-18). Mouse (BALB/c 3T3 embryonic fibroblasts and cultures from bone marrow- BMC and bone explants- BEC) and human (Lep-3 and BJ embryonic fibroblasts, SAOS-2 osteosarcoma) cells were used as model systems in our investigations. Direct experiments (DE) with cells seeded on the surface of the materials were performed by MTT test and Scanning electron microscopy (SEM) after 3–6 days (MTT test) and 7–10 days (SEM). In indirect experiments (IDE) the cells were cultivated for 72 h in culture medium pre-incubated for 3, 6 and 21 days in the presence of the examined material and cell viability was estimated by MTT test. The results obtained demonstrate the ability of cells to adhere and proliferate on materials' surface. Direct experiments with ACP-TA reveal cell viability ≥ 100% as compared to the control (BALB/c 3T3, BECs, Lep-3, SAOS-2). Longer pre-incubation period of culture medium with this material leads to increased cell viability (IDE). Viability of Lep-3 human fibroblasts estimated in both DE and IDE with ACP-CA-5 and ACP-CA-18 on 3rd day was ≥ 100% as compared to the control and then it starts to decrease but retains >70–80%. In contrast, the viability of human osteosarcoma SAOS-2 cells is around 50% in the same conditions. Better viability was observed with ACP-CA-5 than ACP-CA-18. In conclusion, among the materials investigated ACP-TA exhibit the most promising cytocompatibility proved in direct and indirect experiments with human and murine cells.

P.09-178-Mon
The new approaches to understanding the mechanism of immunosuppression in brain glioma patients

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The correlation between the peripheral blood cells aggregation level, measured by surface plasmon resonance method (SPR), and chromosomal abberations of peripheral blood lymphocytes (PBL) was investigated in patients with brain malignant gliomas. The results show that SPR indices of blood cells brain gliomas patients gradually decrease with increasing of the gliomas malignancy grade and for certain ones are lower than that of healthy persons. The decreasing of SPR data correlate with the increase of chromosomal aberration frequency in PBL of glioma patients. Cytogenetic characteristics of patients with brain tumors under treatment by Verapamil and Ketamine in vitro were investigated. The decrease of the chromosome aberrations frequencies just like decreasing of the aneuploid and polyploid cells quantity in patient's malignant tumors was shown under the Verapamil action. The substantial reduction in aneuploid cells number among tests of patients with malignant tumors under Ketamine exposure was shown. Patients with benign or malignant tumors had the very lowest magnitudes of multiaberrant cells amount and of the extent of aberrant cell injury under the Verapamil influence. The diamine oxidase (DAO) and polyamine oxidase (PAO) were excreted by PBC of such patients in culture medium during transformation of lymphocytes into blasts under the influence of phytohemagglutinin. The degree of blood cells aggregation was determined in these conditions with highly sensitive method of SPR. Increased activity of DAO and PAO in case of spinal hernia and reduced activity of these enzymes in malignant gliomas was found. The regulatory role of these enzymes on the proliferative activity of lymphocytes in neurosurgical pathology, including brain gliomas with different degree of malignancy, is suggested. The publication was supported by the "RUDN University Program 5-100" and the Ministry of Education and Science of the Russian Federation (the Agreement No. 02.A03.21.0008).

P.09-179-Tue
Effects of reduced tumor burden by selective treatment of lung cancer on muscle mass loss in cachectic mice

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Cancer-cachexia is a syndrome characterized by the pronounced loss of body weight and the wasting of muscle and adipose tissue, which leads to impaired quality of life and reduced survival. To what extent tumor burden influences muscle mass independently of specific treatments for cachexia remains to be known. To assess whether reduced tumor burden by selective treatment of the cancer cells may exert any effects on muscle mass loss and function in mice. Body and muscle weight, grip strength, muscle morphometry, apoptotic nuclei, systemic levels of troponin-I, proteolysis, and apoptosis markers were determined in diaphragm and gastrocnemius muscles of lung cancer (LP07 adenocarcinoma cells) mice (BALB/c) treated with monoclonal antibodies (mAb, CD-137, CTLA-4, PD-1, and CD-19) against immune check-point molecules (N = 10/group). Non-treated lung cancer cachectic mice were the controls. Compared to non-treated cachectic mice, final body weight, body weight gain, and grip strength significantly increased in the mAb-treated animals. In diaphragm and gastrocnemius muscles of mAb-treated mice, the number of apoptotic nuclei, tyrosine release, proteolysis and apoptosis markers significantly decreased compared to non-treated cachectic mice. Systemic levels of troponin-I significantly decreased in treated cachectic mice compared to non-treated animals. We conclude that reduced tumor burden as a result of selective treatment of the lung cancer cells also elicits beneficial effects on muscle mass loss through attenuation of several biological mechanisms that lead to increased protein breakdown and apoptosis.

P.09-180-Wed
Assessment of photocurable biodegradable hydrogels for the regeneration of hollow organs

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Despite increasing quality of medical care and technological advances, damage to hollow organs still often causes lethal outcome. Xenogeneic and allogenic grafts are currently preferred in clinical practice, yet their application is associated with multiple risks. Development of bioartificial implants to reconstruct defects of hollow organs is an important biomedical challenge. Success of reconstruction surgery largely depends on biocompatibility and biodegradability of the implant material, as well as on the spatial correlation between the implant and tissues. Therefore, development of the methodology that would allow fabrication of biocompatible personalized implants for hollow organs is of utmost importance for tissue engineering. Hydrogels, obtained by photocrosslinking using derivatives of biopolymers can be utilized for the development of personalized implants for tissue engineering and regenerative medicine applications. Silk fibroin/gelatin scaffolds appear to be a promising biomaterial for this purpose. Based on photocrosslinkable derivatives of methacrylated fibroin and methacrylated gelatin (ratio 7:3), tube-shaped hydrogel scaffolds were fabricated. These tubes are characterized by uniform structure and high elasticity. Spatial dimensions (length = 5 mm, D = 3.8 mm, wall width 115 μm) allow the usage of these tubes for rat small intestine reconstruction. Rat circumferential defect was closed with tubular hydrogel implant with 3 mm distance between the afferent and efferent intestine segments. One month after the implantation full reparation of the jejunal wall defect was evident. Our data provide evidence for good perspectives for application of such hydrogels for reparation of walls of hollow organs. This work was supported by the Ministry of Education and Science of the Russian Federation under the Agreement on the provision of subsidies No. 14.604.21.0167 from September 26, 2017 (RFMEFI60417X0167).

P.09-181-Mon
Myelin-specific autoantibodies induced in vivo by exposure to Epstein-Barr viral antigen are caused by epitope spreading and only partially are cross-reactive

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Multiple Sclerosis (MS) is an autoimmune chronic inflammatory disease of central nervous system (CNS). Crossreactivity of neuronal proteins with exogenous antigens is considered as one of the possible mechanisms of MS triggering. Previously we showed that monoclonal myelin basic protein (MBP)-specific IgGs are cross-reactive with Epstein-Barr virus (EBV) protein LMP-1. Here we report evidence that exposure of mice to LMP1 may result in induction of myelin-reactive autoantibodies in vivo. Only part of such anti-MBP antibodies are cross-reactive towards LMP1 but majority is occurred as a result of epitope spreading. We suggest that chronic contact with viral antigen rather than multiple rapid exposures to it is more sufficient in inducing switch of B cells from viral to myelin antigen. Moreover, even in inbred animals being almost identical in terms of genome, such switch was observed only in 20% of animals, indicating that this evidence is occurred by a chance rather than systematically. Our findings provide novel insights into still enigmatic link between EBV infection and MS development, determining several criteria that are beneficial for induction of self-reacting antibodies on the background of viral infection. Current study was supported by Russian Science Foundation grant #17-74-30019.

P.09-182-Tue
Characterization of factors influencing subcellular localization of NPAS4

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Neuronal Per-Arnt-Sim domain protein 4 (NPAS4) is class I bHLH-PAS transcription factor discovered first in neurons of mammalian hippocampus and later in β-cells of pancreas. Functionally, NPAS4 is connected with response to an ischemic insult and maintaining the homeostatic balance between synaptic excitation and inhibition. Also, it was shown that NPAS4 in pancreatic β-cells, reduces insulin content, blunts the responsiveness to glucagon-like peptide 1 (GLP-1) and protects cells from ER stress. Although NPAS4 was proposed as therapeutic target for neurodegeneration diseases, type 2 diabetes and pancreas transplantation, no detailed biochemical and structural characterization of this protein was presented. Functional activity of the bHLH-PAS proteins is often correlated with shuttling between nucleus and cytoplasm which is a consequence of masking or unmasking subcellular localization signals by interacting partners as answer for environmental condition, ligand binding and post-translational modifications. Until now, no cellular localization signals of NPAS4 were described. In order to determine the sequences of nuclear localization signals (NLSs) and nuclear export signals (NESs) in NPAS4 we used series of deletion and point mutants tagged with yellow fluorescence protein (YFP) expressed in COS-7 and Neuro2a cells. We demonstrate that NPAS4 possess unique mosaic of partially overlapping localization signals located in different parts of protein. Additionally, we present results of performed in silico analysis of the structural and biochemical properties of NPAS4. Our final conclusion is, that NPAS4 is a shuttling protein with a complicated system determining localization of this protein in response to different stimuli. Participation in Congress supported by Wroclaw Centre of Biotechnology, programme The Leading National Research Centre (KNOW) for years 2014–2018.

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P.09-183-Wed
Novel "photoinks" leading to photocrosslinked composites based on interpenetrating networks of methacryloyl gelatin and silk-like structural proteins

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Hydrogels formed from natural polymers are very attractive and useful in tissue engineering, because they provide cells with the environment that is similar to the extracellular matrix. Protein polymers fibroin (SF) and recombinant spidroin 1 (rS1) are widely used in bioengineering as materials for scaffolds fabrication for regeneration of damaged tissues and organs, as well as for making biodegradable carriers for localized drug delivery. Photocrosslinkable materials can be polymerized under biocompatible conditions and therefore can be used in order to create hydrogels of specific micropatterns and 3D structures, depending on particular application. By combining SF or rS1 with photocrosslinkable gelatin methacryloyl (GM), we created novel biocompatible bioresorbable materials with unique physical properties. The acquired materials can be used for creating micropatterns of desired shapes with certain elements not wider than 5 μm. Biocompatibility of obtained materials was tested on both immortalized (3T3) and primary murine fibroblasts (MEF). MTT data demonstrate that growth of fibroblasts on photocrosslinked MG-fibroin and MG-spidroin scaffolds did not differ from their growth on reference scaffolds. Fibroblast viability remained high throughout the culturing period. Therefore, hydrogels, consisting of interpenetrating networks of chemically crosslinked MG and physically crosslinked SF and rS1 are biocompatible and characterized by better mechanical properties in comparison to pristine MG. We suggest that due to their versatility and biocompatibility the SF-MG and rS1-MG based materials can be utilized for stereolithography and bioprinting in the future. This work was supported by the Ministry of Education and Science of the Russian Federation under the Agreement on the provision of subsidies No. 14.604.21.0167 from September 26, 2017 (RFMEFI60417X0167).

P.09-184-Mon
Mitochondriotrophic agents triphenylphosphonium alkyl-benzoates derivatives induce antimetastatic effect in metastatic colorectal cancer cells in vitro

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Resistance to cell death, high invasiveness and metastasis, induction of angiogenesis and metabolic reprogramming are specific features of cancer cells. Metabolic alterations are especially relevant in the search of new treatment, such as, mitochondrial-higher transmembrane potential due to an inner membrane modified composition, highly glycolytic activity and reduced mitochondrial mass. Mitochondria have became in a convincing pharmacology target in the search and design of new molecules targeting to bioenergetics of cancer cells. Recently, we describe new anticancer compounds able to reach mitochondria, triphenylphosphonium alkyl-benzoates derivatives (TPP^+C_{10} and GA-TPP^+C_{10}), and induce energy stress conducing to selectively apoptosis *in vitro* in breast, colorectal and oral cancer cells. In this study we investigate the antimetastatic effect of TPP^+C_{10} and GA-TPP^+C_{10} in metastatic colorectal cancer cells. We test these compounds inducing apoptosis in COLO-205, SW620 and CT26 cells measuring Annexin V-FITC marker and caspase 3 activation by flow cytometry and cell viability by MTT assay. For determination of antimetastatic effect, we evaluate ERK phosphorylation and VEGF levels by western blot. Besides, we assess cell migration by wound healing method and transwell assay. We compare the effect of these new compounds with standard drug treatment for colorectal cancer. The results showed that our compounds induced apoptosis. Moreover, they were able to reduce ERK phosphorylation and decrease VEGF levels, and also decrease cell migration. TPP^+C_{10} and GA-TPP^+C_{10} were more potent and effective than standard drug treatment. In conclusion, the results showed that triphenylphosphonium alkyl-benzoates derivatives are effective and potent drugs on metastatic colon cancer cell, being a new pharmacological approach and promising strategy for colorectal cancer. The work was supported by FONDECYT de iniciación 11160281.

P.09-185-Tue
Different expression of the nucleolar protein SURF6 in human normal and activated peripheral blood lymphocytes

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The level of many nucleolar proteins including major rRNA processing factors is known to vary in cell cycle progression being increased in actively proliferating cells. Quantitative analysis of these proteins is routinely used for evaluation of cell proliferative potencies in clinical oncology. In lymphoproliferative disorders, the particular value has quantification of Ki-67-positive cells as far as the nucle(ol)ar Ki-67 protein is absent in resting lymphocytes. In order to examine whether other nucleolar proteins may also serve as a diagnostic oncomarkers, in the current study we examined the level of expression of an evolutionary conserved rRNA processing factor SURF6 in resting and PHA-activated lymphocytes obtained from a statistically significant number of healthy donors. Two specific anti-SURF6 antibodies were used to assess the SURF6 level by quantitative immunocytochemistry and on immunoblots. Activation of lymphocytes was monitored by FACS analysis after 16 h (G1 period), 24 h, 48 h and 72 h, which correspond to the most high proliferation activity of lymphocytes. In parallel, the expression level of a multifunctional nucleolar protein NPM1/B23, the Ki-67 and PCNA proteins were also analyzed. Our results show that similar to Ki-67 and PCNA but unlike NPM1, the SURF6 protein remains undetectable in resting (or G0) lymphocytes. Its expression commences around 16 h of PHA-activation when neither Ki-67 nor PCNA can be detected. Further activation of lymphocytes is accompanied by augmentation of the number of SURF6-positive cells as well as by an increase of specific SURF6 signals on

immunoblots. However, even after 72 h of PHA activation, the number of SURF6-positive lymphocytes exceeds that of Ki-67-positive cells. Based on these results we conclude that the nucleolar rRNA processing factor SURF6 is a novel and early marker of lymphocyte activation for proliferation. The study was supported by the Russian Foundation for Basic Research (grant 18-34-00767).

P.09-186-Wed
Changes in expression of miRNAs associated with nuclear factor kappa B in experimental acute respiratory distress syndrome

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Acute respiratory distress syndrome is a disease that have a high mortality ratio. microRNAs are small, non-coding RNAs that regulate gene expression by binding to specific target sites on mRNA. In recent years, it has been reported that miRNAs have an important role in a number of basic physiological and pathological processes. This study aimed to determine the expression response of nuclear factor kappa B-associated miRNAs in experimental acute respiratory distress syndrome (ARDS) induced by lipopolysaccharide (LPS) in rats. Expressions of 9 miRNA were determined by quantitative Real-Time PCR in each group. Expressions of 7 miRNA were significantly changed in the study group compared to control group. While rno-miR-7a-5p, rno-miR-7b, rno-miR-9a-5p, rno-miR-21-5p, rno-miR-29a-3p and rno-miR-138-5p ($P < 0.05$) upregulated, only rno-miR-124-3p ($P < 0.05$) were also found to be down-regulated. This study suggests that these miRNAs may have a role in the pathogenesis of ARDS related to NF-κB. However, this relationship needs to be examined in new studies by evaluation of pathways and target genes.

P.09-187-Mon
Phenotypic characteristic of a new model of frontotemporal degeneration caused by aggregation of the human FUS protein in the nervous system of transgenic mice

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The investigated line of transgenic mice was obtained and maintained based on the Center for Collective Use of the Institute of Biology of the Gene of the Russian Academy of Sciences. The genetic model was based on the functional analysis of the FUS protein involved in neurodegenerative processes. The FUS protein, like TDP-43, was associated with the pathogenesis of both amyotrophic lateral sclerosis and front-temporal frontal degeneration. It has been suggested that the key role in the pathological process associated with these proteins is played by the disorder of RNA metabolism, since both these proteins are DNA / RNA binding. During the work on the grant, it was possible to clarify the time limits for the development of the pathological process associated with neurodegeneration against the background of the overexpression in the brains of mice of the mutant form of the human FUS protein. It was shown that in the early stages of the course of the disease, it is possible to detect disturbances in the spatial memory function of transgenic mice when passing the Morris water labyrinth. Thus, 30 transgenic males were tested in 3 age groups - 45, 85, 105 days and the corresponding control group. The obtained results indicate the presence of statistically significant ($P < 0.1$) differences in the time of the mice in the platform waiting sector. The difference in average time spent in the target sector was 7.8 s or 13% of the total time of the test voyage. The results between the groups of 45 and 85 days were not statistically different (in the 85-day group of transgenic mice a decrease in the total distance traveled during the experiment was noted). The model under investigation seems promising for rapid testing of potential neuroprotective strategies and drugs for the therapy of amyotrophic lateral sclerosis and front-temporal frontal degeneration The reported study was funded by RFBR according to the research project No. 16-04-01805 A.

P.09-188-Tue
A heterozygous mutation NM_000518_c.7C>T (p.His3Tyr) in exon 1 in the HBB gene causing HbA1c interference: Hb Fukuoka

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Mutations and / or deletions result in genes encoding hemoglobin variants alpha and beta chains. There are more than 700 known variants, but half of them are silent. Variants that do not present any clinical findings usually come about as a result of interference in the HbA1c assay and cause measurement difficulty. The HbA1c result of a patient in our laboratory by ion exchange high performance liquid chromatography (HPLC) method estimated as %49. Rerun and dilution of the sample revealed the same result. The same sample's HbA1c estimation made by capillary electrophoresis (Sebia Minicap) in another laboratory, no abnormal Hb detected, also reanalysing the sample with dilution showed the same result. HbA1c value of the sample via another ion Exchange HPLC analyzer (UFLC, Shimadzu Prominence) was 39.4%. This analyze also did not revealed the true result of the HbA1c. The HbA1c value of the patient was studied in another cation-exchange HPLC method (Tosoh G8). HbA1c is 3.8% but does not reflect the correct result. Also a variant peak found as 41.6% in Tosoh. The sample was given for further analyze at genetic department. The sequence analysis of the HBB gene revealed a heterozygous mutation NM_000518_c.7C>T (p.His3Tyr) in exon. In addition, the patient was found to have a c.9T> C (rs713040) heterozygote substitution in the same codon, which did not lead to amino acid changes. Hemoglobin variants cause interference in the measurement methods of glycohemoglobin (HbA1c). This is the first case in Turkey Fukuoka Hb was identified by type The HbA1c and molecular analyzes of different measurement methods.

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P.09-189-Wed
Impact of long-distance mutations on properties of beta-lactamases conferring bacterial resistance towards beta-lactam antibiotics

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Beta-lactamases are enzymes responsible for bacterial resistance to beta-lactam antibiotics. TEM type beta–lactamases represent one of the most extensive subfamilies formed by more than 200 mutants. Mutations are divided to the key, lead to a change in substrate specificity, and the secondary. We have produced *in vitro* a number of recombinant TEM beta-lactamases, bearing two secondary substitutions Q39K and M182T and their combinations with the key mutations in positions 104, 164, 69. Kinetic study revealed that mutation Q39K, neutral by itself, was characterized by decreased K_M towards cephalosporins and loss of protein thermal stability in combination with the key mutations. The role of secondary mutations Q39K, M182T in combination with two key mutations G238S, E240K was investigated by means of molecular dynamics simulation. The trajectories were used to construct Residue Interaction Networks (RINs) in order to analyze the relationships between different residues. The analysis was focused on the effect of long-distance mutations on the mobility of the omega-loop, formed by residues 164–179, which represents catalytically important structural element of beta-lactamases located on the bottom part of the active site. Replacement of the residues 238 and 240 weakened the contacts between omega-loop residues, and its mobility was increased compared to the fixed state in wild type beta-lactamase. Combination of two key mutations with a secondary M182T resulted in the release of R65 and the formation of its new contacts with N175 and D176 which explained an increase in the stability of the beta-lactamases. The long-distance substitution Q39K interferes with the shift in the position of R65, and the mobility of the omega-loop becomes close to that of the parent enzyme. This effect on activity of beta-lactamases can be used as a new potential target for novel beta-lactamase inhibitors. The work was supported by the Russian Science Foundation (Project 15-14-00014).

P.09-190-Mon
An allelic variant of the SNP rs7873784 associated with autoimmune pathologies binds PU.1 and enhances TLR4 expression

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Toll-like receptor 4 (TLR4) is innate immunity receptor involved in development of many complex diseases. Human gene encoding TLR4 contains over 2000 single nucleotide polymorphisms (SNP), most of which are still not characterized. Most of these SNPs are located in non-coding, potentially regulatory regions. One particular SNP rs7873784, is located in the 3'-untranslated region (UTR) of *TLR4* gene and is associated with development of non-alcoholic fatty liver disease (NAFLD) and rheumatoid arthritis (RA). Since TLR4 has been shown to play a role in progression of both disorders, we have studied the effect of rs7873784 variation on enhancer activity of the 3'-UTR of human *TLR4* gene. Our *in silico* and *in vitro* results indicate that minor rs7873784 variant (C) creates a binding site for transcription factor PU.1, a known regulator of TLR4 expression. Increased binding of PU.1 results in a significant increase of enhancer activity of the *TLR4* 3'-UTR region while PU.1 knockdown or complete disruption of the PU.1-binding site eliminates this effect. We hypothesise that additional PU.1 site may increase TLR4 expression in individuals carrying minor rs7873784 variant (C) and aggravate development of NAFLD and RA. This project is supported by grant 14-14-01140 from Russian Science Foundation.

P.09-191-Tue
Release of impregnated protein from polylactide and chitosan granules, depending on their size, porosity, and the presence of a cross-linking agent

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The ability to set the peak of growth factors release from osteoplastic materials 3–5 days after implantation, by the end of the inflammation stage, is an important task of modern pharmacology and bioengineering. Determine the kinetics of BMP-2 release from chitosan granules and polylactide depending on the size of the polylactide and chitosan particles. With prior spraying freeze-drying technique was used for producing chitosan and porous polylactide (PLA) particles from its solvents or emulsions. The impregnation of the studied proteins, BSA and rhBMP-2, into PLA particles was carried out after production and in chitosan particles - *in situ*. Using calorimetry, the kinetics of the accumulation of BSA released from the beads in water was determined daily. Concentration of released rhBMP-2 at the DMEM / F-12 culture medium, with 10% bovine serum was measured by ELISA. The experiment was carried out for 5 days in 3 replicates. Chitosan particles of large size (1–2 mm) provided the maximum release of BSA and rhBMP-2 to the 3rd day, which corresponds to a minimum sufficient time for effective osteoinduction in the post-operative period. Large chitosan particles released 80 $\pm$ 13% of the whole impregnated protein to the 3rd day, while 100% of the protein left the small particles (0.2–0.7 mm) at the 2nd day. The crosslinked chitosan beads released up to 18 $\pm$ 10% of the impregnated protein with the maximum peak 10 $\pm$ 3% on the 6th day. A statistically significant difference in the release of BSA and BMP-2 between large and small particles from polylactide granules of different porosity was not detected. The release of 100% protein from polylactide granules was revealed at 2rd day. The effectiveness of impregnation in polylactide granules was 15% of the total protein weight compared to chitosan beads, where this index was 92 $\pm$ 7%. The research was supported by the RSF [grant n. 16-15-00298] and RFBR state assignment for RCMG.

P.09-192-Wed
Platelet rich plasma treatment may be effective in the protection of bladder oxidant-antioxidant balance in experimental spinal cord injury

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Platelets have many growth factors and cytokines which start wound healing. Growth factors are released from platelets by stimulation with various agents. Platelet rich plasma (PRP) provides rapid improvement in both soft and hard tissues. Spinal cord injury (SCI) can lead to bladder problems. In this study, the potential protective effect of PRP in experimental spinal cord injury was investigated for on bladder. Sprague-Dawley male rats were divided into three groups; control group, SCI group and PRP applied SCI group. Modified weight-drop model was performed for SCI induction. PRP was prepared by two consecutive centrifugations and applied to spinal cord. A week after SCI induction, rats were decapitated and bladders were taken. Bladder homogenate was prepared in saline to examine tissue biochemical parameters. Malondialdehyde, myeloperoxidase, and sialic acid increased significantly in SCI group compared to control group ($P < 0.01$, $P < 0.01$ and $P < 0.001$, respectively). Glutathione-S transferase, superoxide dismutase and catalase activities decrease significantly compared to control group ($P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively). Application of PRP improved almost all parameters significantly compared to SCI group. PRP treatment may have beneficial effects against SCI induced oxidative damage in bladder through its anti-inflammatory and antioxidant effects.

P.09-193-Mon
Effects of BF142 glycogen phosphorylase inhibitor on β cell model

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Glycogen phosphorylase (GP) enzyme catalyzes glycogen breakdown, hence, contributes to hepatic glucose production during fasting. Therefore, GP is considered as potential target to modulate glucose levels in type 2 diabetes, and pharmacological GP inhibitors are regarded as potential antidiabetic agents. The natural product FR258900 isolated from the cultured broth of the fungal strain No. 138354 was discovered a decade ago, and had been proven a potent glucose lowering agent in diabetic mice. Based on FR258900 we synthesized a potent GP inhibitor 2,3-bis [(2E)-3-(4-hydroxyphenyl)prop-2-enamido] butanedioic acid that was termed BF142. The aim of the current study was to investigate the effects of BF142 on MIN6 insulinoma cells, a well-established model for β cells. BF142 treatment slightly increases the pathway of insulin secretion, indicated by enhanced glycolysis, mitochondrial oxidation, ATP production and calcium signaling in MIN6 cells, however, the glucose-induced insulin secretion did not change. BF142 induced the phosphorylation of p70S6K indicating the enhanced protein translation. In treated cells we observed increased nuclear translocation of pancreatic and duodenal homeobox 1 (PDX1), as well as insulin production. These data suggest that BF142 can influence the function of β cells in some aspects, but not as coherently as the previously studied glucose analogue GP inhibitor KB228, or the CP-316819 GP inhibitor reaching phase II clinical trial.

P.09-194-Tue
Impact of novel UQCRC2 mutation on respiratory chain supercomplexes in cultured skin fibroblasts

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In the patient with psychomotoric delay, encephalomyopathy and lactic acidosis, whole-exome sequencing revealed a homozygous missense mutation (c.665G>C; p. Gly222Ala) in *UQCRC*2 coding for Core 2 protein, the structural subunit of mitochondrial respiratory chain complex III (CIII). CIII together with complexes I and IV (CI and CIV) associates into a variety of supramolecular structures known as supercomplexes (SCs). Biogenesis of CIII relies on coordinated expression of assembly factors and 11 structural subunits one of which is encoded by mitochondrial DNA. BN-PAGE immunoblot of lauryl maltoside-solubilized skin fibroblast mitochondria, condition in which SCs are disrupted, revealed severe decrease in amount of CIII as well as C I and normal levels of CIV and CV in the patient. However, BN-PAGE immunoblot analysis of digitonin-solubilized fibroblast mitochondria, condition in which SCs are preserved depending on used digitonin concentration, revealed normal level of free CI as well as CIV and decreased level of free CIII. Amounts of SCs composed of CI-III-IV and CIII-IV were substantially decreased in the patient. The data corresponds to impaired accumulation of SCs due to CIII depletion and supports suggestion that SCs function to confer stability to CI. In the study of Zara and colleagues, the yeast strain carrying deletion of Core 2 homolog revealed accumulation of new subcomplex made up of yeast homologs of Core 1 and CYC1 structural subunits. Nevertheless, no new subcomplexes were detected in our patient, furthermore, free Core 1 subunit was observed to be moderately decreased compared to control in digitonin-solubilized samples. Until now, also the mutation (c.547C>T; p.Arg183Trp) has been described in *UQCRC2* in patients with CIII deficiency and recurrent lactic acidosis with glycemic disturbance. Supported by UNCE 204064, GAČR 14-36804G, Progres Q26/LF1, RVO-VFN64165/2012.

P.09-195-Wed
Portulaca oleracea L. extract alleviates inflammation on dextran sulfate sodium-induced ulcerative colitis mouse model

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Portulaca oleracea L. (*P. oleracea*) is a widespread medicinal plant that is used to treat hypotension, diabetes and bacillary dysentery in many countries. Many studies were reported that *Portulaca oleracea L.* extract (PLE) alleviated inflammatory disease including inflammatory bowel disease (IBD). However, the functional mechanism of anti-inflammatory effects of

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IBD is not clear. IBD is a group of inflammatory disease including Crohn's disease and ulcerative colitis. We used dextran sulfate sodium (DSS)-induced ulcerative colitis mice model. In this study, the ameliorating efficacy of PLE on DSS-induced colitis mice was evaluated. 3% DSS and sulfasalazine (50 mg/kg/day) or PLE (30, 100, 300 mg/kg/day) were treated mice and then body weight change, colon length, histological colon injury and productions of cytokines in the serum were evaluated. The results showed PLE treated groups significantly ameliorated in weight loss, colon length reduction and histological changes. Histologically, the colon of PLE treated groups exhibited less epithelial cell shedding, crypt destruction and infiltration of inflammatory cells compared to DSS treated mice. Furthermore the concentrations of interferon-γ, interleukin (IL)-6, IL-1β and tumor necrosis factor-α in serum were decreased in PLE treated groups. Consequently, these results showed that PLE ameliorated colitis in DSS treated mice. PLE could be a therapeutic natural material for ulcerative colitis.

P.09-196-Mon
Disruption of protein folding machinery by pathogenic cystathionine beta-synthase mutants

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Pathogenic missense mutations in cystathionine beta-synthase (CBS) cause structural perturbations, which result in misfolding of the mutant enzymes with subsequent aggregation or rapid degradation of the proteins. Loss of CBS activity leads to homocystinuria (HCU), an inherited metabolic disorder characterized biochemically by severely elevated plasma homocysteine and methionine levels and clinically manifesting with connective tissue defects, thromboembolism, stroke and premature death. It was shown previously in a yeast model of HCU that HSP26:HSP70 ratio determines whether misfolded CBS I278T mutant will be either refolded or degraded via ubiquitin/proteasome-dependent mechanism. Here, we examined expression of molecular chaperones in mammalian cells recombinantly expressing pathogenic CBS mutants and in patient-derived fibroblasts. Furthermore, we evaluated whether overexpression of HSP70 or a global heat shock protein (HSP) transcription regulator HSF1 impacts steady-state levels and activity of CBS mutants. In comparison to the appropriate negative controls, expression at mRNA and protein levels of several molecular chaperones by RT-PCR and Western blot, respectively, was impaired in models of HCU, namely BiP, HSP27 or HSP40. Higher abundance of HSP27 and its phosphorylation on residues Ser78 and Ser82, but not Ser15, in cells expressing CBS mutants suggest that this small HSP plays important role in deciding the fate of mutant CBS and thus may represent a mammalian ortholog of yeast HSP26. Levels and cellular localization by confocal microscopy of BiP may serve as an indicator of severity of folding defects caused by particular CBS missense mutations. Overexpression of HSP70 and HSF1 resulted in an increased abundance of mutant CBS protein, such as R125Q and T191M, which, however, lead only to a modest rescue of CBS activity, if any. Taken together, targeted manipulation of HSP levels may represent a viable therapeutic approach for the certain cases of HCU.

P.09-197-Tue
Association of serum myoglobin level and histological myocardial changes in short-term isoproterenol model of the myocardial damage in Wistar rats

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Isoproterenol is a non-selective agonist of beta adrenergic receptors that causes myocardiocyte damage in a dose-dependent manner due to calcium overload. We investigated association between myoglobin serum level (MSL) and myocardial changes induced by isoproterenol administration (100 mg/kg of rat body weight) in a short-term model of myocardial damage in Wistar albino rats. Thirty adult male Wistar rats were randomly distributed into groups: control group (CG) received saline (n = 6) and ISO group treated with ISO. ISO group was divided into 4 groups according to the time of MSL determination after ISO administration: ISO I (n = 6; 30'), ISO II (n = 6; 60'), ISO III (n = 6; 120') and ISO IV (n = 6; 240'). Blood for MSL determination was taken from abdominal aorta. MSL was determined with ELISA. Histological sections of the heart were stained with hematoxylin-eosin and graded as grade 1 (very few myocardiocytes damaged), grade 2 (multiple myocardiocytes damaged), grade 3 (extensive focal damage of myocardiocytes) and grade 4 (most of myocardiocytes damaged). Median MSL values were higher in all ISO groups compared to CG with plateau within first hour. In the next 2 h, further increase was noted. There were statistically significant differences in MSL between ISO I and ISO III; ISO I and ISO IV ($P = 0.04$, respectively); ISO II and ISO III ($P = 0.009$) and ISO II and ISO IV ($P = 0.01$). No differences were found in MSL between ISO I and ISO II and between ISO III and ISO IV. Histologically, myocardiocytes evolved from eosinophilia and granular degeneration at 30' post-ISO to necrosis with complete loss of nuclei at 240' post-ISO. There was a moderate correlation between MSL and myocardial lesion score (rho = 0.549, $P = 0.01$). Isoproterenol is a potent marker to induce myocardial damage evolving from degeneration to myocardial necrosis. Those changes are in a moderate correlation with myoglobin in serum because of its short half-life and rapid removal by kidney.

P.09-198-Wed
7-Methylguanine: a natural DNA repair inhibitor and a promising anticancer compound

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7-Methylguanine (7-MG) is a natural compound which inhibits DNA repair protein poly(ADP-ribose)polymerase 1 (PARP-1) *in vitro* and thus may be considered as a potential anticancer drug candidate for a combination chemotherapy. We previously demonstrated the ability of 7-MG to suppress the activity of recombinant human PARP-1 using the radiolabeled substrate NAD^+ and activated DNA, and here we present in more detail analysis of its inhibitory and anti-proliferative effects. (1) The inhibitory properties of 7-MG against purified PARP-1 were

confirmed by a novel fluorescent method for the real-time measurement of the enzyme activity. 7-MG was shown to be a competitive inhibitor of NAD^+ binding to the catalytic fragment of PARP-1. (2) The effect on PARP-1 binding to nucleosomes was studied using spFRET microscopy. 7-MG exhibited a minimal effect on the nucleosome structure, but induced PARP-1 trapping in non-productive complexes. (3) 7-MG cytotoxicity was evaluated on human cancer cell lines HCT116 (colorectal carcinoma) and U2OS (osteosarcoma) by measuring Sub-G1 (apoptotic) population of cells with flow cytometry. At the PARP-1 inhibitory concentrations, 7-MG itself was not cytotoxic, but it was able to accelerate apoptotic cell death in combination with cisplatin (a DNA-damaging drug). (4) QSAR-modeling of the ADMET profile of 7-MG was done with ACD/Percepta. The safety of 7-MG was predicted and confirmed by preliminary *in vivo* tests on mice. Despite the fact that 7-MG is a weaker inhibitor than olaparib and some other PARP inhibitors, this natural compound may possess better pharmacokinetics and an adverse-effect profile compared to synthetic inhibitors and become a promising new constituent of anticancer therapy. This work was supported by RFBR grants No. 17-08-01614 and No. 17-00-00163 (17-00-00132, 17-00-00097).

P.09-199-Mon
ER-driven anti-aggregate activity toward pathogenic alphaB-crystallin mutants

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Mutations within the alphaB-crystallin (aBC) gene are linked to alphaB-crystallinopathy, an autosomal-dominantly inherited muscular disease, histologically characterized by intracellular accumulation of protein aggregates involving aBC mutants. We previously showed that enforced expression of wild-type aBC on the ER membrane prevents aggregate formation mediated by a pathogenic aBC mutant with an Arg 120 to Gly (R120G) substitution, and that the anti-aggregate activity toward the R120G mutant depends on physical interactions between the ER-anchored aBC (ERaBC) and CLN6, an ER transmembrane protein. Symptoms and age of disease onset vary from one aBC mutant to another, implying mutant-specific mechanisms underlying the pathogenesis of alphaB-crystallinopathy. In this study, we thus explored if the use of ERaBC is effective to repress aggregate formation by aBC mutants such as D109H, G154S and R157H, all of which cause phenotypes distinct from that associated with the R120G mutant. When individually transfected into HeLa cells, all those aBC mutants aggregated similarly. Meanwhile, coexpression with ERaBC profoundly inhibited the aggregate formation, indicating that ERaBC can antagonize diverse aBC mutants with aggregate-prone properties. In our previous report, ERaBC's preventive effect on the R120G mutant was attenuated in HeLa cells challenged with the lysosomal inhibitor bafilomycin A1. Unexpectedly, bafilomycin A1 treatment did not significantly affect ERaBC's anti-aggregate activity toward the D109H, G154S, or R157H mutants, demonstrating that ERaBC operates in both bafilomycin A1-sensitive and -insensitive manners according to amino acid replacement. Taken together, we conclude that ERaBC can manipulate the microenvironment surrounding the ER membrane not in a single way, providing potential therapeutic strategies for countering a wide range of pathogenic aBC mutants.

P.09-200-Tue
Identification of irreversible IMPDH inhibitors by luminescence-based high-throughput screening

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IMPDH is a fascinating enzyme that converts inosine monophosphate to guanosine monophosphate, thus controlling the gateway for guanine nucleotide biosynthesis. Due to its vital role, the utilization of IMPDH as a drug target has been considered for various therapeutic domains, such as immunosuppression in graft transplantation, antineoplastic, and anti-infection. Given the chronic nature of some of these therapies, it is necessary to develop irreversible inhibitors of IMPDH which could provide prolonged therapeutic duration. With this in mind, a luminescence-based high-throughput-screening study of 3,200 compounds was conducted for IMPDH inhibitory activity. The screening identified three known compounds as irreversible IMPDH inhibitors: disulfiram, bronopol, and ebselen. Subsequent evaluation of inhibition kinetics revealed that the compounds are indeed potent *Cryptosporidium parvum* and human type 2 IMPDH inhibitors with k_{on} values up to 9 x 10^4 M^{-1} s^{-1}. Elucidation of inhibition mechanism showed that these compounds bind to the IMP binding site of IMPDH, most likely by reacting with the catalytic cysteine residue. A mouse model of *C. parvum* infection, parasitic protozoa that rely on IMPDH for its guanine production, was utilized to test the effect of these inhibitors *in vivo*. A significant reduction *C. parvum* oocyst shedding was observed, indicating parasite growth inhibition.

P.09-201-Wed
Interferon regulatory factor 4 regulates the expression of ANP1 gene in beige-like 3T3-L1 and X9 cells

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Brown adipose tissue is a key location of heat production in mammals. Brown adipocytes in brown adipose tissue express thermogenic genes in the defense against a cold in nonshivering thermogenesis. Inducible 'brown-like' adipocytes, also known as beige cells, develop in white fat to promote thermogenesis in response to various activators. The activities of brown and beige adipocytes reduce metabolic disease including obesity. Interferon regulatory factor 4 (IRF4), a key regulator of thermogenesis, is induced by the cold in brown and beige adipocytes. Here, we show that IRF4 transcriptionally activates expression of atrial natriuretic peptide (ANP) in brown and beige adipocytes. The level of ANP expression in beige-like-differentiated 3T3-L1 cells was increased. The expression pattern of ANP was similar to that of IRF4 during the differentiation of beige adipocytes. Overexpression of IRF4 in cells increased the level of ANP protein, whereas knockdown of the IRF4 gene results in a reduced basal level of the ANP expression. A putative IRF4-binding site in the ANP promoter was identified. By chromatin immunoprecipitation and luciferase reporter assays, we observed that IRF4 interacts with the -1187/-1163 region of the ANP promoter in beige-like adipocyte. Our results also confirmed in X9 mouse brown adipocytes. The level of ANP expression in X9 cells was induced by overexpression of IRF4. Taken together, our findings show

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that IRF4 is essential for the transcriptional activation of ANP during brown and beige cells differentiation, and may reveal a potential therapeutic effect of ANP on obesity-related disease.

P.09-202-Mon
Characterization of type II toxin-antitoxin systems in *Agrobacterium tumefaciens*

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In bacterial and archaeal genome or plasmids, there are toxin-antitoxin (TA) systems which are consisted with toxin and its antitoxin counterpart. TA systems were first described to function as post-segregational killing mechanisms to avoid loss of plasmids, and then they have been reported to decrease energy consumption by reducing cell growth as stress responses. These TA systems are classified into 4 to 6 categories by how their antitoxins inhibit toxins' activities. In type II TA system, one of the most studied, toxins and their cognate antitoxins are located in the same operon, and antitoxins prevent their toxins from being active. In stress conditions, however, relatively unstable antitoxins are quickly degraded, toxins become active, and toxins make cells stop growing by various mechanisms. Until now, it has been unknown whether *Agrobacterium tumefaciens* have TA systems. In this study, we isolated 6 type II toxin-antitoxin system candidates, Atu0939-Atu0940, Atu2018-Atu2017, Atu0674-Atu0673, Atu0934-Atu0933, Atu8169-Atu-8168, and Atu-1004-Atu1003 in the genome of *Agrobacterium tumefaciens*, and we found that these are homologous to mRNA interferases of *E. coli*, MazF, YeoB, YafO, and YafQ, by amino acid sequence alignment. To find out if these isolated genes belong to TA system, we overexpress putative toxin-antitoxin pairs in pBAD system with 0.2% arabinose on the same plates. All of 6 pairs did not inhibit cell growth, while toxins by themselves showed cell growth inhibition. Therefore, we confirmed that these genes are TA systems. Furthermore, we performed Northern blot assay with *ompF* to determine if these toxins are mRNA interferases that cleave mRNAs as substrates Here, we report TA systems in *Agrobacterium tumefaciens* for the first time, and the understating of TA system in this bacteria can help to develop the method of prevention and the therapeutic agents of related diseases, such as crown gall diseases.

P.09-203-Tue
The percentage of acute myocardial infarction diagnosis of patients who applied to hospital by chest pain complaint

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In this study, it was aimed to investigate the extent of acute MI (AMI) and biochemical laboratory parameters of the patients with chest pain in the emergency department of our hospital. Archive records of biochemistry, cardiology and emergency departments of Afyon Kocatepe University Ahmet Necdet Sezer Application and Research Hospital were retrospectively reviewed. Demographic information (age, sex), biochemical markers (CK MB, Troponin I, Myoglobin) levels were determined by the research. Statistical analysis was performed with the information obtained. A total of 2820 patients were admitted to our emergency department with complaints of chest pain. Only 140 (4.96%) of these patients had acute myocardial infarction (AMI) diagnosed by cardiology. Of the AMI-diagnosed patients, 113 were male (80.70%) and 27 were female (19.30%). The mean age of the patients who were admitted to our clinic with an acute chest pain complaint was 63.50 ± 11.56 (32–86). CK-MB was found in 2752 cases of the patients who complained of acute chest pain, and 1256 (45.63%) of them were found. Troponin I (Tp I) was found in 2706 patients and 598 (22.09%) were found to be high. The myoglobin levels were measured in thirty-five patients and myoglobin levels were found higher in 9 patients (25.7%). Troponin I was measured in 136 of AMI-diagnosed patients and 133 (97.79%) were high, in 124 of 136 patient CK-MB levels were high (90.51%); in 4 of 6 patients myoglobin levels were found high (66.6%). 4.96% of patients with chest pain complaints were diagnosed as AMI. The most specific biochemical markers used for AMI were Tp I (97.79%) and CK-MB (90.51%) in the second.

P.09-204-Wed
The influence of selected metal ions on the oligomerization of cystatin C and its variants

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Neurodegenerative diseases are one of the major challenges to modern medicine. The lack of effective drugs and therapy systems results in the growing number of individuals suffering from different forms of dementia and other dysfunctions arising from the central nervous system degeneration. The Icelandic type of hereditary cerebral amyloid angiopathy is caused by aggregation of a protein called cystatin C (hCC). Mutation in the hCC gene leads to the replacement of the leucine residue in the 68th position of the chain with the glutamine residue. The result of the change is a production of highly amyloidogenic variant L68Q. Mutant forms deposits that accumulate in the blood vessels of the brain. Weakened arteries burst due to blood pressure resulting in massive haemorrhages and strokes leading to the patient's death. Many studies on amyloidogenic proteins such as amyloid β (Alzheimer's disease) and α-synuclein (Parkinson's disease) indicate the important role of metal ions in the oligomerization process. Copper and zinc, which are found as trace elements essential for proper functioning of the body, probably accelerate brain degeneration. In our project, we have studied the impact of metal ions on the oligomerization and aggregation of human cystatin C. The wild-type protein and its mutants with increased or lowered propensity for oligomer formation were incubated with copper at different ratios. The results indicate a strong impact of copper ions on hCC oligomerization process, in most cases its substantial acceleration connected with protein precipitation. The effect of copper-induced protein aggregation may also apply to other transition metals and affect the cystatin C oligomerization *in vivo*. A thorough examination of the deposits formation mechanism is necessary for proper understanding of hCC and other amyloidogenic proteins pathogenicity. Work supported by National Science Centre NCN OPUS 11 grant UMO-2016/21/B/NZ1/02823.

P.09-205-Mon
UV-emitting upconversion nanoparticles for the treatment of estrogen-dependent tumors

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We have previously obtained ultraviolet-emitting upconversion nanoparticles (UV-UCNP) and applied them for deep cancer treatment with near infrared (NIR) light *in vivo*. The method is based on active UV-UCNP accumulation in tumor and its emission in UV region upon NIR irradiation. Different endogenous chromophores such as riboflavin or DNA accept UV, leading to cytotoxic reactive oxygen species or pyrimidine dimers formation. As a result, we observed tumor regression upon soft and deep-penetrating NIR-irradiation. Here we demonstrated the enhanced accumulation of riboflavin in estrogen-dependent cell line MCF-7 when supplemented with the hormone. This correlates well with the literature review suggesting that riboflavin binding protein RfBP gene expression is under estrogen control. The fluorescence signal of blue-light photoexcited riboflavin has been also 5 times higher in hormone-supplemented MCF-7 comparing to fibroblasts which was detected by flow cytometry. We assume it to be due to enhanced glycolysis of estrogen-dependent cells and propose to take advantage of high enough riboflavin accumulation by estrogen-dependent tumors and to apply UV-emitting upconversion nanoparticles, which match excitation of riboflavin when irradiated with NIR light for tumor therapy. This scientific work was supported by Grants RSF no. 16-13-10528 (in the part of UCNP synthesis) and no. 14-24-00106 (in the part of *in vitro* experiments).

P.09-206-Tue
Antioxidant modifications of new metformin derivate HL156A regulate metabolic reprogramming of SAMP1/klotho (−/−) mice

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Aging is characterized by a reduced ability to defense with stress, an inability to maintain homeostasis, and an increased risk of disease. This study aimed to identify novel metabolic pathways perturbed in accelerated aging mice (SAMP1/kl −/−) using metabolomics approach and to gain new metabolic insights into the metformin derivative, HL156A. In SAMP1/kl −/− MEFs, we observed that HL156A induces the FoxO1 fork head transcription factor that is down-regulated by insulin/IGF-1 signaling and inhibits the IGF1/AKT/mTOR signaling pathways. HL156A treatment leads to decreased reactive oxygen species (ROS) production and enhanced mitochondrial transmembrane potential in SAMP1/kl −/− MEFs. In metabolome profile analysis, the reduced glutathione was importantly decreased in the kidney of SAMP1/kl −/− mice (8–12 week). However, treatment of HL156A (30 mg/kg) for 4 weeks improved survival and enhanced the level of glutathione (GSH) and decreased significant elevation in GSSG, along with induction in GSH/GSSG ratio in kidney of SAMP1/kl −/− mice. In histological sections, GSH and FOXO1 increased in HL156A-treated SAMP1/kl −/− mice compared to untreated controls. In addition, the excessive calcification was inhibited by HL156A treatment in the kidney. Based on the above findings, we conclude that the new metformin derivate HL156A may protect kidney from oxidative damage through modulating antioxidant pathways via induction of glutathione metabolism. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2018001260).

P.09-207-Wed
Possible role of LPA SNPs on future cardiovascular risk prediction in patients with preeclampsia

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Cardiovascular disease (CVD) and preeclampsia share several common metabolic and structural abnormalities such as, lipid abnormalities, oxidative stress, and inflammatory response and acute atherosis of the placental bed and atherosclerotic plaque. Further, there is an increased neutrophil activation with elevated C-reactive protein levels and an increased leukocyte adhesion to endothelium may be the other contributor of the preeclampsia. The similarity between the pathogenesis of preeclampsia and the atherosclerosis has led researchers to investigate the role of Lp(a) in pre-eclampsia because of its act as an acute phase protein and plays a role as a vehicle for cholesterol deposition at the site of abnormal placentation. It is well-known that Lp(a) levels have an impact on increased risk of CVD which is affected by LPA gene. Further, LPA genotypes which carry high CVD risk are not evaluated along with cardiovascular risk. Our study tested whether cardiovascular corresponding LPA risk genotypes improve preeclampsia and cardiovascular disease (CVD) risk prediction beyond conventional risk factors. We were included 200 pregnant Turkish women into the study. We stratified the preeclamptic (PE) group: early (EOP) (28.7 ± 3.0 weeks) and late onset(LOP) (36.0 ± 1.4 weeks). 14 LPA SNPs were evaluated in the study. rs9355296 and rs3798220 were found an independent risk factor for preeclampsia by logistic regression analysis. A positive correlation was found between rs9355296 and the diagnostic criteria of preeclampsia. Further rs9355296 G/* carriers have higher vascular inflammation rather than AA carriers. The findings reveal that LPA genetic variability with high inflammatory response might be an indication of future cardiovascular events.

P.09-208-Mon
Kinetics of regulation of muscle proteolysis signaling in a slow-twitch hindlimb muscle of mice exposed to immobilization and reloading

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Many chronic diseases are associated with muscle mass loss (sarcopenia). We hypothesized that the kinetics of regulation of

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muscle proteolysis signaling (including acetylation of transcription factors) may differ between early- and late-time points in a slow-twitch muscle of mice exposed to hindlimb immobilization (I) and recovery following I. In soleus of mice exposed to periods (1, 3, 7, 15 and 30 days, I groups) of immobilization or reloading (1, 3, 7, 15 and 30 days, R groups) following 7-day immobilization, food intake, total body and soleus muscle weights, levels of markers of proteolysis, muscle-specific microRNAs (miR-1, miR-206, miR-133, miR-486), autophagy, and acetylated levels of myogenic transcription factors were assessed. Compared to non-immobilized control muscles, in the 7-day I, 15-day I and 30-day I mouse cohorts, soleus muscle weight, FoxO1 and PGC-1alpha acetylation levels, and miR-1 and miR-206 expression levels were reduced, while those of acetylated FoxO3, Bax, Bcl-2, and p62 were increased. Muscle reloading following splint removal increased soleus muscle weight and Sirtuin-1 protein levels, whereas E3 ligases atrogin-1 and MuRF-1 protein levels were significantly decreased. In this mouse model of disuse muscle atrophy, FoxO3 acetylation may drive muscle mass loss during immobilization in a slow-twitch muscle of the hindlimbs.

P.09-209-Tue
Nardilysin, a new PER2-interacting protein, regulates the mammalian circadian clock

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The circadian clock is a timing system that allows organisms to keep metabolic, physiological, and behavioral rhythms in resonance with daily environmental cycles. Thus, circadian disruption may lead to a number of diseases, including metabolic syndrome, cancer, and inflammation. In mammals, the molecular clock is composed of transcriptional/translational negative feedback loops. The transcription factors CLOCK:BMAL1 activate transcription of the *Period* (*Per*) and *Cryptochrome* (*Cry*) genes through E-box elements. The translated PER and CRY proteins repress CLOCK:BMAL1 activity to inhibit their own expression. Although fundamental, the underlying mechanism of negative feedback still remains unclear. Here, we show that nardilysin (N-arginine dibasic convertase; *Nrd1* and NRDC) interacts with PER2 and modulates the mammalian circadian clock. NRDC-deficient mice exhibited significantly shorter circadian period than wild-type littermates. ChIP-seq analysis for NRDC-DNA binding in hepatic chromatin and motif analysis of the NRDC-binding sites revealed a significant enrichment for USF1 (E-box) motif. These results suggest that NRDC interacts with clock proteins at E-box. Co-immunoprecipitation assays showed that NRDC forms a complex with PER2, but not CLOCK and BMAL1. To investigate whether NRDC regulates PER2 function, reporter assays were carried out. While PER2 dose-dependently suppressed CLOCK:BMAL1-induced transactivation, NRDC expression significantly inhibited PER2-mediated suppression of the transactivation. Our findings demonstrate that NRDC regulates the mammalian circadian rhythm via modulating PER2 function.

P.09-210-Wed
MPK38 Enhances MST1-mediated apoptotic signaling through direct interaction

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Mammalian sterile 20-like kinase 1 (Mst1) is a serine/threonine kinase that is activated by caspase-mediated cleavage and phosphorylation in response to apoptotic stimuli, such as cisplatin. Here, we identify MPK38 as regulators of Mst1 in apoptosis. Direct binding of MST1 and MPK38 can be detected when co-expressed in HEK293 cells and was also confirmed by an immunoprecipitation analyses in various cell types. During cisplatin-induced apoptosis in Jurkat cells, the physical association between MPK38 and Mst1 is increased. Overexpression of MPK38 increased the apoptotic activity of Mst1 in intact cells, suggesting that regulation of Mst1 by MPK38 involves more than the simple association of the two proteins. Both the activation of Mst1 and the incidence of apoptosis induced by cisplatin were reduced in cells depleted of MPK38 by RNA interference and were increased by overexpression of MPK38 in cells. Taken together, our data indicated that MPK38 can act as novel potential regulators of Mst1 activation by direct interaction and thereby promotes apoptosis induced by apoptotic signaling.

P.09-211-Mon
Effect of freezing time on tissue factor activity of breast milk

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Breast milk is a miracle that contains both nutritional components and non-nutritive bioactive factors that promote survival and healthy development. Breast milk also has clot-promoting activity and this activity is attributed to the presence of tissue factor. Tissue factor is a receptor factor in the extrinsic coagulation system and is expressed in various tissues and body fluids. Although scientific evidence related to the effects of milk storage conditions on nutritional components is present, tissue factor activity changes with the storage conditions have not been studied. Therefore in this study, the effect of freezing time up to 6 months on the breast milk tissue factor activity, fat, protein, lactose, water, pH and energy levels were determined. For this purpose, breast milk samples were collected from healthy women and pooled. Breast milk was divided into aliquots and stored at -20°C. On day 0, tissue factor activity, fat, protein, lactose and pH levels were also determined. In the experiment days, milk samples were divided into 6 aliquots and the nutritional parameters were measured in all aliquots. Tissue factor activity and lactose level did not significantly change during the first 2 months storage but then gradually decreased. Protein, fat, water and energy levels also decreased with freezing but this decrease was present in all time intervals. Breastmilk pH did not significantly change with freezing. As a conclusion; the role of tissue factor in breastmilk is not clear but its activity loss during the storage time should be accounted as the higher coagulative activity of tissue factor in fresh breast milk may have some protective role in the oral tissues of babies.

P.09-212-Tue
Confirmatory and orthogonal assays for eliminating artefactual drug bioactivities

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In the search for potential novel drugs, thousands of extracts derived from endemic and indigenous Philippine plants have undergone primary screening against important disease indications including cancer, cardiometabolic diseases and inflammation. As part of the national drug discovery pipeline, this study has developed and performed specialized sets of orthogonal and secondary assays to validate primary hits and to rule out potential artefactual drug bioactivity. For cancer, a suite of multi-hallmark assays evaluating effects on cell proliferation, apoptosis, migration, and invasion was established. Given the multiple manifestations of cancer, this strategy offers the advantage of providing a more comprehensive bioactivity profile against the disease. Notably, DDHP-1508 has emerged to be a principal hit due to its antagonistic effects on all the hallmarks tested. For cardiometabolic diseases, glucose uptake assay and high-content imaging-based assays measuring LDL-cholesterol uptake were performed. The orthogonal assays conducted had successfully confirmed bioactivity in 64 compounds out of the initial 918 primary hits for peripheral glucose uptake, and 8 compounds out of the initial 770 for increased LDL-cholesterol uptake. For inflammation, a sequential workflow was developed to identify dual COX-2/5-LOX inhibitors. Reactive Oxygen Species (ROS) activity visualization is followed by a COX-2 activation reporter assay, then by PGE2 and 5LOX immunostaining. Validity of top-tier hits were then confirmed by inflammation markers via qPCR. From a pool of 263 extracts, DDHP-0175, DDHP-1975, and DDHP-900.10 were recognized as the top candidates for dual COX-2/5-LOX inhibition. Taken together, the set of methods used for each of the disease indications has been proven to be effective for validating primary assay hits, narrowing down the potential novel drug candidates, and providing a prioritization scheme for downstream purification, structure-ID, and clinical testing.

P.09-213-Wed
A novel receptor in *Clostridium saccharobutylicum* that recognizes the quorum sensing signal autoinducer-2

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Quorum sensing is a mechanism for cell-to-cell communication that, through the exchange of small chemical molecules called autoinducers, allows bacteria to monitor their population density and regulate gene expression accordingly. Autoinducer-2 (AI-2) is a quorum sensing signal that is unique in facilitating inter-species communication. While it is produced and recognized by a wide variety of bacteria, to date only two classes of AI-2 receptors have been identified: the LuxP-type, in the *Vibrionales,* and the LsrB-type, found in a number of phylogenetically distinct bacterial families. Recently, AI-2 was shown to affect the colonization levels of a variety of bacteria in the microbiome of the mouse gut, including members of the genus *Clostridium*, but no AI-2 receptor had been identified in this genus. Here, we demonstrate that *Clostridium saccharobutylicum* possesses a functional LsrB-type AI-2 receptor. The crystal structure of the *C. saccharobutylicum* receptor shows that it binds the same form of AI-2 as the other known LsrB-type receptors, but also reveals that it has two previously unobserved variations in the amino acids of the AI-2 binding site. Isothermal titration calorimetry shows that this receptor binds AI-2 with high affinity (dissociation constant in the submicromolar range). Altogether, this work represents the first identification and characterization of an AI-2 receptor in the *Clostridium* genus and shows previously unseen variations in the binding site of LsrB-type AI-2 receptors. These findings are important for the identification of novel AI-2 receptors in therapeutically relevant microbes in the mammalian gut microbiome, a niche where inter-species interactions are highly prevalent. Moreover, they provide valuable structural details to aid in the design of agonist or antagonists active against a wider range of quorum sensing receptors.

P.09-214-Mon
Up-regulation of the proapoptotic mediator PMAIP1/NOXA by heat shock

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PMAIP1/NOXA is a pro-apoptotic protein, a member of the Bcl-2 family. We found that *Pmaip1* was the most induced gene in testes of transgenic mice expressing constitutively active, mutated HSF1 (Heat Shock Factor 1). HSF1 is the main mediator of the heat shock response typically inducing cytoprotective Heat Shock Proteins (HSPs). However, cell death is induced by active HSF1 in mouse spermatocytes, possibly via PMAIP1. Based on genomic studies we stated heat-induced HSF1 binding to its consensus binding sites (HSEs), interestingly located in the *Pmaip1* introns, and up-regulation of the *Pmaip1* transcription in spermatocytes and in certain somatic human and mouse cell lines and tissues subjected to heat shock. We found the PMAIP1 protein accumulation in so-called heat-sensitive organs. TUNEL test demonstrated that apoptosis was rapidly induced by heat shock in tissues exhibiting the highest *Pmaip1* activation, i.e. testes, thymus, and spleen. Among cell lines, the highest *Pmaip1* induction was observed in HECa10 murine endothelial cells. Using HECa10 cells with decreased HSF1 expression (shRNA) we confirmed that HSF1 is important for *Pmaip1* activation. Hemideletion of HSE from the second *Pmaip1* intron (CRISPR/Cas9) also led to diminished activation of *Pmaip1* by heat shock, however, we cannot exclude a clonal selection effect. Using live imaging microscopy we proved that PMAIP1 overexpression led to apoptosis. Furthermore, we deleted the *Pmaip1* gene in HECa10 cells to validate its participation in the regulation of apoptosis after heat shock. Our finding supports the idea that HSF1 may play a dual role in response to heat shock: cytoprotective, mediated by HSPs, or proapoptotic, mediated by PMAIP1. The final cell response to stress could be determined by the balance between antiapoptotic and proapoptotic factors differentially regulated by HSF1. This work was supported by the Polish National Science Centre (grant no 2014/13/B/NZ3/04650)

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P.09-215-Tue
Targeting isocitrate lyase for the treatments of tuberculosis

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The enzymes isocitrate lyase (ICL) isoforms 1 and 2 are essential for the survival of *Mycobacterium tuberculosis* within macrophages during tuberculosis infection. ICLs are not present in humans and are therefore attractive therapeutic targets for the treatment of tuberculosis. In this talk, we describe our work in the development and design of new ICL inhibitors. A particular focus is the application our combined high-throughput virtual screening, nuclear magnetic resonance spectroscopy and thermal shift assay strategy, which has led to the discovery of several novel ICL inhibitor scaffolds that are the subject of ongoing medicinal chemistry studies in our laboratories. Finally, we will also describe our recent efforts in studying the structure and catalytic mechanism of ICLs. We hope our work will inspire the development of the next generation of ICL inhibitors that may be used to treat tuberculosis.

P.09-216-Wed
Toluidine blue O reduces APLP2 and APLP2 CTF levels in Hs766T cells

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Cancer and neurodegeneration are two different pathological diseases but they share common mechanisms in many ways. Amyloid precursor-like protein 2 (APLP2) and its homologous family member, amyloid precursor protein (APP) are overexpressed in many cancers and they are linked to abnormal growth, migration, and invasion. A recent study has revealed that APLP2 has a role in the growth of pancreatic cancer cells. Besides, inhibitors that prevent APLP2 cleavage, leading to low APLP2 C-terminal fragments (CTFs), decrease the viability of pancreatic cancer cells. According to our recent studies, a phenothiazine-derived compound, toluidine blue O (TBO) was found to mitigate amyloid pathology by reducing APP and Aβ peptide levels in Chinese hamster ovary cells stably expressing wild type human APP and PS1. In present study, we aimed to investigate whether TBO may reduce the APLP2 and APLP2 CTFs, which have effective roles in pancreatic cancer growth and viability. Pancreatic cancer cells (Hs766T) were treated with a dose range of TBO (0–15 μM) or vehicle control for 24 h. After treatment of Hs766T cells with TBO without any side effect on cell viability, the levels of APLP2 and APLP2 CTFs in cell lysates were analyzed using Western blot and normalized to total protein levels. We observed a significant decrease in both intracellular APLP2 and APLP2 CTF levels in a dose-dependent manner compared to vehicle-treated cells. APLP2 levels were reduced by 26% ($*P < 0.05$) at 5 μM and 42% ($***P < 0.01$) at 10 μM TBO. Also, APLP2 CTF levels were decreased by 43% ($*P < 0.05$), 48% ($**P < 0.01$) and 66% ($***P < 0.001$) at 5 μM, 10 μM and 15 μM TBO. In conclusion, these results support the idea that TBO may be used as a therapeutic drug in pancreas cancer. Supported by a grant from the Scientific Research Unit of Hacettepe University (HUBAB, TSA-2017-13929).

P.09-217-Mon
Biological activity of South African macrofungi against respiratory and lung disease

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Macrofungi represent an untapped source of natural bioactive compounds for various diseases, which have been targeted as potential therapeutic agents. Respiratory disease and lung cancer places a global burden on health due to antimicrobial resistance, non-specific drug targeting and damaging side effects. This study investigated the antimicrobial activity and cytotoxicity of 21 South African macrofungi against respiratory pathogens (e.g. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Streptococcus pneumoniae*, *S. pyogenes*, *Staphylococcus aureus*) and human lung adenocarcinoma A549 cells. Ethanol and aqueous extracts were screened for antimicrobial activity using the ρ-iodonitrotetrazolium chloride assay and the effect on bacterial morphology determined using transmission electron microscopy (TEM). Cytotoxicity was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, and the mechanism elucidated by cell cycle analysis and fluorescent staining. Ethanol extracts showed higher antimicrobial activity against the Gram-positive bacteria than aqueous extracts. The macrofungal extracts of *Fomitopsis lilacinogilva* and *Pisolithus tinctorius* showed to cause cell membrane damage. Ethanol extracts of *Pycnoporus sanguineus, F. lilacinogilva* and *Gymnopilus junonius* and the aqueous extract of *Pseudophaeolus baudonii* showed cytotoxic activity against A549 cells, with IC_{50} ranging between 7.4–69.2 μg/mL. Fluorescent staining confirmed cell cycle arrest and apoptosis induced by extracts. Morphological and biochemical changes included chromatin condensation, membrane blebbing, loss of cytoskeletal structure, caspase activation and phosphatidylserine translocation. This study demonstrated the antimicrobial activity of South African macrofungi and their inhibition of A549 cancer cell proliferation by means of cell cycle arrest and induction of apoptosis.

P.09-218-Tue
Association between serum homocysteine levels and swimming stress in male and female rats

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Stress exposure has been associated with impaired homocysteine metabolism and consequently increased levels of homocysteine. Hyperhomocysteinemia is associated with increased risk of developing atherosclerosis, cardiovascular diseases, Alzheimer disease and other neurodegenerative diseases. Additionally, it was shown that prolonged stress exposure is a risk factor and female gender shows protective role for development of stress-related diseases. Aim of this study was to examine gender differences in serum homocysteine level (SHL) in rats exposed to swimming stress. Adult Wistar rats were distributed into three groups: control group (n = 12; CG), repeated swimming stress (n = 12; RSS) and single swimming stress (n = 12; SSS). Each of the rat groups were further equally divided by gender (n = 6; female and male control group – FCG and MCG, female and male repeated swimming stress – FRSS and MRSS, female and male single swimming stress – FSSS and MSSS). Rats exposed to repeated swimming stress

were forced to swim for 5 days, each day 5 min longer than previous day, in total 25 min on 5th day. Rats exposed to single swimming stress swam only once for 25 min. RSS and SSS rats were sacrificed on the 5th day immediately after swimming, while CG rats were sacrificed at rest. Blood samples were obtained from abdominal aorta and serum cortisol level (SCL) and SHL were determined by enzyme-linked immunosorbent assay. Stress response SCLs were significantly different between all 3 groups, both in male and female rats ($P = 0.007$ and $P = 0.009$, respectively). Moreover, SCLs were significantly higher in SSS rats of both genders compared to corresponding CGs ($P < 0.05$). There were no significant differences in SHLs between MCG, MRSS and MSSS as well as between FCG, FRSS and FSSS. SHLs and SCLs showed no gender differences in tested groups ($P > 0.05$). In this rat model, cortisol is a sensitive marker of acute swimming stress in both genders, whereas homocysteine is not.

P.09-219-Wed
Evaluation of nuclear factor-kB with oxysterols and clinical/biochemical characteristics of type 1 and type 2 diabetes mellitus

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Nuclear factor-kappa B (NF-kB) is a transcription factor that is strategic at the crossroad between oxidative stress and inflammation in Diabetes Mellitus (DM). This study describes the relationship between NF-kB and new oxidative stress biomarkers oxysterols in type 1 DM (DM1) and type 2 DM (DM2). Clinical and biochemical characteristics of 26 DM1, 80 DM2 and 205 age- and gender-matched healthy controls were determined. Oxysterols (7-Ketocholesterol and Cholestane-3β,5α,6β-triol) were quantified by LC-MS/MS. NF-kB was measured by ELISA. Significant higher levels of NF-kB and oxysterols that were observed in both types of DM compared to healthy controls, had also strong positive correlations [NFκB and 7-Ketocholesterol ($r = 0.919$, $P < 0.001$), NFκB and Cholestane-3β,5α,6β-triol ($r = 0.812$, $P < 0.001$)]. Positive and strong correlations of NF-kB with HbA1c ($r = 0.907$, $P < 0.001$), glucose ($r = 0.885$, $P < 0.001$), total cholesterol ($r = 0.627$, $P < 0.001$), and with the number of coronary risk factors ($r = 0.915$, $P < 0.001$) were found in DM2. Significant positive correlations of NF-kB with HbA1c, glucose, total cholesterol, and the number of risk factors were also found in DM1 ($P < 0.05$). Oxysterols also showed strong positive correlations with HbA1c, glucose, total cholesterol, and the number of coronary risk factors in both DM1 and DM2 patients. NF-kB and oxysterols were increased significantly in smokers. Oral hypoglycemic drug treatment reduced the levels of NF-kB and oxysterols in DM2. Activated NF-κB exacerbate inflammation, oxidative stress and promote apoptosis. NFκB is also activated by oxidative stress. It is highly likely that inhibition of NFκB will be considered as a logical target for the treatment of DM.

P.09-220-Mon
A comparative study on essential oils compositions and cytotoxic effects of wild and cultured form of *Origanum acutidens* (Hand.-Mazz.) Ietswaart. (Lamiaceae) on H1299 cells

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In this study, cytotoxic effects of essential oils from wild and cultured form of *Origanum acutidens* (Hand.-Mazz.) Ietswaart. (Lamiaceae) were determined on lung cancer cells (H1299). Also, the components of these two forms of essential oils were compared. The air-dried aerial parts of plants were powdered and subjected to hydro-distillation for 3 h using the Clevenger-type apparatus. The oils were investigated by GC and GC-MS. Cytotoxic effect of essential oil on H1299 cells were determined by Cell Titer-Blue^{R} Cell Viability. Nineteen components in wild form of *O. acutidens* and twenty four components in cultured form of *O. acutidens* essential oil were identified. The main components of the oils were found as carvacrol and p-cymene. The amount of carvacrol (72.65%) and p-cymene (15.19%) for wild form were found different than cultured form carvacrol (67.98%) and p-cymene (16.01%). IC_{50} values (concentration that kills 50% of cells) of wild form essential oil in H1299 cells were calculated as 149.5 μg/mL, 130.5 5 μg/mL and 109 5 μg/mL for 24, 48 and 72 h respectively. On the other hand, IC_{50} values of cultured form essential oil were found 83.3 μg/mL, 75.2 μg/mL and 64.5 μg/mL for 24, 48 and 72 h respectively. Cytotoxic effect of essential oil increased depend on concentrations and incubation times. As a result, essential oil from cultured form of *O. acutidens* had more cytotoxic effect on H1299 cells than wild form essential oils. It can come from amount of ratio of oil compositions.

P.09-221-Tue
Sphingolipidomic analysis reveals decreased levels of very long chain sphingomyelins and ceramides in sickle cell disease patients

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This study aimed to identify levels of C16-C24 sphingomyelin (CerPCho) and C16-C24 ceramide (CER) in serum obtained from SCD patients and controls. Circulating levels of neutral sphingomyelinase activity (N-SMase), ceramide-1-phosphate (C1P), sphingosine-1-phosphate (S1P) were also determined. Blood was collected from hemoglobin (Hb)A volunteers and homozygous HbSS patients. Serum levels of C16-C24 CerPCho and C16-C24 CER were determined by an optimized multiple reaction monitoring method using ultra fast-liquid chromatography coupled with tandem mass spectrometry. Serum activity of N-SMase was assayed by standard kit methods, C1P and S1P levels were determined by enzyme-linked immunosorbent assay. A significant decrease was observed in serum levels of C18-C24 CerPCho and very-long-chain C22-C24 CERs in SCD patients compared to controls. A significant positive correlation was found between

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serum total cholesterol levels and C18-C24 CerPCho, C22-C24 CERs in SCD patients. Patients with SCD had significantly elevated serum activity of N-SMase, increased circulating levels of C1P and S1P compared to controls. Erythrocytes are an important source for circulating sphingolipids. A probable decrease of sphingomyelin content in SCD red cell membranes can therefore lead to increased susceptibility to hemolysis. This work was supported by a grant (ID: 2557) from Akdeniz University Research Foundation.

P.09-223-Mon
New insights into allergenic relationship between red meat and cow's milk

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Red meat allergy is a novel form of food allergy with severe delayed allergic reactions where IgE antibodies are directed against the carbohydrate galactose-α-1,3-galactose (α-Gal) epitope. The α-Gal epitope is abundantly expressed on glycolipids and glycoproteins from non-primate mammals, but not in humans. Many red meat allergic patients report allergic reactions after drinking cow's milk. We investigated molecular basics of IgE reactivity to milk proteins among red meat allergic patients. Milk proteins, namely α-casein, β-casein, κ-casein, α-lactalbumin, β-lactoglobulin, bovine γ-globulin (BGG) were tested by immunoblot and inhibition ELISA using anti-α-Gal antibody and red meat allergic patient's sera. Basophil activation test was performed with milk and milk proteins. All the tested proteins were negative for the presence of α-Gal epitope except BGG. BGG was also shown to bind IgE antibodies from a pool of 15 red meat allergic patients, and the binding was almost completely abrogated by thyroglobulin (a glycoprotein rich in α-Gal epitope). Additionally, ELISA experiment showed that BGG exerts a dose-dependent inhibition of red meat allergic patients' IgE binding to α-Gal. Inhibition ELISA with raw milk and commercially available milk preparations showed that raw milk was a more potent inhibitor of the IgE binding than the commercially available milks. Importantly, activation of red meat allergic patient's basophils by BGG and milk was demonstrated. In this study we identified BGG as carrier of the α-Gal epitope in milk that bound IgE antibodies and furthermore activated basophils of red meat allergic patients. Taken together, the results highlight the importance of BGG as a clinically relevant milk allergen among the red meat allergic population.

P.09-224-Tue
Apoptotic and antiproliferative processes from dysplastic and metastatic prostate cells, modulated by proteolytic enzymes of entomological origin

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Proteolytic enzymes have important medical and pharmaceutical application due to their key role in developmental and tissue homeostasis during the life-cycle of many organisms. The delicate balance between cell proliferation and cell death is regulated by the activation of caspases, a conserved family of cysteine proteases, but also non-caspase proteases have their role in programmed cell death. Caspases from many organisms have been characterized, including several insects species. Although several basic strategies are used to activate and regulate caspase-dependent cell death, mammals and insects emphasize distinct points of control. The aim of our study was to explore the apoptotic process and its correlation with the distinct proteolytic activity of two types of entomological complexes. Also, we investigated their impact on one of the first hallmark of carcinogenic progression: the anchorage-independent growth, „in vitro" expressed by the soft agar colony formation assay. The medical transposition of this research will be in prostate's proliferative disregulation (benign hyperplasia, prostate cancer) generated by the stromal processes of transdifferentiation and cellular senescence, implicated in stromal modulation. The experimental models targeted standardised cell lines PWR-1E and DU-145 cells, relevant for prostate dysplasia and adenocarcinoma metastasis respectively. We used the flow cytometry technique for early and late apoptosis detection (annexin V and PE detection), soft agar colony formation assay for clonogenic capacity evaluation and gelatin- zymography for protease activity quantification. Results correlate a specific proteolytic activity of the entomological complex with its pro-apoptotic and anti-proliferative action. We highlighted one of the basic mechanisms that interrelates these processes and may substantiate therapeutic action in prostate dysfunctions.

P.09-225-Wed
Active and passive transport of carnosine and its derivatives into neurons

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The dipeptide L-carnosine is a recognized neuroprotective agent effective both in animal models and in cultured neurons. Carnosine has been shown to enter neurons and exert an antioxidant effect. Exogenously introduced carnosine is mainly transported into neurons via the oligopeptide transporter PEPT2. Since exogenous carnosine is rapidly cleaved by serum carnosinase, we are developing carnosinase-resistant carnosine derivatives to be used as neuroprotective drugs. Carnosine in a nanomicellar complex with α-lipoic acid is able to enter neurons, becomes more resistant to carnosinase, and shows neuroprotective activity at substantially lower concentrations. The study of the effectiveness of PEPT2-mediated transport of both carnosine and its derivatives into neurons is an important aspect of said drug development. To determine the proportion of passive and PEPT2-mediated active

transport of carnosine, we added carnosine (20 μM – 62.5 mM) to the culture of rat cortical neurons and determined its content within the cells after 5, 15 and 30 min. To determine the intracellular content of carnosine, the cultures were washed from external carnosine and lysed. The content of carnosine in the lysates was determined using liquid chromatography–mass spectrometry. The dependence of intracellular content of carnosine on the incubation time was linear for all concentrations except for the maximum one. The dependence of the rate of carnosine entry into the cells on its concentration in the medium can be decomposed into two components: the enzymatic one, described by the Michaelis-Menten equation (K_M = 118 μM), and the nonenzymatic one ($K_{ne} = 0.21 \times 10^{-4}$ min^{-1}). The nonenzymatic component of the transport rate prevailed over the enzymatic one at high concentrations of carnosine (over 12.5 mM). Thus, at physiological concentrations of carnosine (0.1–1 mM), the rate of its active transport into neurons is an order of magnitude higher than that of the passive transport.

P.09-226-Mon
Defining how formaldehyde regulates the functions of biomolecules

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Formaldehyde (HCHO) is a highly reactive small molecule that is toxic and carcinogenic above threshold concentrations. Humans are exposed to HCHO as a consequence of its widespread use in industry, including in plastics manufacturing, embalming, cosmetics, and as a sterilising agent. In addition, HCHO is routinely produced during enzymatic demethylation reactions within cells, such as those catalysed by histone and nucleic acid demethylases. It is therefore apparent that cells are constantly exposed to HCHO. Despite the detrimental effects of HCHO exposure, it is unclear how HCHO induces toxicity at the molecular level. Adducts between HCHO and biomolecules, e.g. DNA and proteins, are reported, although there is a paucity of systematic biochemical studies. It is also unclear in many cases whether such reactions occur in cells, or whether the reactions lead to functional changes. I am interested in profiling HCHO biochemistry with a view to understanding the mechanisms underpinning HCHO-mediated disease. Here, I will discuss biochemical studies characterising the mechanisms of HCHO production and metabolism, as well as work profiling the reactions of HCHO with amino acids and nucleic acids. The combined results reveal that HCHO is an important endogenous metabolite with the potential to regulate the functions of multiple cellular processes.

P.09-227-Tue
Microcin C-like peptide-adenylate antibiotic from *Rubidus massiliensis*

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Microcin C (McC) from *E. coli* is a founding member of the growing family of ribosomally-synthetized post-translationally modified peptide-nucleotide antibiotics. The peptide portion of McC is bound to adenosine monophosphate though a non-hydrolyzable phosphoramidate link. Taken into the cytoplasm of sensitive bacterium through the peptide transporter, YejABEF, McC is processed by aminopeptidases, resulting in the release of an aspartamide-adenylate. Asp-AMP binds to aspartyl tRNA-synthetase, thus causing translation inhibition and cell death. Recently we have shown that genomes of various microorganisms contain functional *mcc* gene clusters. Most of the *mcc* clusters are comprised of only three (*mccABC*) genes that are sufficient for toxic peptidyl-adenylate production. Many of the newly identified *mcc* clusters contain additional genes, the products of which are responsible for additional post-translational modifications on the compound, or provide immunity to the producing cell. Here we describe an expanded *mcc* cluster present in the genome of the amoeba-associated bacterium, *Rubidus massiliensis*. The *mcc* (Rma) cluster consists of five genes (*mccABCFE2*) organized into an operon. We show that MccB(Rma) is a *bona fide* adenylyltransferase that modifies the C-terminal asparagine residue of the MccA(Rma) heptapeptide with an adenosine monophosphate. The product of *mccC*(Rma) encodes an export pump belonging to the MFS family. Two additional genes, *mccF*(Rma) and *mccE2*(Rma), are homologous to *E. coli mccF* and *mccE* and provide immunity to a wide variety of McC-like compounds. This work was supported by Russia Science Foundation RSF 16-14-10356.

P.09-228-Wed
Association of methylenetetrahydrofolate reductase (MTHFR) gene variants with dental caries and gingivitis in Czech children

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Oral bacteria (cariogenic bacteria or periopathogens), host immune system and genetic predisposition play a role in the etiopathogenesis of dental caries and gingivitis. Susceptibility to inflammation and host-pathogen interactions may be modified by functional polymorphisms in genes linked with the folate metabolic pathway, such as methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and methionine synthase reductase (MTRR). The aim of our study was to analyze four single nucleotide polymorphisms (SNPs) in the genes for these enzymes in Czech children with known oral status. This case-control study included 592 children aged 13–15 years from the European Longitudinal Study of Pregnancy and Childhood (ELSPAC). Oral status was recorded using DMFT (decay-missing-filled teeth) index and gingival index, the presence of 10 selected oral pathogenic bacteria was analyzed, and MTHFR C677T (rs1801133), MTHFR A1298C (rs1801131), MTR A2756G (rs1805087) and MTRR A66G (rs1801394) SNPs were determined by qPCR. Children with dental caries (N = 446) more often suffered by gingivitis than caries free children (controls, N = 145), thus gingivitis was associated with dental caries ($P < 0.001$). There were no significant differences in the allele or genotype frequencies in both MTR and MTRR SNPs between patients and controls. However, MTHFR 1298 AA genotype vs. AC+CC genotypes was found as protective factor for dental caries development ($P < 0.01$) and MTHFR 677 CC genotype vs. CT+TT genotypes was associated with the non-presence of *Prevotella intermedia*, an important periopathogen. It seems that children with common variants in MTHFR gene, and thus normal ability to utilize folic acid, are

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protected against dental caries and gingivitis. The study was supported by Ministry of Health of the Czech Republic, grant nr. 17-30439A, funds provided by the Faculty of Medicine MU to junior researcher Petra Borilova Linhartova, grant GACR GB14-37368G, and the project MUNI/A/1008/2017.

P.09-229-Mon
The ectonucleotidase CD73 is a target of profibrotic TGF-β signaling during diabetic nephropathy

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Progression of diabetic nephropathy (DN) is linked to tubule-interstitial fibrosis conducted by TGF-β. The nucleoside adenosine increases with the progression of DN. Extracellular adenosine production is dependent on the activity of ectonucleotidase CD73 in tubule epithelial cells. Our aim was to identify CD73 induction downstream of TGF-b signaling and demonstrate its potential as a molecular marker of renal tubule injury. Cells from the human renal epithelial tubule cell line HK2 were treated with TGF-β 10 ng/mL, adenosine 10 mM or NECA 1 mM for 48 h. CD73 induction was determined by RT-qPCR. The recruitment of transcription factors and changes in the histone code at the CD73 gene promoter were determined by ChIP-qPCR. Cohorts of healthy, diabetics and diabetics with renal injury were established. CD73 content was measured in urinary exosomes and AMPase activity mediated by CD73 was quantified in urinary sediment from cohorts. We found Smad3 positioned at the CD73 promoter region proximal to the transcriptional start site, in contrast to the collagen 1a2 promoter which exhibit heterochromatinic marks in HK2 cells. The expression of CD73 and markers of profibrotic activation of cells, fibronectin and collagen 1a2, were upregulated after exposure of HK2 cells to TGF-b. The epigenetic environment in the CD73 promoter was also modified by TGF-β, promoting transcriptional active chromatin. CD73 content was increased in urinary exosomes from diabetic patients and correlated with tubular injury. CD73 activity was also increased in urinary sediment from DN patients. The ectonucleotidase CD73 gene is a target of profibrotic TGF-β signaling in epithelial tubule cells. Induction of CD73 is evidenced by increased activity in the urinary sediment of DN patients, being a potential marker of tubule-interstitial fibrosis.

P.09-230-Tue
T cell repertoire profiling after hematopoietic stem cell transplantation with CD19/αβT cell depletion and donor lymphocyte infusion

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Depletion of αβT cells substantially decrease risk of GVHD, increasing the safety of allogeneic hematopoietic stem cell transplantation. During HSCT, patient experienced a period of profound immunodeficiency, which is caused by decrease of T cell repertoire diversity and total number of T-cells. To substitute the deficiency and to protect patient's organism from pathogens on early stages of immune system reconstitution infusion of $CD45RA^-$-lymphocytes from mature donor (DLI) can be used. In our study we investigated clonal repertoire of pediatric patients after allogenic HSCT with CD19/αβT and DLI by TCR β-chains deep sequencing. For each patient we collected two sets of blood samples: in 3 and 12 months after HSCT, and sample of $CD45RA^-$-lymphocytes (DLI) from matched donor. Every set of samples include total fraction of periphery T-cells, fraction of CD4+ and CD8+ T lymphocytes. TCR-β repertoire profiling was performed using original technology with molecular barcode-based normalization and PCR and sequencing error correction. Here we found, that 1 year after HSCT patient T-cell diversity of peripheral blood reached up to more than half of a healthy repertoire, while was lower than in age matched healthy donors. We showed, that some of the recipient T cell clonotypes matched with clonotypes from DLI-sample repertoire persist in patient's repertoire at least for the year after HSCT. Furthermore, some of these clones expanded in a period between 3 and 12 months after HSCT. T-cells from DLI make up from small percent of total patient's peripheral blood T-cells to almost half of them one year after HSCT. Funding: RFBR grant 16-04-01881.

P.09-231-Wed
Vitamin B12 status in patients with graves thyroiditis

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Graves' disease is an autoimmune disease that leads to over activity of whole thyroid glands and it is the most common cause of hyperthyroidism. Vitamin B_{12} is necessary for hematopoiesis and normal neuronal function. In humans, it is obtained only from animal proteins. We aimed in this study to compare the level of vitamin B_{12} in Graves' Thyroiditis and healthy control groups. Plasma samples were collected from 50 healthy control and 50 patients with Graves Thyroiditis who apply to the Selcuk University Hospital between in 01.04.2017–01.10.2017, prospectively. The mean age for controls and patients were 38.8 ± 11.5 and 37.4 ± 11.2, respectively ($P = 0.9$). Patients with other chronic disease, inflammatory disorders and patients using Vitamin B_{12} consisting drug were excluded. Vitamin B12 levels calculated in. Roche Cobas e170 with ECLIA method. Analysis was performed with IBM SPSS v20. Vitamin B_{12} levels were statistically lower in patients with Graves Thyroiditis compared to control group [327.8 ± 130.3 ng/L and 398.6 ± 185.8 ng/L] ($P = 0.03$). Our results showed that Vitamin B_{12} levels are lower in Graves disease compared to control group. These findings can cause hyperhomocysteinemia that may lead increased cardiovascular risk in patients with Graves Thyroiditis. Further studies are needed.

P.09-233-Tue
Assessment of active DNA demethylation pathway during chemotherapy of acute myeloid leukemia

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Patients with acute myeloid leukemias frequently harbor mutations in genes involved in the DNA hydroxymethylation pathway. Loss-of-function mutations in *TET2* have been described in various hematological malignancies and also TET1 has contrasting roles in myeloid and lymphoid transformation. To assess whether active demethylation pathways are affected by standard induction treatment (cytarabine and an anthracycline), we compared broad spectrum of endogenously generated DNA modifications (5-methylcytosine, 5-hydroxymethylcytosine, 5-formylcytosine, 5-carboxylcytosine, 5-hydroxymethyluracil and 8-oxoguanine) and expression of TET dioxygenases as well as TDG glycosylase in peripheral blood nuclear cells of patients with acute myeloid leukemia before and after first course of therapy. Peripheral blood nuclear cells were isolated from heparinized blood samples with Histopaque 1119 solution, according to the manufacturer's instruction. DNA was extracted from frozen cells by modified phenol method. Isolated material was hydrolysed to deoxynucleosides and analyzed using stable isotope dilution two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry. The indirect multicolor flow cytometry-based method was used to assess intracellular expression of proteins in specific subpopulations of peripheral blood nuclear cells. Monocytes, lymphocytes and granulocytes after antileukemic treatment presented with lower expression of TET1 and higher expression of TET2 than cells before therapy, while TET2 and TDG remained unaltered. The levels DNA modifications were not altered by therapy. This suggest, that overexpression of TET3 may compensate lower expression of TET1 in the course of chemotherapy of acute myeloid leukemia. This work was supported by the Polish National Science Centre (grant number 2015/19/B/NZ5/02208).

P.09-234-Wed
Farnesoid X receptor mRNA expression in non-alcoholic fatty liver disease is inversely associated with the severity of liver damage and degree of insulin resistance

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Non-alcoholic fatty liver disease (NAFLD) is a group of pathological changes which are associated with obesity and characterized by abnormal lipid accumulation in liver cells. NAFLD arises as one of the most important problem of public health in developed countries. The first two stages of NAFLD are non-alcoholic fatty liver (NAFL, steatosis) and non-alcoholic steatohepatitis (NASH) which can progress to fibrosis and even liver cirrhosis. Recent studies demonstrated the role of farnesoid X receptor (FXR) in the regulation of glucose and lipid metabolism. Here we performed an expression analysis of FXR in core needle biopsies obtained from patients with different NAFLD stages (n = 20). FXR expression in the group of NASH patients inversely correlates with the severity of liver damage and degree of insulin resistance as assessed by NAFLD Activity Score (NAS) and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), respectively. Our results suggest the protective role of hepatic FXR-mediated signaling against the progression of NAFLD in humans and provide a biochemical framework for the use of FXR agonists in treatment of NAFLD and associated metabolic disorders. This work is supported by the Russian Science Foundation under grant No. 17-15-01475.

P.09-235-Mon
Bacterial L-asparaginases: old friend and persistent foe

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Bacterial type II L-asparaginases (ASNases, EC: 3.5.1.1.) catalyze hydrolysis of L-Asn to L-Asp and, to a lesser extent, of L-Gln to L-Glu. Two ASNases, from *E. coli* and *Erwinia chrysanthemi*, are used clinically in treatment of blood cancers and were also recently shown to inhibit metastases of breast cancer (and possibly other solid tumors). In all cases, therapeutic properties of ASNases are related to the dependence of cancer cells on the circulating L-Asn, with depletion of this amino acid leading to their starvation. Despite the clinical importance of these enzymes, their detailed catalytic mechanism and the basis of their substrate specificity are still a matter of vigorous dispute. In contrast to the recently reported model of catalysis proposing a single displacement mechanism, our data support a double displacement mechanism with Thr12 (EcAII, *E. coli*) acting as the primary nucleophile. Based on extensive studies of EcAII utilizing X-ray crystallography, NMR spectroscopy, kinetic measurements, and

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MD/QM-MM computations, we have been able to describe all major steps of the catalytic mechanism. In this project we determined over 30 crystal structures of different mutants with different ligands under various conditions. Structural studies have been complemented by kinetic measurements for over 100 different mutants of EcAII, as well as by multiple NMR experiments in solution, which allowed careful monitoring of the progress of reaction. Models of the catalytic mechanism generated with these data were evaluated by computational methods. MD simulations revealed specific patterns of hydration and local electrostatics involved in catalysis, whereas QM-MM calculations supported the energetic feasibility of major stages in the proposed catalytic mechanism. We will present progress of our efforts with the aim of resolving the disagreement between the two competing models of catalysis by type II L-asparaginases.

P.09-236-Tue
Puerarin suppress on atopic dermatitis-like skin lesions through regulation of inflammatory mediators

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Atopic dermatitis (AD) is one of the common inflammatory immune disorders. Puerarin is the main isoflavonoid obtained from the root of Pueraria lobata and has been known have ameliorative effects on diverse inflammatory diseases. However, the effects of puerarin on AD have not been uncovered. 2,4-dinitrochlorobenzene (DNCB) was used to induce atopic dermatitis (AD)-like skin lesions on BALB/c mice for 17 days. Further, the BALB/c mice were orally administered puerarin. Puerarin ameliorated DNCB-induced AD-like symptoms in the mice by regulating skin thickness, degranulation of mast cells, and serum immunoglobulin E (IgE). Human keratinocytes (HaCaT cells) were also used to clarify the effects of puerarin on the secretion of pro-inflammatory cytokines. Puerarin inhibited the secretion of inflammatory cytokines and chemokines. The aim of this study was to investigate the protective and alleviative effect of puerarin on AD in vitro and in vivo. The results in this study indicated that puerarin ameliorates AD-like skin lesion and skin inflammation via regulation of various atopic and inflammatory mediators. Therefore, puerarin might be useful in treating AD and other skin diseases.

P.09-237-Wed
Iron loading can induce both pro- and anti-inflammatory changes in cultured human THP-1 monocytes

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Cardiovascular diseases due to atherosclerosis remain a dominant medical problem worldwide. Macrophages play a crucial role both in the atherosclerosis progression and recycling of body iron. Epidemiological data point to elevation of the body iron stores as one of the non-classical cardiovascular risk factors, and we know the iron must be within macrophage to be atherogenic. Presumably the iron within macrophages turns the cells to a more pro-inflammatory phenotype, but experimental evidence for such relationship remains limited. In this study, human monocytic THP-1 cells were fed with micromolar iron in three forms: a) transferrin, b) ferric-ammonium citrate (FAC) and c) hemin, for 2 and 24 h. According to lactate dehydrogenase release only hemin was toxic to the cells. All kinds of iron loading elevated the labile iron pool (spectrofluorometric assay with calcein) at 2 h, as well as expression of ferritin (Western blot) at 24 h. As expected, hemin dramatically induced expression of hemoxygenase-1 (qPCR assay). Further qPCR analyses of selected scavenger receptors (MSR-1, CD36, TLR2) and cytokines (IL-1beta, IL-6, TNFalpha, TGFbeta, IL-10) so far revealed only effects of hemin (decreased TNFalpha, MSR1, CD36) corresponding to the anti-inflammatory M(hem) phenotype. Only when the acid-base indicator and antioxidant Phenol Red was omitted from the medium used for iron loading, induction of MSR-1 (Western blot) in response to iron, especially in transferrin form, could be observed. Degree of this induction was quite variable, ranging from zero to 5-fold. At this stage we conclude that iron loading can induce both anti-and pro-inflammatory changes in the THP-1 monocytes, depending on the form of iron and also on other factors, such as peroxidation of cellular lipids, whose identification seems critical for our understanding when and how iron within macrophages contributes to the progression of atherosclerotic lesions.

P.09-238-Mon
The effects of Rooibos (*Aspalathus linearis*) on mitochondrial DNA depleted 3T3-L1 preadipocytes

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Rooibos (*Aspalathus linearis*), a well-known South African fynbos plant, has become increasingly popular as a cosmetic ingredient, however little scientific information exists to support such use. Human aging is characterised by a loss of subcutaneous adipose tissue resulting in a redistribution of fat to visceral depots, a feature strongly associated with many age related diseases. Adipocyte progenitor cells, preadipocytes, appear to be the most vulnerable and account for the age related decline in dermal adipose tissue mass. Mitochondrial dysfunction is recognised as a contributory factor in aging process and is identified as a potential therapeutic target to preserve tissue function. In the present study the anti-aging potential of rooibos was investigated using 3T3-L1 preadipocytes depleted of mitochondrial DNA (mtDNA) through long-term exposure to sub-lethal concentrations of ethidium bromide. MtDNA depleted 3T3-L1 preadipocytes showed a significantly reduced rate of proliferation, delayed cell cycle progression (G0/G1 arrest), decreased mitochondrial membrane potential (MMP), as well as an increase in glucose utilization and lactate production, confirming mitochondrial dysfunction. Treatment with rooibos stimulated cell growth which was reflected in an attenuation of the G0/G1 arrest as well as an improved MMP, indicating that rooibos improves mitochondrial function. However, glucose utilization and lactate production were not diminished as would be expected for improved mitochondrial function raising the possibility that rooibos increases glycolytic ATP production to sustain favourable cell function and resistance to mitochondrial dysfunction. Cellular ATP levels and AMPK activation provide support for this hypothesis. Taken together the data indicates that rooibos treatment alters preadipocyte cellular energy metabolism providing some degree of resistance to mitochondrial dysfunction and may thus have potential to recover adipose tissue health.

P.09-239-Tue
Antitubercular and HIV-1 enzyme inhibitory activities of South African macrofungi

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The medicinal properties of African and South African macrofungal species, including mushrooms/toadstools, brackets and puff balls, are poorly documented or had not been investigated. Globally, ±36.7 million people live with the human immunodeficiency virus (HIV), and ±10.4 million people became infected with tuberculosis (TB) in 2016. HIV and TB are two diseases with high prevalence in Sub-Saharan Africa. TB is an opportunistic infection in HIV/AIDS infected individuals with impaired immune systems. Increased antimicrobial resistance is a global problem and growing concern. Ethanol (80%) extracts of 21 macrofungal species were screened for antitubercular activity against the *Mycobacterium tuberculosis* H37 strain, and the endpoint determined using the CellTiter-Blue® cell viability assay. The ethanol extracts were also screened for HIV-1 reverse transcriptase (RT) and protease inhibition using colorimetric and fluorometric assay kits, respectively. *Fomitopsis lilacinogilva* (MIC: 31.25 μg/mL) and *Gymnopilus junonius* (MIC: 62.5 μg/mL) inhibited *M. tuberculosis*. *Pisolithus tinctorius* (IC_{50}: 1.35 μg/mL) inhibited HIV-1 RT activity, which was comparable to the non-nucleoside RT inhibitor, nevirapine (1 and 10 μg/mL), used in antiretroviral therapy. *P. tinctorius* (IC_{50}: <2.5 μg/mL), *Pycnoporus sanguineus* (IC_{50}: 26.30 μg/mL) and *Chlorophyllum molybdites* (IC_{50}: 49.7 μg/mL) inhibited HIV-1 protease activity. South African macrofungal species contain biological active compounds with antitubercular and HIV-1 enzyme inhibitory activities, which might play an important role in the future development of novel antibiotics and antiretroviral drugs against TB and HIV/AIDS, respectively.

P.09-240-Wed
A suitable system for studying the functionality of a plasmodial protein in mammalian cell lines

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Malaria is one of the most significant causes of morbidity and mortality worldwide. In *P. falciparum* phospholipid biosynthesis has an essential role in the synthesis of membranes. The most prevalent way of *de novo* biosynthesis is Kennedy-pathway, where the reaction catalysed by CTP:phosphocholine cytidylyltransferase (CCT) is rate-limiting. Because of the persistent need of Plasmodium for membrane synthesis during its life cycle, *de novo* phospholipid biosynthesis emerges as a target for new generation antimalarial drugs. CHO-MT58, a mutant cell line was proved to be an appropriate tool for investigating intracellular function of CCT. In this cell line, the endogenous CCT activity decreases dramatically at 40 °C, blocking membrane synthesis and ultimately leading to apoptosis. We have studied the rescuing potential of *Pf*CCT in CHO-MT58 cells with the isogenic CHO-K1 cells as a control. Cells after transient transfection were incubated at 40 °C and then analysed by FACS using the fluorescence of EGFP fused to *Pf*CCT. The proportion of cells undergoing apoptosis was determined by propidium-iodide staining. We have demonstrated for the first time that heterologously expressed *Pf*CCT is able to complement endogenous CCT activity in mammalian cells. Thus, a suitable system has been established for functional investigation of structural elements of *Pf*CCT. In order to reveal the role of different protein sequences in enzymatic function, we redesigned the structural gene of *Pf*CCT obtaining a modular system where different domains are easy to be removed or exchanged. Here we designed a series of different truncation and deletion constructs to reveal the role of Plasmodium specific sequences. In parallel, heterologous expression experiments of different constructs in the mutant CHO-MT58 and the wild type control cell lines are performed to validate the reported model system.

P.09-241-Mon
Enzymatic characterization of selected somatic variations in glucokinase

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Variations in the glucokinase (*GCK*) gene can lead to progression of several diseases. Inactivating variations are one of the causes of maturity onset diabetes of the young and activating variations are associated with persistent hyperinsulinemic hypoglycemia of infancy. In recent years, cancer genomic studies have revealed somatic-cancer variations in *GCK* (103 missense substitutions identified so far). The role of *GCK* somatic variations in cancer onset and progression remains enigmatic. Particularly, it is unclear, whether *GCK* somatic variations should be considered driver or passenger variations. We hypothesized that some *GCK* somatic variations lead to substantial changes in enzyme kinetics. We prepared a series of 16 GCK expression constructs carrying somatic variations, expressed them in *Escherichia coli* and analysed the enzyme kinetics of recombinant proteins. We selected a half of the variations to be located to the loop interacting with allosteric activators, whereas another half of variations was preselected from the rest of the molecule by applying the combination of prediction algorithms (SNAP2 and EVmutation) that was designed to distinguish possibly activating variations from the inactivating ones. The GCK activity was measured spectrophotometrically using coupled reaction with glucose-6-phosphate dehydrogenase under glucose and ATP concentration gradients, and we analysed the effects of variations on thermostability. The GCK thermal stability was affected in six somatic variations: S433N, V338L, R345H, Q24H have increased stability, whilst K104H and R358P were less stable compared to the wild-type. Three of the analysed GCK variations from the loop (S64F, T65I, G68S) had significantly activating effect and other 12 were neutral in this regard. In conclusion, we validated the recently revealed settings of prediction algorithms and provide the first data on the enzyme kinetics of somatic cancer-associated *GCK* variations.

P.09-242-Tue
Biocatalytic properties of hypothetic proteins from amicoumacin biosynthetic gene cluster involved in antibiotic activation and self-resistance

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The increasing incidence of bacterial multidrug-resistance requires discovery of new antibiotics and investigation of the mechanisms

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of their drug resistance. However, classical microbiological techniques of antibiotic screening became inefficient due to the problem of "antibiotic rediscovery". Hence, implication of alternative approaches seems to be the only essential pathway to defeat pathogens resistant to common antibiotics. Ultrahigh-throughput screening in microfluidic double emulsion allows direct isolation of bacterial strains with prominent inhibiting properties against reporter pathogens. We applied this particularly efficient approach to select bacterial strains highly active against common pathogen *Staphylococcus aureus*, using microbiota samples as a source of different bacterial strains. The combination of whole-genome sequencing and metabolomic analysis enabled us to reveal the interconnection between the genome and functionality of the selected bacteria. Bioinformatic analysis was subsequently used to identify hypothetic proteins involved in amicoumacin production and predict their potential biocatalytic activity. This relationship was clearly confirmed by heterologous expression and functional analysis of the respective biocatalysts involved in the process of antibiotic biosynthesis and inactivation. This work was supported by grant RFMEFI60716X0145 from the Ministry of Education and Science of Russia.

P.09-243-Wed
Medicinal plants and natural compounds targeting mitochondrial function

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The recent discovery of mitochondrial signalling mechanisms and the involvement of mitochondrial function in cellular stress responses has not only revolutionized the way in which we view mitochondria but has also highlighted the increasing number of human diseases associated with changes in mitochondrial activity and the processes they regulate. Consequently, pharmacological approaches to preserve, restore or enhance mitochondrial function has emerged as attractive therapeutic strategies to target a wide range of seemingly unrelated diseases. Furthermore, identification of molecular signalling pathways associated with increased longevity and health span has provided a unified principle linking longevity to energy metabolism through mitochondrial function. This is of major importance seeing as significant overlap exist between these signalling pathways and the extensive biological activities of polyphenols. It is therefore of prominent interest to evaluate medicinal plants within the context of mitochondrial function, and explore potential health benefits such effects may have. *Aspalathus linearis*, *Sutherlandia frutescens*, *Cyclopia intermedia* and *Camellia sinensis* were selected based on their reported involvement in energy metabolism and age-related disease mechanisms. Possible mechanisms of action were investigated through the evaluation of anti-oxidant capacity (DPPH, FRAP, NO scavenging), protection against the induction of oxidative stress, metabolic reprogramming of L6 myotubes (glucose / galactose cultured) as well as changes in mitochondrial content, membrane potential, and glucose consumption / lactate production. Results indicated diverse effects on mitochondrial signatures, with no plant exhibiting the same overall activity. Fermented *C. intermedia* conformed to expectations of the model the most through increased metabolic activity and viability, mitochondrial content and membrane potential in galactose, relative to glucose cultured cells.

P.09-244-Mon
Purine-2,6-dione derivatives as a promising phosphodiesterases inhibitors – new approach in the search for compounds that modulate remodeling response of airway smooth muscle cells

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Airway wall remodeling is one of the most important consequences of chronic inflammation in diseases such as asthma or chronic obstructive pulmonary disease (COPD). The nature of this process is remarkably complicated, mainly due to the fact that many cells, both structural and immune participate in it, as well as many different factors released by them. Undoubtedly, a significant role during airway remodeling is attributed to airway smooth muscle cells (ASMC) particularly to their overgrowth (hyperplasia) and increase of their size (hypertrophy). Many growth factors and cytokines are involved in airway smooth muscle thickening but among them transforming growth factor type β (TGF-β) is considered to be one of the most prominent in induction of ASMC remodeling response. Most of the currently used drugs do not affect airway wall remodeling therefore the need for specific therapy aimed at counteracting or preventing processes associated with lung fibrosis is very urgent. Here we used newly synthesised purine-2,6-dione derivatives (compounds 1 and 2) and investigated their potency to mitigation of TGF-β induced remodeling response of ASMC. Both compounds revealed to be a phosphodiesterases (PDE) inhibitors. ASMC were pretreated with increasing doses of 1 and 2 and then stimulated with TGF-β. We observed significant reduction in TGF-β induced ASMC proliferation and expression of profibrotic genes (α-smooth muscle actin, calponin, collagen I, fibronectin, and versican) in the response to 1 and 2. Our compounds (showing simultaneously PDE1, PDE3, PDE4 and PDE7 inhibition) were able to significantly reduce hyperplasia and hypertrophy of the ASMC, in contrast to the reference drug - roflumilast (PDE4 inhibitor). This data indicates that multiple PDE inhibitors can act more effective then single and specific one. This may provide a new and valuable alternative in the future searching for new effective anti-fibrotic compounds.

P.09-245-Tue
Mcc-like gene cluster from *Streptococcus equinus*

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Microcin C-like compounds (McC) are a family of peptide-nucleotide antibiotics, with some of having additional post-translational modifications. Bioinformatic searches revealed *mcc*-like gene clusters in a diverse range of Gram-negative and Gram-positive bacteria. One of these discovered clusters is from *S. equinus*. There are four genes in the cluster: $mccB^{Seq}$, $mccA^{Seq}$, $mccE_2S$ and $mccC^{Seq}$. *A* 19 amino-acid peptide precursor molecule is encoded by $mccA^{Seq}$. The N-terminal domain of $MccB^{Seq}$ demonstrates an ability to attach a terminal cytidine monophosphate to the cognate precursor peptide $MccA^{Seq}$. The C-terminal domain of $MccE_2S^{Seq}$ is a carboxy-S-adenosyl-L-methionine (cx-SAM) synthetase. $MccS^{Seq}$ is responsible for the synthesis of cx-SAM in the presence of SAM and prephenic acid. Next, the C-terminal domain of $MccB^{Seq}$ uses cx-SAM as a donor of a carboxymethyl group for the modification reaction of the cytidine residue of $MccA^{Seq}$-cytidylate. The entire biosynthetic pathway of the carboxymethylated peptide-cytidylate ($MccA^{Seq}$-cxCMP) from *S. equinus* was reconstructed *in vitro*. However, this compound did not demonstrate any antimicrobial activity against *E. coli*. $MccC^{Seq}$ is a member of the superfamily of membrane transport proteins, and is responsible for microcin export from producing cells. The N-terminal domain of $MccE_2S^{Seq}$ is a predicted acetyltransferase, and may possibly play a role in self-immunity to produced microcin. To test this, cells overexpressing $MccE_2^{Seq}$ from *S. equinus* were obtained, and conferred immunity to McC from both *E. coli* and *Y. pseudotuberculosis*. This work was supported by Russia Science Foundation RSF 16-14-10356.

P.09-246-Wed
D-2-hydroxyglutarate as a possible biomarker of progressing inflammatory bowel diseases to colorectal cancer. The clinical approach

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It is known that sustained inflammation can promote tumorigenesis. Inflammatory bowel diseases (IBD) and colorectal cancer (CRC) illustrate the association between chronic inflammation and cancer. However, the molecular mechanisms that drive progression from colitis to cancer are obscure. The most common forms of IBD are ulcerative colitis (UC) and Crohn's disease (CD). D-2-hydroxyglutarate (D2HG) is normally found in human urine. D2HG is produced by hydroxyacid-oxoacid transhydrogenase enzyme (HOT). In this reaction, the formation of D2HG from 2-ketoglutarate (2KG) is coupled to oxidation of γ-hydroxybutyrate. Human intestines have high levels of γ-hydroxybutyrate, which could cause a high production of D2HG. Under physiological conditions the cellular level of D2HG is maintained by D-2-hydroxyglutaric dehydrogenase (D2HGDH) which recycle D2HG back to 2KG. D2HGDH is most active in colon, liver, kidney and brain in mammals. Therefore, it is thought that high activity of D2HGDH in the intestine could have an important role in preventing an increase in D2HG concentration. Cells exposed to hypoxia exhibited transient increased Hif-1α protein levels. Theiss's research team proved that endogenous Hif-1α regulates D2HG via transcriptional control of D2HGDH expression. Our study included patients in whom, depending on clinical diagnosis CRC, UC, CD was found as against controls. The essence of study was analysis of 2HG enantiomers urinary level by UPLC-MS/MS method. The mean values of D2HG were 6.50 ± 7.97; 4.51 ± 2.90; 5.36 ± 2.81 (μmol/mmol creatinine) in patients with IBD, CRC and controls, respectively. Our results are consistent with Theiss's, where elevated D2HG was during colitis, but not after advanced neoplasia formation. In conclusion, D2HG level in urine may provide a biomarker to predict IBD patient's risk of progressing to cancer. This work was financially supported by NSC grant No. 2017/25/N/NZ5/00378.

P.09-247-Mon
Characteristics of qualitative changes of T-reg lymphocytes in chronic obstructive pulmonary disease and bronchial asthma patients

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Chronic obstructive pulmonary disease (COPD) and bronchial asthma (BA) are two chronic diseases of respiratory system. Despite the differences between COPD and BA in mechanisms of development and clinical manifestation, the diseases share common features which may create certain difficulties in diagnostics and therapy. In case of complicated and therapy-resistant BA, diagnostic significance of bronchodilator test in distinguishing between BA and COPD is reduced. In these cases symptomatic therapy is usually applied. Here, we sought to understand the mechanism of development of the mentioned diseases in order to find a therapy aimed to pathogenesis. Studying the T-reg lymphocytes in peripheral blood has revealed higher level of T-reg cells in patients with broncho-obstructive diseases when compared to healthy controls ($P < 0.05$). Only minute part of human CD4+ cells exhibit high expression of CD25 antigens, so we studied the level of $CD4^+CD25^{h1gh}$ in COPD patients, which was significantly higher than the one in healthy control and BA patient groups (2.87% vs 1.97% and 1.61% respectively; $P < 0.05$ for both comparisons). We also demonstrated that the FOXP3 protein is expressed in T-reg lymphocytes. We employed flow cytometry to demonstrate that the $CD4^+CD25^+$, but not $CD4^+CD25^-$, population of BA patients have become resistant to apoptosis. Finally, we observed reduction of FOXP3 protein expression in T-reg lymphocytes when compared to control groups. In summary, activation of $CD4^+CD25^+$ T-lymphocytes is characteristic to both BA and COPD. At the same time, and low percentage of T-reg cells may indicates the abrogation of cell-mediated mechanisms of immunosuppression in BA patients. High level of natural $CD4^+CD25^+$ T-cells in COPD patients points on activation of suppression pathway of immune response regulation, which may lead to chronization of inflammatory response during this disease.

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P.09-248-Tue
Features of energy metabolism in patients with essential tremor

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Essential tremor (ET) is the most common movement disorder, characterized by a postural and kinetic tremor of the upper limbs and sometimes other body parts. Recently there has been a discussion of neurodegenerative nature of the changes observed in ET. Mitochondrial disorders are reported to be involved in the pathogenesis of neurodegeneration. Therefore, the aim of this study was to determine the parameters of energy metabolism in patients with ET. Patients and methods. The study population consisted of 43 ET patients, aged 19 to 81. The diagnosis is based on Movement Disorder Society criteria (1998). The control group consisted of 40 healthy individuals. In the serum of patients with ET and control group measurement of the concentration of lactate, pyruvate and lactate dehydrogenase (LDH) usage measured by colorimetric method. Results and discussion. The average concentration of pyruvate in the blood serum of patients with ET was significantly lower than in the control group and amounted to (0.73 ± 0.02) mg/dl and (0.85 ± 0.03) mg/dL, respectively ($P < 0.001$). The blood lactate level of patients with ET was on average (10.31 ± 0.60) mg/dl and did not differ from the control group - (8.68 ± 0.88) mg/dl. Analysis of the lactate/pyruvate ratio revealed a significant increase in patients with ET (14.7 ± 0.79 vs. 10.1 ± 1.0 in the control group, $P < 0.001$). When comparing the LDH activity in the blood of patients with ET and the control group, which was (181 ± 6.5) U/L and (148 ± 22) U/L, respectively, there were no differences. Perhaps in patients with ET, ways to compensate for energy metabolism disorders are activated, for example, by activating adenine nucleotide transferase and creatine kinase or, less likely, by reducing the functional activity of cells.

P.09-249-Wed
The specificity of MccC to peptide-nucleotide antibiotics

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Microcin C (McC)-like compounds are a family of peptide-nucleotide antibiotics with complex post-translational modifications. All proteins that are responsible for McC synthesis are encoded in *mcc*-like gene clusters. Many of the validated *mcc* clusters contain three genes: *mccABC*. Such minimal arrangement is sufficient to produce a toxic peptide-nucleotide and export it outside the producing cells. The *mccA* gene encodes a peptide precursor. All McC-like compounds have nucleotide monophosphates attached to the C-terminal Asn residue of MccA via a phosphoramidite bond, carried out by MccB. MccC provides immunity to McC by its export from cells. Here, we investigated the specificity of MccC from *E. coli* ($MccC^{Eco}$) and *Y. pseudotuberculosis* ($MccC^{Yps}$) to McC-like compounds. $McC^{Eco-1177}$ is a seven amino acid-long peptide-adenylate with an additional aminopropyl moiety attached to the phosphate group. $McC^{Eco-1177}$ and its non-aminopropylated intermediate ($McC^{Eco-1120}$) are active against *E. coli* strains, but $McC^{Eco-1177}$ has greater toxicity. *E. coli* cells overexpressing $MccC^{Eco}$ have resistance to McC^{1177}, but not to McC^{1120}. According to our data, $MccC^{Eco}$ more efficiently exports the aminopropylated form of McC^{Eco}. It is probably due to the fact that McC^{1177} is the final product of McC biosynthesis. McC^{Yps} is an 11 amino acid long carboxymethylated peptidyl-cytidylate that has activity against *E. coli* strains. *E. coli* cells overexpressing $MccC^{Yps}$ have resistance to McC^{Yps} along with McC^{1177}, but remain sensitive to McC^{Yps}. Our study reveals that $MccC^{Yps}$ effectively exports not only its cognate microcin, but also McC from *E. coli*. This work was supported by Russia Science Foundation RSF 16-14-10356.

P.09-250-Mon
Generation of stable reporter cell lines to study the transcriptional and translational regulation of the human ABCG2 multidrug transporter

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The human ABCG2 is a plasma membrane transporter that actively extrudes toxins and drugs from cells and causes multidrug resistance in cancer. To investigate the xenobiotic-induced regulation of ABCG2, we generated two types of fluorescent reporter cell lines in A549 lung tumor cells, using the CRISPR-Cas9 genome editing system. To follow the transcriptional regulation of ABCG2 via its native regulatory elements, we replaced the initial coding region of *ABCG2* by a green fluorescent protein (eGFP). Previous results from our laboratory demonstrated that an N-terminally eGFP-tagged ABCG2 protein is membrane localized and functional. Hence, to examine the effect of xenobiotics on ABCG2 protein expression, we have generated A549 cells in which the native ABCG2 coding region was modified to express an eGFP-ABCG2 fusion protein. The CRISPR-edited A549 cells were sorted by flow cytometry and single-cell cloned to provide homogenous reporter cell lines. Sequence analysis and gene copy assessment of the eGFP knock-in ABCG2 reporter cell lines showed that the engineered genetic regions contained one copy of the eGFP cDNA, following the original ATG of ABCG2. Xenobiotic treatment of these stable cell lines demonstrated that the eGFP knock-in cell lines can be efficiently used as highly sensitive reporters to examine the endogenous or pharmacological modulation of ABCG2 transcription. Genetic analysis the eGFP-ABCG2 fusion protein-expressing cells revealed that the modified A549 cells express the N-terminally tagged protein on both alleles. In the eGFP-ABCG2 fusion cells, the tagged protein correctly localized to the plasma membrane and the cells actively extruded the fluorescent ABCG2 substrate, DCV. In addition, eGFP-ABCG2 fusion protein expression was modulated by known regulators of ABCG2 expression. Therefore, this eGFP-ABCG2 expressing stable cell line may be used to examine the combined transcriptional, translational and post-translational regulation of ABCG2 expression.

P.09-251-Tue
The impact of highly active antiretroviral therapy on adverse pregnancy outcomes

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Combination antiretroviral drugs such as Highly Active Antiretroviral Therapy (HAART) are now being widely used by HIV-infected pregnant women for reducing mother-to-infant HIV transmission and improving maternal health. Recent studies associate in utero HAART exposure with adverse pregnancy outcomes such as pre-eclampsia, premature delivery, low birth weight and small for gestational age births. However, there is limited research on the molecular mechanism for these HAART induced pregnancy toxicities. In order to investigate whether these toxicities are due to placenta insufficiency secondary to the impact of HAART on trophoblast cell proliferation and migration, the first trimester extravillous trophoblast cell line, HTR8SVneo, was used as model trophoblast cell line. The cells were exposed to Tenofovir, Emtricitabine and Efavirenz the individual drugs of first-line HAART Atripla® and Zidovudine, Lamivudine, Lopinavir and Ritonavir the individual drugs of the second-line HAART drug Aluvia®. The effects of HAART on trophoblast proliferation were investigated by counting viable cells and by MTT assays. Cell migration was assessed by wound-healing assay. Our results show that first and second line HAART drugs, individually and combined inhibited trophoblast proliferation in a dose and time dependent manner, in addition, the drugs reduced trophoblast migration. The inhibition of cell proliferation and migration was observed in the presence of physiological concentrations of the HAART drugs. These results suggest that the adverse pregnancy outcomes of HAART may be due to placenta insufficiency secondary to the inhibition of trophoblast cell proliferation and migration by HAART.

P.09-252-Wed
Monitoring of changes in serum antioxidative system parameters in different gestation periods of cows

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The purpose of this study was to provide a unique perspective into the oxidative status of cows in different gestation periods. Thirty Holstein cows were assigned to this study. Blood samples were collected during different periods; 4–6 weeks before dry period (D4-5W), at the beginning of dry period (D), first month of dry period (D1), antepartum day (AP- 8), postpartum (PP) day 3 (PP-d3), PP day 8 (PP-d8), PP between 3–4 weeks (PP3-4w) and PP between 80–90 days (PP-d80-90). Glutathione peroxidase (GPx) and Superoxide dismutase (SOD) activities were measured in serum by using commercial assay kits (Randox, UK). "Repeated Measures Define Factors" test and X2 test was performed. The values were classified as low (A; GPx = 300.67–594.68 U/L; SOD = 0.009–0.099 U/mL), medium (B; GPx: 600.22–798.19 U/L; SOD = 0.101–0.150 U/mL) and high (C; GPx:831.44–2666.83 U/L; SOD = 0.133–0.998 U/mL) groups and the percentage changes of the group were compared. Comparing the mean values of GPx obtained before dry period with the values obtained in the following periods, it was determined that a significant increase in the D1 ($P < 0.05$) started and these values reached the highest on PP-d3 and PP-d8 ($P < 0.0001$). It was determined that the ratio of the C classified in all the obtained GPx values was very low at D4-5W and D periods, respectively 6.7% and 13.3%; on the other hand, this rate increased significantly at D1 ($P < 0.05$) and was highest on PP-d3 and PP-d8, respectively 80% and 70% ($P < 0.0001$). However, it was determined that the ratios in category A dropped significantly in PP-d3 and PP-d8 periods ($P < 0.01$). SOD values in the period of PP3-4w show a significant decrease compared to the other periods ($P < 0.05$; $P < 0.01$). The mean values of SOD significantly decreased in PP3-4w compared to D4-5W period. In conclusion, the results obtained show that GPx levels increased significantly in early PP period. Cows especially undergoes oxidative stress in this period compared to other periods.

P.09-253-Mon
Antioxidant effects of *Ribes nigrum* tincture against oxidative stress induced in human colon cells

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Natural antioxidants found in the various parts of plants have attracted an increased interest from consumers and scientific community. This study aimed to evaluate the antioxidant effects of *Ribes nigrum* tincture on human colon cells (C2BBe1 clone of Caco-2). Cells were grown in standard culture conditions and treated firstly with an oxidant agent (H_2O_2) in order to induce oxidative stress and then with different concentrations of tincture (5, 25, 50 and 100 μg/mL) for 24 and 48 h. The cell viability was tested by MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. The reactive oxygen species (ROS) were measured fluorimetrically using 5, 6-carboxy-2, 7-dichlorodihydrofluorescein-diacetate (carboxy-DCFDA) as reagent. Malondialdehyde (MDA) and reduced glutathione (GSH) were assayed by a fluorimetric respectively colorimetric method. MTT tests showed values similar with control cells after the treatment with *Ribes nigrum* tincture for both time intervals. After 24 h, a slight increase of GSH level was noticed for all tincture concentrations (in the absence and presence of oxidant), while at 48 h this remained at the level of control. Moreover, the levels of MDA and ROS production were unchanged in human colon cells, for all conditions studied. In conclusion, our results suggest that *Ribes nigrum* tincture may be used as a natural therapeutic agent and functional food ingredient to support treatment of ROS – mediated colon maladies, such as inflammatory bowel disease. Acknowledgements to Project Bridge Grant 12BG/2016.

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P.09-254-Tue
Hypoxia-induced epithelial-mesenchymal transition enhanced glutathione-S-transferase expression in HT-29 cell line

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Colorectal cancer (CRC) is the most common type of malignancy in the gastrointestinal tract and drug resistance remains a major clinical problem in the current treatment of CRC. Cancer-associated epithelial-mesenchymal transition (EMT) has been found to play a critical role in drug resistance but the nature of these intrinsic links remains unclear. It has been suggested that oxidative stress (OS) had a direct role in promoting EMT and mesenchymal cells equipped with flexible pathways in response to OS tend to gain resistance for chemotherapeutic drugs. The π and μ classes of Glutathione S-transferases (GSTs) play a regulatory role in cancer development. High levels of GSTs have been reported in some tumor types. Although there are some studies identified the resistance differences of EMT, the relationship between resistance profile differences of epithelial and mesenchymal forms in CRC is not yet been elucidated. Thus, the aim of the present study was to evaluate if OS generation by hypoxia inducing agent can have a role in EMT of CRC and to compare the expressions of GST isozyme levels for epithelial and mesenchymal phenotype. To determine the effect of hypoxia inducing agent on EMT, HT-29 cells were treated with 50–200 μM of cobalt chloride for 24 to 72 h. The expression of the mesenchymal markers α-SMA, vimentin, and the epithelial marker E-cadherin was analyzed by real time qRT-PCR. The GST-π and μ isozyme levels of epithelial and mesenchymal forms were also quantified by qRT-PCR. As a result, vimentin and α-SMA expression increased whereas E-cadherin expression was decreased. The expression of GST-π in mesenchymal cells were significantly higher but no significant changes in GST-μ expression were observed. In summary, this study clearly demonstrated that GST-π had a functional importance in drug resistance of mesenchymal cells. Further studies for inhibition of GSTs in mesenchymal phenotype to overcome drug resistance will be conducted.

P.09-255-Wed
Expression and purification of signaling receptor BTLA followed by initial crystallization trials of the protein complex with inhibitors of BTLA/HVEM interaction

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Many strategies have been developed for the treatment of infectious diseases or cancer. One of the promising approaches to activate the therapeutic antitumor immunity is the blocking of immune checkpoints. The immune checkpoints are crucial for functioning of the body under normal physiological conditions. They prevent of autoimmunity and protect tissues from damage when the immune system is responding to pathogenic infection. The expression of immune checkpoints proteins can be dysregulated by tumors as a major mechanism of the immune response, especially against T cells that are specific for tumor antigens. In the immune response against melanoma is involved BTLA (B- and T- lymphocyte attenuator) receptor, which inhibit the activation of $CD4^+$ T cells, as a result of complex formation with HVEM protein (herpes virus entry mediator) present on the surface of melanoma cells. Blocking the interaction between BTLA and HVEM proteins can be a way of stimulating the immune response. The crystal structure extracellular domains of BTLA/HVEM complex shows specific details of the proteins interaction. This structural information was used to design and characterize peptide-based inhibitors of BTLA/HVEM complex formation. In this project we plan to determine crystal structure of BTLA protein in complex with peptide-based inhibitors of BTLA/HVEM complex formation, using X-ray crystallography. Protein used for crystallization was expressed in *E. coli* and purified using affinity column followed by size exclusion chromatography. Peptides were synthesized by solid-phase peptide synthesis with a semiautomated peptide synthesizer Millipore 9050 Plus PepSynthesizer, (Millipore Corporation, Burlington, VT, USA) using standard conditions of solid-phase peptide synthesis. Crystallization trials were performed using vapor diffusion hanging drop method. This work was supported by Polish National Science Center grant no. 2016/21/D/NZ1/02777.

P.09-256-Mon
Molecular mechanisms of presentation of autoantigens peptides on MHC class II, catalyzed by HLA-DM

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The major histocompatibility complex class II (MHC II) plays an important role not only in the adaptive immune response to foreign pathogens, but also in the development of a number of autoimmune diseases. Complexes MHC II, located on the surface of antigen-presenting cells, present the antigenic peptides to T-cell receptors on the surface of T cells, forming the trimolecular complexes, which in turn trigger the cascade of the immune response. The most studied allele HLA-DR2b (HLA-DRA, HLA-DRB1 1501) is one of the main molecules associated with the development of Multiple Sclerosis (MS), chronic neurodegenerative autoimmune disease. HLA-DR2b presents the immunodominant autoantigenic peptide of the myelin basic protein (MBP), one of the antigens in MS - MBP85-99. The group of Favorova O. carried out genetic analysis of Russian healthy and MS donors with genotyping by groups of HLA-DR1 alleles. From this analysis, we identified two groups of alleles associated with the disease, HLA-DRB1 15 and HLA-DRB1 03, as well as two potential protective groups of alleles, HLA-DRB1 01 and HLA-DRB1 11. We showed that protective HLA-DR1 allele (HLA-DRA, HLA-DRB1 0101) can bind C-terminal fragment of MBP but it kinetically discriminates between myelin and viral antigenic peptides during the peptide exchange acceleration by HLA-DM, non-classical MHC II. This project is supported by Russian Science Foundation 17-74-30019.

P.09-257-Tue
Alanyl-glutamine in peritoneal dialysis fluid counteracts GDP-induced inadequate activation of HSF-1 in mesothelial cells

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Exposure of mesothelial cells (MC) to peritoneal dialysis fluids (PDF) during peritoneal dialysis results in cellular injury. Studies suggest that PDF blocks the heat shock response, one of the evolutionary most important cellular stress responses. Protective stress responses may be inadequate in MC exposed to standard heat sterilized PDF (H-PDF) due to the presence of glucose degradation products (GDP), resulting in increased vulnerability against PDF. Human MC were exposed to H-PDF or filter-sterilized PDF (without GDP) with or without concomitant heat stress (42°C) as the gold standard of stress induction. In addition single components of PDF, like individual GDP and additives were investigated. Effects of GDP on cellular viability, stress proteome and activation of the heat shock transcription factor HSF-1, heat shock proteins (HSPs) and stress kinases were assessed with and without addition of alanyl-glutamine (AG). Exposure to H-PDF resulted in cellular damage but inadequate stress responses, with dampening of the stress proteome and reduced Hsp72 expression. Compared to heat, PDF leads to increased lethality but decreased Hsp72 expression. A concurrent blockage of the nuclear shift, phosphorylation and DNA-binding of HSF-1 with reduced activity of the promotor was found. GDP blocked HSF-1 transcriptional activity in MC which was also associated with an inadequate increase of the activating phosphorylation of HSF1 at S326. The inadequate HSF-1 activation could be unblocked by filter-sterilized PDF or addition of AG. AG restored HSF-1 activation and improved survival in a cell line and in patient-derived primary MC via a specific glycosylation of upstream stress kinases of HSF-1. GDP mediated dampening of cellular stress responses via suppressed HSF-1 activation represents a novel pathomechanisms in PDF exposed MC, and an attractive therapeutic target in PDF induced peritoneal damage.

P.09-258-Wed
New diagnostic marker for multiple sclerosis based on fluorescence peptide-based sensor of the myelin-specific abzymatic activity

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Multiple sclerosis (MS) is one of the most socially significant autoimmune diseases spread all over the world. This neurological dysfunction is characterized by chronic inflammation, demyelination, axonal and oligodendrocyte loss, caused by activation and migration of immune cells into the central nervous system. Early diagnostics is one of the necessary requirements for the successful MS treatment. The phenomena of natural antibodies with catalytic activity is widely studied during last few decades. These antibody-enzymes or abzymes may either protect organism or contribute to the development of the autoimmune abnormalities. Previously we showed that myelin-reactive autoantibodies from patients with multiple sclerosis had an ability to recognize and hydrolyze encephalitogenic MBP peptide 81–103 flanked by two fluorescent proteins, designated EPeFRET (*Encephalitogenic Peptide Fluorescence Resonance Energy Transfer*). Here we report next generation of this biomarker for MS based on the antibody-mediated degradation of the novel chemically synthesized FRET substrate representing fluorophore Cy5 and quencher QXL680 interconnected by the MBP peptide 81-99 – Cy5-MBP_{81-99}-QXL680. This substrate is degraded being incubated with purified antibodies from mice with experimental autoimmune encephalomyelitis and patients with MS in contrast to those from non-immunized mice and healthy donors. Analysis of fluorescence increase rate revealed statistically significant difference between rate of hydrolysis of Cy5-MBP_{81-99}-QXL680 by IgG isolated from relapsing-remitting MS patients compared to secondary progressive MS patients and healthy donors. Data presented herein suggest elaboration of additional specific, rapid and sensitive diagnostic criteria for early diagnostics of MS. This study was supported by Russian Science Foundation grant #17-74-30019.

P.09-259-Mon
Evolution of inhibitor-resistant variants of HIV protease analyzed by pre-steady state kinetic analysis

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Pre-steady state kinetic analysis of HIV protease substrate binding and processing is crucial for insight into the evolution of inhibitor-resistant forms of HIV-1 protease. These data may provide a correct vector for rational drug design assuming possible intrinsic dynamic effects and should also give some clues to the molecular mechanism of protease action and resistance to inhibitors. Here we report pre-steady state kinetics of the interaction of wild type or naturally generated mutant forms of HIV-1 protease with a FRET-labeled peptide. The three-stage "minimal" kinetic scheme with two reversible substrate-binding steps and peptide cleavage adequately described experimental data. For the first time, a set of "elementary" kinetic parameters of wild type HIV-1 protease and its natural mutant inhibitor-resistant forms MDR-HM, ANAM-11 and prDRV4 were compared. Inhibitors of the first and second generation were used to estimate the inhibitory effects on HIV-1 protease activity. The resulting set of kinetic data supported that the mutant forms are kinetically unaffected by inhibitors of the first generation, proving their functional resistance to these compounds. The second generation inhibitor darunavir inhibited mutant forms MDR-HM and ANAM-11 but was ineffective against prDRV4. Our kinetic data revealed that these inhibitors induced different conformational changes in the enzyme and, thereby they have different mode of binding in the enzyme active site. These data confirmed hypothesis that the driving force of the inhibitor-resistance evolution is

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disruption of enzyme-inhibitor complex by changing of the contact network in the inhibitor binding site. This work was supported by Russian Science Foundation grant # 14-50-00131.

P.09-260-Tue
Effect of the ubiquitin-proteasome system inhibitors on the energy metabolism of nerve cells

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Different nerve cells have specific metabolic status to maintain brain energy metabolism. Neurons were shown mainly generate energy through oxidative phosphorylation, while astrocytes also produce significant levels of ATP through glycolysis. The proteasome system plays a key role in modulating metabolism via regulation of protein degradation and cell signaling processes. However, the role of the ubiquitin-proteasome system (UPS) in the regulation of nerve cell metabolism is little studied. The aim of this investigation was to analyze the metabolic changes in nerve cells under the UPS inhibitors treatment using confocal fluorescence microscopy and fluorescence lifetime imaging (FLIM). Neurons and astrocytes were isolated from freshly dissected embryonic mouse hippocampus (E18). For metabolism study, primary hippocampal culture was treated with the UPS inhibitors (MG132 and PR619) before fluorescence imaging. Detection of fluorescence intensity and fluorescence lifetime of the metabolic cofactors (NAD(P)H) and FAD) was performed using the FLIM system (Becker&Hickl GmbH., Germany) on LSM 710 microscope (Carl Zeiss, Germany). NAD(P)H fluorescence lifetime imaging revealed an increase in bound NAD(P)H amplitude in hippocampal neurons upon PR619 treatment. It can be associated with a shift towards more oxidative metabolism. At the same time, the amplitude of bound NAD(P)H was slightly elevated under MG132 action. Astrocytes did not show significant changes in energy metabolism in comparison with intact cells after the UPS inhibitors treatment. These results demonstrate the different mechanisms of action of MG132 and PR619 on cellular metabolism. Modulation of the UPS activity may be a useful therapeutic strategy for regulation of the energy metabolism of nerve cells and treatment of neurodegenerative disease. This work was supported by the Russian Science Foundation (grant number 17-75-10202).

P.09-261-Wed
Use of hormone-sensitive fluorescent biosensors and in vitro assays for characterization of steroidal compounds with antiproliferative activity against breast and prostate cancer cell lines

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Hormone-dependent cancers, including breast and prostate cancers, are leading causes of mortality. Because hormonal steroids influence proliferation of hormone-sensitive cancers, reduction of circulating estrogen and androgen levels is an important treatment strategy. Some anticancer drugs reduce steroid hormone levels by inhibition of enzymes involved in steroidogenesis, or by targeting steroid receptors, such as estrogen receptor. However, breast and prostate cancers often become resistant to these treatments, making development of new compounds a priority. Our group has synthesized steroidal compounds with antiproliferative activities against human tumor cells. In addition, local medicinal plants traditionally used for reproductive or hormonal problems have been collected. To screen for compounds or plant extracts with potential for treatment of hormone-dependent cancers, we developed yeast-based hormone-sensitive fluorescent biosensors and in vitro enzymatic assays. Yeast vectors encoding steroid receptor ligand binding domains for estrogen receptor isoforms, androgen receptor or glucocorticoid receptor, were created fused to a yellow fluorescent protein biosensor. Yeast treated with cognate ligand display a dose-dependent increase in fluorescence. Compounds/extracts with specific affinity for estrogen receptor beta, estrogen receptor alpha or glucocorticoid receptor have been identified. Other drug targets involved in steroidogenesis (human aromatase, human aldoketo reductase 1C3/AKR1C3) have been expressed in *E. coli* for purification, screening and X-ray analysis. Steroidal inhibitors of AKR1C3 have been identified. Human AKR1C3 has been crystallized in complex with a steroidal inhibitor and the X-ray structure is undergoing refinement. Molecular docking simulations and molecular dynamics support our in vitro screening results. This combined approach is low-cost, reusable, and has identified compounds and plant extracts with affinity for anticancer targets.

P.09-262-Mon
Identification of CC2D1A homozygous mutation as a cause of Joubert Syndrome with obsessive compulsive disorder

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Joubert syndrome (JS) is a rare autosomal recessive disorder with partial or complete agenesis of cerebellar vermis. This syndrome is identified mainly by the presence of molar tooth sign in magnetic resonance imaging of the brain since it has a varied phenotypic presentation. Obsessive-compulsive disorder (OCD) is a neuropsychiatric disorder, commonly having an early age of onset, and is characterized by the presence of obsessions and compulsions that can become incapacitating. Here in, we report a 17 year-old male patient with JS who shows the symptoms of OCD. He was admitted to the neurology department with the chief complaint of imbalance and abnormal eye movements since his birth. He had also delayed motor and cognitive milestones. His mother described neonatal episodic hyperpnea and hypotonia for the early childhood period. On neurological examination he had oculomotor apraxia and truncal ataxia. The brain MRI revealed typical molar-tooth sign. The patient presented to child and adolescent psychiatry department, 2 years duration of illness characterized by repetitive questions, extreme anxiety and anger when non-answered by his mother and worrying about getting sick. Neuropsychological testing performed after clinical interview using the Wechsler Intelligence Scale for Children, revised edition, he demonstrated moderate intellectual disability overall. There is increasing understanding that the interconnectivity between association neocortex, limbic cortex and the cerebellar hemispheres may play a key role in the processing of multimodal information. A missense homozygous mutation (c.1739C>T; p.Thr580Ile) within the *CC2D1A* gene has been identified by sequencing. This mutations (rs202057391) has been associated with mental retardation before. To the best of our knowledge,

this is the first case of Joubert syndrome with OCD associated with *CC2D1A*. Studies focusing on psychiatric symptoms in JS are necessary for clarifying their relation.

P.09-263-Tue
Partially oxidized glyceraldehyde-3-phosphate dehydrogenase is inactivated by alpha-synuclein

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Colocalization of glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with alpha-synuclein in Lewy bodies was demonstrated by immunofluorescent analysis in brain of patients with Parkinson's disease. Co-expression of GAPDH and alpha-synuclein in COS-7 cells results in the formation of Lewy body-like inclusions, suggesting possible GAPDH involvement in Lewy body formation in synucleinopathies *in vivo*. However, there is still no direct evidence of the interaction between these proteins. Our molecular modeling data predicted the binding of alpha-synuclein to the positively charged groove in the tetrameric GAPDH molecule that comprises NAD^+-binding pocket. Direct interaction between alpha-synuclein and GAPDH was confirmed by different experimental approaches. It was shown that the binding of alpha-synuclein to partially oxidized GAPDH results in the inactivation of the enzyme, but does not lead to dissociation of the tetrameric GAPDH into dimers or monomers. At the same time, the addition of the partially oxidized GAPDH to alpha-synuclein prevents its amyloid transformation. Thus, the interaction between GAPDH and alpha-synuclein could play a role in the onset and development of synucleinopathies. Further investigation of specific interaction between these two proteins could lead to better understanding pathogenetic mechanisms of synucleinopathies and help in searching for drug targets for prevention and curing of these disorders. Acknowledgments: This work was supported by the Russian Science Foundation (project No. 16-14-10027).

P.09-264-Wed
Cinnamaldehyde protects oxidative stress and inhibits TNF-α-induced inflammatory response by heme oxygenase-1 induction in human umbilical vein endothelial cells

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Oxidative stress and inflammation play a critical role in the development of cardiovascular diseases. Cinnamaldehyde (CA) is a natural compound from *Cinnamomum cassia* which is widely studied regard to its anti-cancer, anti-microbial, anti-inflammatory, and anti-oxidant activities. In this study, we investigated the cytoprotective effects and anti-inflammatory properties of CA in hydrogen peroxide- and TNF-α-treated human umbilical vein endothelial cells (HUVECs). The results showed that CA did not have any cytotoxicity or cause morphological changes at concentrations up to 50 μM. CA treatment strongly up-regulated the cellular protein level of heme oxygenase-1 (HO-1) and promoted Nrf2 translocation to the nucleus. CA-mediated Nrf2/HO-1 activation reduced the level of ROS and protected the HUVECs from hydrogen peroxide-induced oxidative stress, which induces apoptosis. Moreover, HO-1 depletion by siRNA attenuated the CA-mediated cell protection against oxidative stress. More interestingly, CA largely inhibited the adhesion of U937 cells to HUVECs by decreasing the expression level of VCAM-1. These results suggest that CA protects HUVECs dysfunction under oxidative stress conditions, and this effect is mediated by Nrf2 activation and the up-regulation of HO-1. Overall, these observations suggest that CA has the potential for use as an anti-atherosclerotic agent.

P.09-265-Mon
Localization of LEA proteins in the cells of anhydrobiotic insect

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The larvae of *Polypedilum vanderplanki* and its relative *P. pembai* are the only known insects and the most complex animals with an ability to survive complete body desiccation, termed anhydrobiosis. Desiccation of *P. vanderplanki* larva is associated with induction of some protective proteins, including LEA (Late Embryogenesis Abundant) proteins. LEA proteins stabilize vitrified sugar glasses which preserve cell components in dried state. High redundancy of LEA genes in *P. vanderplanki* genome (27 members) suggests their functional difference. In this study, we aimed to examine the subcellular targeting of LEA proteins in *P. vanderplanki* cells. Genes encoding 27 LEA proteins were cloned in fusion with GFP in pP121K vector under control of *P. vanderplanki*-specific promoter. *P. vanderplanki*-derived cultured cells Pv11 were transfected with LEA-containing vectors using NEPA 21 electroporator. After incubation for 24 h., cell membranes and nuclei of Pv11 cells were stained with CellVue Claret Far Red and Hoechst stain, respectively. Cells were also stained with Cytopainter kit containing a mixture of Golgi-selective, ER-selective and nucleus-selective dyes. Then cells were investigated with a confocal laser-scanning microscope LSM 780 (Carl Zeiss). We found that three LEA proteins have very specific localization in Pv11 cells. These are targeted to cell membrane LEA1 protein, localized in endoplasmic reticulum LEA3 protein and localized in vacuole-like compartment LEA22 protein. Other LEA proteins are uniformly distributed across the whole cell or localized in cytosol with exclusion from nucleus or both from nucleus and vacuole-like compartment. Thus, we revealed different localization of LEA proteins in *P. vanderplanki* cells, suggesting their different spatial roles and different functions in anhydrobiosis. This study was supported by Russian Science Foundation grant No 17-44-07002.

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P.09-267-Wed
Three-spined stickleback of the White Sea is a new source of long-chain omega-3 oils

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Eicosapentaenoic fatty acid (EPA), and docosahexaenoic fatty acid (DHA), are the most well- known essential polyunsaturated fatty acids (PUFA) for humans. Their function as beneficial health constituents of lipids from aquatic organisms, especially fish, has been reported and proofed in much research: brain and eyes systems development, preventing atherosclerosis, dementia and more. Throughout the last decades, the third fatty acid – docosapentaenoic acid (DPA), has been included in the group of essential fatty acids, which is because of its biological role in wound-healing processes and combined effect together with EPA. The White Sea is considered as an ecologically clean marine ecosystem with almost no human impact, in addition the resources of the sea are still unstudied and its bioresources are misjudged from the position of potential sustainable sources of products for biotechnology such as high quality long-chain omega-3 oils. Three-spined stickleback, *Gastrosteus aculeatus*, is the most abundant non-commercial fish species in the White Sea. In our study, we accessed the lipids and their fatty acids constituents profiles with focus on the amount of EPA, DPA and DHA in juveniles and adults of the three-spined stickleback from the White Sea in summer. Shared Equipment Facilities of KarRC RAS was used to analyze lipid classes and fatty acids, and the technological platform for the research was HPTLC and GC. In flesh, juveniles had higher amounts of PUFA (up to 45% sum FAs), due to omega-3 acids, than adults in muscle (28% sum FAs), in liver (35% sum FAs), and in female gonads (up to 45% sum FAs). Among omega-3 PUFA, EPA and DHA were dominating, the levels were high and accounted for 8% and 24% in juveniles and less in adults – 6% and 13%. The amount of DPA was 3.5% and 4.3% in juveniles and adults respectively, which is higher than commonly reported for other fish – from 1% to 3%. The research was carried out in the frame of the budgetary theme № 0221-2017-0050.

P.09-268-Mon
Influence of 5-substituted 3-pyridylisoxazoles on human platelet membrane receptors and platelet activation

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Design and synthesis of the series of 5-substituted 3-pyridylisoxazoles with the substituent variation in positions 3 (2-, 3- and 4-pyridyl) and 5 (various groups, including alkyl C3-C8, Ph, C(=O)NH_2, etc.) of isoxazole ring as potential anti-platelet agents were done by us earlier [1, 2]. The main goal of this work is the study of relative selectivities of 5-substituted 3-pyridylisoxazoles with respect to the platelet membrane receptors and the influence of this compound class on the platelets activation induced by thrombin and collagen-relative peptide (CRP) with the generation of two platelets subpopulations. The influence of a series of the most active compounds, 5-alkyl-3-(3-pyridyl)isoxazoles, on the platelet membrane receptors was studied using the samples of platelet-rich plasma and washed human platelets suspensions. The experimental data indicate that 5-substituted 3-pyridylisoxazoles are not the selective inhibitors of ADP, epinephrine, PAF and thrombin receptors. These investigations allowed to identify the role of tested 5-substituted 3-pyridylisoxazoles in the platelet signal transduction as the thromboxane A_2 receptor antagonists. All tested compounds were shown to partly inhibit the formation of serine-positive platelets subpopulation induced by thrombin and CRP. The possibility of tested compounds to influence on the pro-coagulating serine-positive activated platelets subpopulation demonstrates their availability as possible new drugs. All results allow to understand platelet responses formation and to predict the possible cell response controlling. This work was partly supported by Russian Foundation of Basic Research (grant No 17-04-01326a).

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P.09-269-Tue
Determination of toxicity of ammonia on narrow clawed crayfish (*Astacus leptodactylus* Esch. 1823)

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In this study, the toxic effects of ammonia on fresh water crayfish were investigated. Four different measuring times (24, 48, 96 and 168 hour) and two different sub-lethal doses of ammonia (12.3 mg/L and 60.25 mg/L) exposed to fresh water crayfish (*Astacus leptodactylus*). Changes in biochemical parameters hemolymph of fresh water crayfish, total hemocyte counts and

gill and hepatopancreas histology were examined. As a result of the experiments, all biochemical parameters except total protein were found statistically significant ($P < 0.05$). Glucose and lactic acid which are known as a secondary stress parameters were increased significantly ($P < 0.05$). As a result of the histological examinations on the gill tissues hyperemia, infiltration of hemolymph and melanisation were determined. In the hepatopancreas tissues; degeneration in the lumen, the lumen enlargement and edema were determined. Total number of hemocyte and hemolymph glucose used to determine the health status of crayfishes. The count of total hemocyte on hemolymph samples that were taken from fresh water crayfish was compared to the control group. We conclude that, due to the fact that fresh water crayfishes (*Astacus leptodactylus*) OECD test guidelines are standard test organisms and feeding network, our findings contribute to the literature can be found in critical position.

P.09-270-Wed
Changes in reactive oxygen species in roots of pumpkin (*Cucurbita pepo* L.) genotypes with known drought tolerance levels

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In this study, it was aimed to gain knowledge regarding yield losses due to drought conditions that greatly affect the agriculture in not only our region and country, but also entire world. In plants, water deficiency occurs as a result of drought that consequently causes the formation of various ROS such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH·) and singular oxygen ($^1O_2$) species. Reactive Oxygen Species (ROS) develop as a result of the metabolic reactions that occur in chloroplasts and mitochondria under stress conditions. Specifically, ROS are produced during aerobic metabolism as a result of interaction between O_2 and electrons leaked from the electron transport systems in chloroplasts and mitochondria. ROS damages the membrane lipids, nucleic acids, proteins, chlorophyll and other macromolecules in the cell. Lipid peroxidation occurs due to the effect of free oxygen radicals on the cell membrane. The lipid peroxidation, which leads to destruction of the cell membrane, produces malondialdehyde (MDA) as a result of several reaction steps. In this context, two pumpkin genotypes with different tolerance levels to drought were grown in Hoagland's solution with 6% PEG 6000 supply for 10 days and their responses was investigated. In the experiment, drought stress increased the radical formation in roots and the highest increase was observed in sweeping activity values of OH radical. It was determined that radical scavenging activity of roots of A-18 genotype was increased by 332% as compared to the control; while that of strain B-33, a tolerant genotype, increased by 214% compared to the control. It was found that the H_2O_2 radicals of plants increased with the drought application and the increase rates of both genotypes were close to each other.

P.09-271-Mon
Effect of enhanced external counterpulsation on oxidative sress capacity

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Although, the mechanisms responsible for anti-ischemic benefits of enhanced external counterpulsation (EECP) remain unknown, we hypothesize that improvements in oxidative stress (OS) capacity and other extracardiac parameters are valuable markers to see the efficiency. So, the current study was designed to evaluate a new OS capacity test under the control of EECP theraphy in order to trace the extracardiac effects of EECP. Seventeen symptomatic patients with coronary artery disease were randomized to thirty-five 1-hour sessions of either EECP (n = 17) or sham EECP (n = 10). Plasma levels of OS capacity, HDL-cholesterol, LDL-cholesterol, Glycated hemoglobin (HbA1c), insülin resistance (HOMA-IR), high-sensitivity C-reactive protein (hsCRP) and NtproBNP were measured. Measurement of OS capacity includes Free Oxygen Radical Test (FORT) and the Free Oxygen Radical Defence (FORD). There were no differences between the two groups at study entry with respect to blood pressure, drug therapy, prior cardiovascular history and/or procedures, or cardiovascular risk factors. Following the intervention, patients that received EECP demonstrated an improvement in FORT (417 U vs 322 U, $P < 0.05$) and FORD (1.04 vs 2.11, $P < 0.01$). Except HbA1c and HOMA-IR, there were also significant beneficial changes in HDL-cholesterol, LDL-cholesterol, hsCRP and NtproBNP ($P < 0.05$). Our findings provide a novel evidence that the beneficial clinical effect of EECP could be also evaluated by a new OS capacity test. We report that the use of this test is practical, but studies involving a larger sample size are necessary to confirm our findings.

Cancer biology

P.10-001-Mon
Novel benzenesulfonamides as selective carbonic anhydrase IX inhibitors exhibit functional effects to reduce hypoxia-induced acidification and clonogenicity in cancer cell lines

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Human carbonic anhydrase (CA) IX has emerged as a promising anticancer drug target and an attractive diagnostic biomarker for a broad range of tumors. Recently, significant efforts have been spent to design agents against CA IX with therapeutic/imaging applications and two of them, SLC-0111 and girentuximab, progressed to clinical trials. However, their progress appears to be limited indicating the demand for different, more efficient CA IX-targeting strategies. In this study, novel benzenesulfonamides against CA IX were designed. They exhibited K_d toward CA IX

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of 160 pM, 50 pM, and 1.1 nM for inhibitors VR16-09, VD11-4-2, and VD12-09, respectively. VR16-09 was the most selective for CA IX. Crystal structures of CA IX with VD11-4-2 and VD12-09 showed a conventional coordination bonds. Western blot analysis demonstrated increased CA IX expression in response to hypoxia in HeLa, H460, MDA-MB-231, and A549 cells. Mass spectrometric gas-analysis revealed nanomolar IC_{50} for tested inhibitors to inhibit extracellular CA activity in MDA-MB-231 cells. Compounds significantly reduced hypoxia-induced extracellular acidification in a dose-dependent manner, whereas the effect on extracellular pH in normoxia was negligible. Functional effect was the most pronounced for VR16-09. Viability EC_{50} values were lower in normoxia than hypoxia in 2D cells. In contrast, hypoxia-dependent effects on clonogenic survival of VR16-09 were observed in H460 spheroids. In conclusion, novel inhibitors exhibited high affinity and strong selectivity toward recombinant CA IX and reached nanomolar functional effects in hypoxic cancer cells. Interestingly, hypoxia-dependent reduction of clonogenicity was observed only in spheroids, highlighting the importance of testing CA IX-targeting compounds in 3D cell models resembling naturally occurring hypoxic microenvironment with clonogenic survival as endpoint. Overall, our newly designed compounds appear to be promising agents for CA IX-specific therapy.

P.10-002-Tue
Lactate-mediated mitoribosomal defects impair OXPHOS and promote hepatoma cell invasiveness

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Impaired mitochondrial oxidative phosphorylation (OXPHOS) capacity, accompanied by enhanced glycolysis, is a key metabolic feature of cancer cells, but its underlying mechanism remains unclear. Previously, we reported that human hepatoma cells that harbor OXPHOS defects exhibit high tumor cell invasiveness via elevated claudin-1 (CLN1). In the present study, we show that OXPHOS-defective hepatoma cells (SNU354 and SNU423 cell lines) exhibit reduced expression of mitochondrial ribosomal protein L13 (MRPL13), a mitochondrial ribosome (mitoribosome) subunit, suggesting a ribosomal defect. Specific mitoribosomal translation inhibition with doxycycline and chloramphenicol or siRNA-mediated MRPL13 knockdown decreased mitochondrial protein expression, reduced the oxygen consumption rate (OCR), and increased CLN1-mediated tumor cell invasiveness in SNU387 cells, which have active mitochondria. Interestingly, we also found that exogenous lactate treatment suppressed MRPL13 expression and OCR and induced CLN1 expression. A bioinformatics analysis of the open RNA-Seq database from The Cancer Genome Atlas Liver Hepatocellular carcinoma (TCGA-LIHC) cohort disclosed a significant negative correlation between MRPL13 and CLN1 expression. Moreover, in patients with low MRPL13 expression, two oxidative metabolic indicators, pyruvate dehydrogenase B expression and the ratio of lactate dehydrogenase (LDH) type B to LDH type A, significantly and negatively correlated with CLN1 expression; this implied that the combination of elevated glycolysis and deficient MRPL13 activity was closely linked to CLN1-mediated tumor activity in LIHC. These results suggest that OXPHOS defects may be initiated and propagated by lactate-mediated mitoribosomal deficiencies and that these deficiencies are critically involved in LIHC development.

P.10-003-Wed
TSG101, a tumor susceptibility gene, bidirectionally modulates cell invasion in an MMP-9-dependent manner

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TSG101 was initially identified in fibroblasts as a tumor suppressor gene. TSG101 has diverse biological functions in endosomal trafficking, transcriptional regulation, and cell proliferation. Recently, TSG101 has received attention in the field of exosome research because of its involvement in multivesicular body formation as a component of the endosomal sorting complex required for transport protein machinery. Since accumulating evidence demonstrates that TSG101 also functions as a tumor-enhancing gene in some epithelial cells, it is assumed that TSG101 functions as a tumor-declining gene or a tumor-enhancing gene in different cell types. However, the precise mechanism underlying the bidirectional and multifaceted functions of TSG101 in carcinogenesis and tumor progression remains unclear. In this study, we found that TSG101 bidirectionally modulates cell invasion through regulating matrix metalloproteinase-9 (MMP-9) expression in different cell types. In HT1080 fibrosarcoma cells, TSG101 depletion promoted cell invasion through enhancing MMP-9 expression. In contrast, in HeLa S3 cervical carcinoma cells, TSG101 depletion repressed cell invasion through reducing MMP-9 expression. To reveal a mechanistic context for the role of TSG101 in cell invasion as a multifaceted gene, we further investigated the molecular mechanism underlying the regulation of MMP-9 expression by TSG101 and found that TSG101 regulated MMP-9 expression at the transcriptional levels.

P.10-004-Mon
Altered miR-17-5p expression pattern in response to chemotherapeutic drugs for metastatic colorectal cancer

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The conventional first-line chemotherapy for metastatic colorectal cancer (mCRC) consists of fluorouracil (5-FU) in combination with either oxaliplatin (FOLFOX) or irinotecan (FOLFIRI). With an overall response rate of approximately 50% for either treatment combination, a major unsolved problem is that there are currently no available predictive markers for these chemotherapeutics. Due to the high tissue specificity and stability of microRNAs (miRNAs) and their altered expression in drug resistance, miRNAs have been suggested as predictive biomarkers for therapeutic response. miR-17-5p belongs to the miR-17/92 cluster which is important in cell cycle, proliferation, apoptosis and other pivotal processes. Furthermore, expression of miR-17-5p is frequently altered in colon tumors. The aim of this study was to determine whether 5-FU, oxaliplatin, irinotecan and their combinations modify the expression of miR-17-5p in colorectal cancer cell lines SW620 and HCT116. Total RNA was isolated after three-day treatment with these drugs and expression level of miR-17-5p was measured by real-time RT-PCR using specific TaqMan miRNA assay. The apoptotic effects of the anti-cancer drugs were studied by flow cytometry using an Annexin V and DAPI staining. It was confirmed that all tested chemotherapeutic

drugs and their combinations promote apoptotic cell death. Results obtained by real-time RT-PCR showed that in SW620 cells miR-17-5p was downregulated in all the treatments while in HCT116 miR-17-5p was upregulated in every treatment tested except oxaliplatin, which was found to downregulate miR-17-5p. Based on our preliminary results, we can conclude that each of the chemotherapeutic drugs alters the miR-17-5p expression and that this effect depends on cellular context. These results support potential use of miR-17-5p as predictive biomarker, but further studies including clinical validation are needed.

P.10-005-Tue
Induction of the immunogenic type of cell death by recombinant lactaptin analogues

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It is known that most of tumors are poor immunogenic and easily evade immune control. New approaches which can activate patient's immune system against cancer are very promising methods for anticancer therapy. One of the successful antitumor strategies is the "double-action" strategy, when the antitumor drug directly induces the death of cancer cells and simultaneously activates the immune system by the induction of immunogenic cell death (ICD) to form a long-term protective antitumor immune response. Genetic modifications of the oncolytic vaccinia virus (VV) improve selective tumor cell infection and death, as well as activation of antitumor immunity. We have engineered recombinant VV (VV-GMCSF-Lact) bearing transgenes of lactaptin and human granulocyte-macrophage colony-stimulating factor (GM-CSF). In this study we investigated the capacity of recombinant analogue of lactaptin (RL2) and VV-GMCSF-Lact to induce the ICD *in vitro* and elicit antitumor immune effect *in vivo*. We observed that intratumoral injections of VV-GMCSF-Lact effectively inhibit the growth of solid tumors in mouse models. Immunohistochemical staining of tumors of non-treated mice and mice treated with VV-GMCSF-Lact was performed to confirm the infiltration of tumor with cytotoxic T-cells. We demonstrated that RL2 and VV-GMCSF-Lact caused all the molecular signatures of the immunogenic type of cell death: calreticulin and HSP70 exposure on outer plasma membrane, ATP and HMGB1 release into the extracellular space. *In vivo* experiments demonstrated visible vaccinating effect after transplantation of the cells treated by RL2. Cells infected by recombinant VV-GMCSF-Lact demonstrated weaker vaccinating effect than cells treated with RL2. Our results may indicate that RL2 and VV-GMCSF-Lact can induce ICD. The work is supported by the RFBR grant No. 16-04-01232.

P.10-006-Wed
Anti-proliferative and apoptotic effects of alone and combined treatment of FK506 with Akt inhibitor on PDGF-mediated prostate cancer cell proliferation

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Cancer cells carry out their specific function with some well-defined signalling as PI3K/Akt/mTOR pathway. Platelet Derived Growth Factor (PDGF), one of the most important growth factor, has roles on this pathway in response to cancer cell proliferation. Besides this pathway also Calcineurin/NFAT signalling effect intracellular events like cell proliferation due increasing calcium overload in cancer cell. So we here stimulated PDGF-mediated cancer cell proliferation and analysed combined inhibition of these two pathways in PC3 prostate cancer cells. Based on this stimulation PC3 cells proliferated and Akt inhibitor decreased the cell proliferation compared to other treatments. We also showed p53, Bax, Caspase 9 and NFKB expressions with RT-PCR. Similar to proliferation results Akt inhibitor showed most significant results in these data. FK506, a Calcineurin inhibitor, decreased NFKB expression but was not sufficient to prevent PDGF-mediated proliferation compared to Akt inhibitor. In addition to these results we analysed apoptosis Annexin V/PI with flow cytometry. We showed apoptotic effect of Akt inhibitor. Akt inhibitor impeded the proliferation of human prostate PC3 cells through the induction of apoptosis but FK506 did not. Our study suggests ongoing cancer cell proliferation by PDGF stimulation can be stopped with Akt inhibition due to apoptotic effect in PC3 cell. Akt and Calcineurin inhibitors create a combined inhibition of intracellular signal pathways during the cancer cell proliferation. And also, our study exemplifies a growth factor based cell proliferation modelling to show different pathways effects in cancer.

P.10-007-Mon
Identification of VRK1 as a new neuroblastoma tumor progression marker regulating cell proliferation and differentiation

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Neuroblastoma (NB) is one of the most common paediatric cancers with an embryonic origin on neural crest cells of the sympathoadrenal lineage. In its aggressive forms, NB presents extensive metastasis, frequent relapses and very low survival rate in affected children. A better cellular and molecular characterization of these tumours is necessary for the identification of proteins with a role in aggressive progression of the disease, and could allow the discovery of new therapeutical targets and prognosis markers. The human protein kinase VRK1 phosphorylates various signalling molecules and transcription factors to regulate cell cycle progression and other processes in normal physiology and pathological situations. Using neuroblastoma tumour expression data and tissue microarrays (TMA) from fresh human

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neuroblastoma samples, we have determined for the first time that VRK1 kinase expression stratify patients according to tumour grade and aggressiveness, representing a new marker for bad prognosis and patient survival. Using specific siRNAs and plasmid on spontaneously immortalized human neuroblastoma cell lines, we have analysed the contribution of VRK1 to neuroblastoma cell biology, establishing this factor as an essential mediator for neuroblastoma cell proliferation and differentiation. We additionally show that VRK1 expression is a prognostic marker in high-grade neuroblastoma tumours, independently of NMYC amplification status or other common prognosis factors. Our study also suggests that VRK1 inhibition may constitute a novel cell cycle-targeted strategy for anti-cancer therapy. Moreover, ongoing studies will help us to elucidate the role of VRK1 in other signalling pathways and functions in neuroblastoma cells providing new therapeutical opportunities for this aggressive cancer.

P.10-008-Tue
The study of calcineurin activity in pathophysiology of uterine cancer

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Calcineurin (CN) is a unique calcium activated protein, serine/threonine phosphatase closely interacting with components of mutually related immune and oxidant/antioxidant systems involved in pathogenesis of cancer. Uterine cancer is among the most abundant gynecologic malignancies and is reported to be associated with both inflammation and oxidative stress. In this study we have investigated the simultaneous changes in activity of mutually related CN, tumor necrosis factor alpha (TNFα) and superoxide dismutase (SOD) in pathophysiology of uterine cancer. CN activity has been determined spectrofluorimetrically in tumor tissue samples of the 33 untreated oncologic patients (range of age 35–76) with the different stages of primary uterine cancer. The peritumoral tissue have been used as a control. The tissue level of TNFα and SOD activity have been studied by ELISA. Results obtained have shown that CN, SOD activity and TNFα level in tumor tissue changes depending on the stage of disease: increases during the 1st stage (15; 2 and 1.5 fold respectively) compared to control and continuously decreases during the 2nd (4; 1.5 and 1.76 fold respectively) and 3rd stages (8.7; 2 and 2 fold respectively) compared to the 1st stage. Moreover, we have found that changes in CN activity depend on histological differentiation as well, but have not found statistically different changes in CN activity depending on the age of patients. In conclusion, CN is involved in pathophysiology of uterine cancer and changes in its activity depend on stage of disease and histological differentiation. Data obtained expand the knowledge about bifunctional nature of CN and mechanisms underlying the interaction between the tumor, immune and antioxidant systems. We thank to National Center of Oncology, Ministry of Healthcare, RA for the provided oncological samples. The study was partially made in the frame of the project #16A-1f10 supported by the State committee of science MES RA.

P.10-009-Wed
Aryl hydrocarbon receptor controls PI3K/AKT/mTOR and ERK signaling and microtubule organization to support liver polyploidization

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Liver polyploidy is a developmentally driven process fundamental for cell growth, metabolic competence, adaptation to stress and even tumor development. Abolishing aryl hydrocarbon receptor (AhR) expression in mice reduced liver size and induced age-dependent portal fibrosis. Here, we have shown than AhR is required for signaling pathways regulating the diploid-to-polyploid conversion taking place during the preweaning-to-adult transition in mouse liver. Thus, we prove that AhR maintains normal hepatocytes size, cellularity and the level of cell cycle regulators in addition to the proliferative potential. So, contrary to wild type mice, *AhR*-/- mice correlated with a compromised polyploidy and enlarged centrosomes from preweaning to adulthood which correlates with a differential p53 regulation between genotypes in the hepatic maturation process. Pathways signaling from the insulin receptor (INS-R) to PI3K/AKT/GSK3b and ERK1/2, and from Wnt/b-Catenin (b-Cat), maintained physiological activation levels during the preweaning (diploid) to adult (polyploid) transition in *AhR*+/+ livers, but were persistently upregulated in their AhR-null counterparts in parallel with their sustained diploidy. Levels of L-Leu and L-Gln were also AhR-modulated, and could converge with those signaling pathways to establish mTOR activation during liver maturation. Moreover, reduced production of mitochondrial oxidative phosphorylation intermediates succinate and fumarate may also influence proper polyploidization and differentiation in adult *AhR*+/+ livers. Thus, AhR contributes to the physiological switch from diploidy to polyploidy in murine liver by integrating survival, proliferation and metabolic pathways. These events seem to result in the development of invasive HCC in AhR-/- after DEN exposure. Since polyploidization seems to be a common property of advanced tumors, AhR antagonists could represent an interesting therapeutic opportunity to treat *hepatocarcinomas*.

P.10-010-Mon
STAT3 is a hub protein of cellular signalling pathways triggered by persistent organochlorine pollutants (POPs)

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Environmental persistent organic pollutants (POPs) as organochlorine pesticides (lindane, dioxin, DDT) are widely distributed in the biosphere. The POPs are known as potential contributors to human carcinogenesis as these compounds may act by destroying key pathways and molecular mechanisms associated with essential cellular processes, development, tissue homeostasis and apoptosis. The STAT3 protein, known as a central linking point for a multitude of cellular signalling pathways and as a carcinogenesis driver, may be involved in the above processes. Our studies were carried out on cell lines corresponding to tissues that are especially vulnerable to damage by environmental

pollutants. In hormone-related cancer cells, POPs act as endocrine disruptors: MCF-7, MDA-MB468, PC3 and LNCaP treated with 10 μM β-lindane (exposure data from environmental-epidemiologic studies) exhibit a delayed activation of the non-canonical pathway of STAT3 as an adaptive response mediated by hormone receptors that is also associated with the occurrence of chemoresistance. In hormone-independent cell lines (HePG2, CaCo, SHSY-5Y) β-lindane induces the rapid activation of the canonical P-Y^{705}STAT3 pathway as well as the overexpression of others pro-inflammatory genes. Preliminary results allow us to hypothesize that the involvement of both canonical and non-canonical STAT3 signalling pathways triggered by POPs influences cellular phenomena such as carcinogenicity and chemoresistance. In fact, the multifaceted role of STAT3 appear to regulate energy metabolism through the altered expression and localization of PKM2 and SHMT2 which lead to a Warburg-like effect.

P.10-011-Tue
Breast cancer treatment capacity of an allomaltol derivative from the standpoint of apoptosis and multidrug resistance

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Despite improvements in the early detection and the pursuing treatment approaches, breast cancer remains a major health issue. This is the consequence of the disorganization of multiple signaling pathways such as apoptotic pathways in addition to multiple drug resistance. That being the case, the pursuit for novel agents to diminish the proliferation of breast cancer cells without causing drug resistance is of interest of the recent studies. Kojic acid is one of the major secondary metabolites of various fungi and bacteria species. Kojic acid and its derivatives such as allomaltol have been proved to demonstrate anti-neoplastic characteristics on different cancer types. In the present study, an allomaltol derivative which was previously synthesized by our team have been chosen to investigate its possible impact on breast cancer cell viability. Results confirmed that the compound discouraged the proliferation of both MCF-7 and MDA-MB-231 cell lines significantly. With the purpose of designating whether the derivative compound triggers p53 apoptotic pathway, RT-PCR was performed following the application of IC_{50} concentration of the compound. Results showed that p53 apoptotic pathway was triggered prominently in MCF-7 cells as pro-apoptotic TP53 and Bax genes were over-expressed while anti-apoptotic Mdm-2 and Bcl-2 genes were suppressed. Bax/Blc-2 ratio was escalated to 3.5 fold in comparison with the control which was another convincing proof that apoptosis was present. In contrast, p53 apoptotic pathway was not activated in MDA-MB-231 cells. The compound also did not provoke drug resistance in terms of Mdr-1 expression and drug efflux in MCF-7 cells while in MDA-MB-231 it led to Mdr-1 activation by 1.4 fold which is way lower rate than most of the chemotherapeutic agents' used to treat breast cancer. These findings suggest that this specific allomaltol derivative offer a novel promising therapeutic approach for breast cancer which needs further investigation.

P.10-012-Wed
Nucleolipidic-based nanosystems for the delivery of an antiproliferative ruthenium complex: pro-apoptotic and pro-autophagic effects in preclinical breast cancer models

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Breast cancer is the second most common cancer worldwide. According to the World Health Organization, its incidence is increasing so that novel anticancer drugs with new molecular mechanisms of action, as well as new therapeutic strategies, are essential to effective kill specific cancer type, and to overcome toxic side effects as well as chemoresistance. Impaired apoptosis seems to play a central role in cancer development and constantly limit the efficacy of conventional therapies. Current research efforts are focused on a deeper understanding of the cellular response to treatments, including the role of cell death pathways activation by metallochemotherapeutics as novel ruthenium-based complexes, recently proposed as safe and effective alternative drugs to cisplatin. Aiming at improving the suitability of Ru-based complexes for biomedical applications, we have developed a novel approach for the *in vivo* delivery of Ru(III) complexes, preparing stable nucleolipidic-based formulations endowed with significant antiproliferative activity. Behind an in-depth microstructural characterization, we have focused on the ability of these Ru-containing nanosystems to inhibit cell proliferation in human breast cancer preclinical models, by both apoptosis and autophagy activation. Using *ad hoc* designed fluorescent formulations and confocal microscopy for targeted studies of intracellular localization, in addition to subcellular fractionation and ICP-MS to assess cellular accumulation, we have detected a wide both cytosolic and nuclear distribution of the active Ru(III) complex, allowing the metal to interact with both mitochondrial and nuclear molecular targets. To limit chemoresistance and counteract uncontrolled proliferation, multiple cell death pathways activation is a promising strategy for targeted therapy development, especially in aggressive cancer diseases such as triple-negative breast cancer.

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P.10-013-Mon
The combined effects of gold nanoparticles and the histone deacetylase inhibitor SAHA on tumor cells

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Due to the moderate success rate and low specificity of routinely applied anti-cancer agents, clinical cancer treatment is mostly accomplished in multimodal manner, where traditional chemotherapy is combined with radiation therapy. Unfortunately, tumor surrounding normal tissues are often injured and severe side effects might develop following such procedures. Radiosensitizing agents are able to enhance the susceptibility of tumor tissues to the injury exerted by irradiation, thus such compounds might help to attenuate the disadvantageous consequences and to augment the potency of radiation treatments. We investigated the applicability and the radiosensitizing efficiency of gold nanoparticles (AuNPs) and of a histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) in individual and in combinational treatment approaches on different cancer cell lines. Due to passive and active targeting, nanomaterials are extremely advantageous in tumor therapy, furthermore AuNPs exhibit unique physicochemical properties that are already exploited in photothermal cancer treatment. We observed significantly increased cancer cell death upon AuNP and SAHA treatments following irradiation with 2 Gy and 4 Gy doses, using colony forming assay. Irradiation-induced DNA double strand breaks were visualized by γH2AX immunostaining. We detected acetylated histones by acetylated-lysine immunostaining and found that AuNPs do not influence the histone deacetylase inhibiting activity of SAHA. The highest radiosensitizing effects were observed when AuNPs and SAHA were applied in combination. Our results suggest that inhibition of deacetylase activity by SAHA leads to the formation of a relaxed chromatin structure, which renders the DNA more vulnerable to the direct damaging effects of ionizing radiation. Furthermore, irradiation can provoke the release of reactive electrons from AuNPs, which ultimately cause DNA breaks and oxidative stress, guiding the targeted cancer cell to apoptosis.

P.10-014-Tue
Role of cystatin F in anti-cancer immune response of cytotoxic T cells

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The potential of anti-cancer immune response to shape and control cancer development is well established and escape of cancer cells from immune surveillance is one of hallmarks of cancer. A crucial role in anti-cancer immune response is attributed to cytotoxic cells, cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, that can directly kill cancer cells. This is achieved by releasing the content of their cytotoxic granules, perforin and granzymes into the synapse formed between cytotoxic and cancer cells. Perforin is a pore-forming protein that enables the diffusion of granzymes into the target cell, while granzymes are peptidases that induce apoptosis of the target cell. Granzymes are synthesised as inactive pro-forms and need to be proteolytically activated by cathepsins C and H. Cystatin F is an inhibitor of cathepsins C and H and, as shown by our group, important regulator of cytotoxicity of NK cells. As both CTLs and NK cells exploit the same cytotoxic machinery, we hypothesized that cystatin F also regulates CTL effector function. Thus, we first established a model system using TALL-104 cell line treated with low concentration of calcium ionophore ionomycin, mimicking unresponsive CTLs after prolonged stimulation with cancer antigens. In our model the cytotoxicity of TALL-104 was reduced, as measured by calcein-AM method, while the viability was unchanged. The protocol also did not activate the cells, however, after appropriate stimulation, the cells released their granule content, as assessed by flow cytometry and LAMP1 labelling and by measuring granzymes B and A levels in cell media. Using western blotting we found increased levels of the inhibitor cystatin F in treated TALL-104 cells. Furthermore, proximity ligation assay and confocal microscopy revealed that cystatin F is co-localized with cathepsin C and granzyme B. Therefore, cystatin F is an important mediator that can impair CTL cytotoxicity and a possible target in cancer immunotherapy.

P.10-015-Wed
NGS of immunoglobulin genes rearrangements for diagnostics of minimal residual disease in pediatric acute lymphoblastic leukemia

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Being the major cause of relapse in leukemia, minimal residual disease (MRD) is considered to be the strongest prognostic factor, allowing to evaluate the efficiency of a treatment, and to make a decision on the subsequent therapy. Detection of MRD via is proven to be one of the most efficient and sensitive techniques. Detection of clonal rearrangements of immunoglobulin (Ig) and T-cell receptor (TCR) genes is widely used for clonality assessment and for MRD monitoring along with RT-PCR of fusion transcripts and flow cytometry, and unlike the latter ones, is applicable in most cases of acute lymphoblastic leukemia (ALL). A cohort of uniformly treated patients consisted of children aged 4 to 13 years with ALL. DNA was extracted from patients' bone marrow (BM) samples on the 1st and 36th day of treatment. Initial detection of patient specific clonal rearrangements was carried out by 8 multiplex PCRs of Ig and TCR loci followed by NGS. MRD detection included targeted NGS of previously detected rearrangements in series of post-treatment BM aliquots. Quantitative analysis was based on digital PCR-like statistical approach. We identified over 500 leukemic rearrangements in 97 patients. In order to evaluate the accuracy of the technique in comparison with other methods we compared the MRD levels of 63 follow-up samples: 21 samples with MRD data obtained by RT-PCR and 42 samples with data obtained by flow cytometry. In both series both negative and positive results were confirmed by NGS. MRD detection by NGS is a sensitive and specific method allowing for clonality evaluation and MRD quantification. Given that RT-PCR of fusion genes and flow

cytometry in some cases are not applicable for MRD detection, NGS-based detection of rearrangements of Ig and TCR loci is likely to become routine procedure for MRD diagnostics. The study was funded by RFBR grant No. 17-29-06052, Russian Scientific Foundation grant No. 17-75-10113 and Russian President's Fellowship SP-671.2018.4

P.10-016-Mon
Simultaneous administration of probiotic lactobacilli and antibiotics displays anti-tumor effect in in vivo colorectal carcinoma model

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Probiotic lactobacilli exhibit various positive effects on human health including immunostimulatory and immunomodulatory effect. In the pilot experiment we aimed to study possible gut microbiome modulating and anti-tumor effect of the *Lactobacillus plantarum* LS/07, a novel isolate from our previous research, using in rat vivo model of the colon carcinoma. Experimental animals (male rats) were divides in three groups (n = 5): control group (CTRL+PRO), colorectal carcinoma group (CRC+PRO) and colorectal carcinoma group treated with antibiotics (CRC+PRO+ATB). All experimental groups received probiotic culture 5 days a week during the 25-week long experiment. We observed no significant differences in feed and water consumption and animal weight changes between three experimental groups. Gut microbiome of the experimental animals was assessed from the feces samples collected at the end of the experiment. Gut microbiome analysis (total bacteria, *Bacteroidetes*, *Firmicutes* and *Lactobacillus*) using PCR-DGGE experiments showed no significant changes between CTRL+PRO and CRC+PRO groups, but we observed reduced diversity of the microbiome between CRC+PRO+ATB and two other groups. Due to the application of antibiotics (amoxicillin and metronidazole), we could not detect any *Lactobacillus plantarum* LS/07 presence using PCR-DGGE in CRC+PRO+ATB group. Surprisingly, we have observed significant reduction of the macroscopic tumors in both CRC+PRO and CRC+PRO+ATB groups when comparing with our data reported in our previous in vivo experiments on the same model. Based on our findings we conclude that alive but also dead lactobacilli could exhibit preventive antitumor effect. Although mechanisms of the preventive effect need to be studied, these data support idea of preventive use of probiotics in humans.

P.10-017-Tue
Dissecting the function of C3G in glioblastoma: from the U87 cell line to patient-derived cells

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C3G is a guanine-nucleotide exchange factor (GEF) for Rap-1, although it can act through GEF-independent mechanisms. The role of C3G in human cancer is controversial, acting as either a tumour suppressor or mediator depending on the context. Similarly, C3G also regulates migration. In colon carcinoma, we found that C3G represses migration and invasion through down-regulation of p38αMAPK activity and promotes tumour growth. Using permanent gene silencing approaches, we have analysed the function of C3G in human glioblastoma (GBM), U87 cell line, as well as in different patient-derived cell lines as *in vitro* models. In U87 cells C3G knock-down enhances invasion by inducing actin-cytoskeleton reorganization. C3G silencing also increases the expression of transcription factors and proteins involved in the epithelial-mesenchymal transition. Moreover, C3G down-regulation increases the number of foci in anchorage-dependent and –independent assays, although with a reduced number of cells within each focus. C3G silencing also modifies the activation of different signalling pathways in U87 cells, such as p38MAPK or ERKs cascades, preventing c-Met and EGFR activation upon stimulation with their ligands. In addition, we have recently characterized the role of C3G in patient-derived cells (named 12Φ12D and HCO1D). U87, 12Φ12D and HCO1D cells differ in their genetic alterations (mutations, amplifications, etc), which are involved in the onset and development of glioblastoma. Therefore, it is important to compare the impact of C3G in all of them. According to the results obtained in U87, C3G silencing enhances cell motility and appears to regulate *in vitro* tumour growth in 12Φ12D and HCO1D cells. C3G also regulates cell signalling in these patient-derived cells. We are currently comparing them with U87 in order to further characterize how C3G regulates GBM initiation, progression and generation of metastasis, to confirm its promising role in clinic.

P.10-018-Wed
Sensitive analysis of somatic mutations in circulating tumor DNA in melanoma patients using biochip-based assay

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Melanoma is a highly aggressive cancer. Analysis of *BRAF* status is mandatory prior to targeted therapy. Circulating tumor DNA (ctDNA) includes the same genetic alterations as an initial tumor. The analysis of ctDNA presents great potential in the process of cancer treatment, including diagnostic and prognostic information before and during therapy, as well as at tumor progression. For detection somatic mutations in ctDNA next generation sequencing and droplet digital PCR most often are used. These methods are very powerful and extremely sensitive but for simple clinical task, they are, probably, too expensive and time-consuming. We developed a biochip for detection somatic *BRAF* mutations (V600E, V600M, V600K, V600R, V600D) in ctDNA. For preferable amplification of mutated DNA we used nested LNA clamp PCR. The PCR product size was 74 bp. PCR fragments were labeled via incorporation of fluorescence-labeled nucleotides during the second round of PCR and hybridized with specific oligonucleotides immobilized on a biochip. Method allows detecting 0.05% mutated DNA in a background of WT DNA. The thirty control DNA samples with known genotype were tested. All genotypes were determined correctly using biochip. Moreover, we tested 36 plasma samples from melanoma patients with known *BRAF* status: 9 tumors were WT and 27 ones were mutated. The ctDNA was isolated from 2–5 ml of plasma collected prior to surgical intervention. In 19/27 *BRAF*-positive patients the consistency of results between ctDNA and tumor DNA was observed. Most of patients (8/9) with discordant genotyping results had no metastases in contrast to patients with coincidental results. We detect no *BRAF* mutations in all ctDNA samples from nine *BRAF* WT patients. The biochip assay is a

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sensitive method for *BRAF* mutation detection in ctDNA. This method can be useful in diagnosis and monitoring of melanoma treatment. This work was supported by Grant of the President of the Russian Federation (#MK-2519.2017.4).

P.10-019-Mon
Subcellular distribution and anticancer activity of 3,6,9-trisubstituted acridine derivatives on selected cancer cell line

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The many of acridines are known cytotoxic or cytostatic agents used in cancer treatment. Unfortunately, selectivity of these compounds is still at a low level. For these reasons a many of teams manipulate with structure of acridine with the aim of increasing the selectivity and at the same time decrease cytotoxic effect on organism. In this study, a series of novel 3,6,9-trisubstituted acridine derivatives (3,6,9-AKR) was prepared with structural modifications in position 9 and then, these derivatives were biologically evaluated for their ability to intercalate into molecule of DNA and for their inhibitory effect on topoisomerase I/II enzymes. The derivatives at different concentrations were analysed against human lung adenocarcinoma A549 cell line, with the aim of detecting the effect of changing the substituent in position 9 on cancer cells. The anticancer activity was assessed using various techniques, such as MMT assay, viability and total cell number measurements, cell cycle distribution analyses and clonogenicity assay, after 24 h and 48 h incubation. The ability of 3,6,9-AKR derivatives to penetrate cell membranes and subsequently enter the cell was monitored using flow cytometry and confocal microscopy. On the basis of these experiments, we can conclude that the changing of shape or length substituent in position 9 lead to significant change in efficiency and the way of antiproliferative effect. This study was supported by Internal Grant Programme of University of P. J. Šafárik in Košice VVGS-PF-2018-754 and VEGA 1/0016/18.

P.10-020-Tue
Dual inhibition of Wnt/β-catenin signaling and histone deacetylation as a new strategy to eliminate breast cancer stem cells by augmentation of apoptosis

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Epigenetic changes play a critical role in the regulation of cancer stem cell (CSC) properties and the development of drug resistance. Modulation of histone acetylation program is closely related to differentiation and apoptosis process. CSCs, a subset of tumor cells, are responsible for disease relapse because of an acquired resistance to apoptosis and the Wnt signaling which is associated with cell survival/self-renewal and differentiation, is re-activated in these cells. Therefore, in the present study, we focused on a possible cytotoxic/apoptotic effect of the combination of niclosamide (Wnt/β-catenin pathway inhibitor) and Valproic acid (VPA, histone deacetylase inhibitor) on breast CSCs. The effect of niclosamide (1 μM, 24 h pre-treatment) and VPA (0.63–5 mM) combination on the viability of MCF-7s cells (CSCs-enriched population) were demonstrated by the ATP assay. Acetylated histone H3 levels at selected doses for the combination were assessed by ELISA. Protein levels associated with the Wnt/β-catenin signaling pathway, EMT and histone modifications were shown by western blotting. Cell death mode was investigated via Hoechst 33342/PI double staining, M30 ELISA, real-time PCR (gene levels associated with apoptosis and autophagy) and western blotting (protein levels associated with apoptosis, autophagy and ER stress). As a result, combination therapy exhibited a marked decrease in cell viability by inducing apoptosis along with the stronger Wnt inhibition and increased histone H3 acetylation in MCF-7s cells. Furthermore, it was found that the epithelial markers were re-expressed in which H3K9ac and H3K4me3 were also increased. In addition, ER stress and blockade of autophagic flux have also been shown to be involved in this process. In conclusion, the future success of this combination approach in targeting CSCs and converted CSCs to non-CSCs may hold significant promise for successful treatment of breast cancer.

P.10-021-Wed
Prognostic impact of immune cytolytic activity and its association with checkpoint molecules and TIL/TAN load in human malignancies

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Immune cytolytic activity (CYT) is determined by the expression of GZMA and PRF1, both secreted by effector CTL and NK cells, and exhibiting high levels upon CD8+ T-cell activation and during a productive clinical response against immune checkpoint blockade therapies. Still, how different tumors induce and adapt to immune responses is not completely understood. Here, we calculated CYT across different cancer types and focused on differences between primary and metastatic tumors. We screened the variation of CYT across 32 different cancer types and 28 different normal tissue types and correlated it with patient overall survival, the expression of several immune checkpoint molecules, and the load of TILs and/or TANs in different tumors. We found diverse levels of CYT across different cancer types, with highest levels in kidney, lung, and cervical cancers, and lowest levels in glioma, adrenocortical carcinoma, and uveal melanoma. CYT was significantly higher in metastatic skin melanoma and correlated significantly to the TIL load. In adrenocortical carcinoma, skin melanoma and bladder cancer, cytolytic activity associated with an improved patient outcome and high levels of both GZMA and PRF1 synergistically affected patient survival in these cancers. In bladder, breast, colon, esophageal, kidney, ovarian, pancreatic, testicular and thyroid cancers, high cytolytic activity was accompanied by upregulation of at least one immune-checkpoint inhibitor, indicating that similar to melanoma and prostate cancer, immune response in cytolytic-high tumors elicit immune suppression in the tumor microenvironment. Overall, our data highlight the existence of diverse cytolytic levels across different cancer types and suggest that along with the existence of complicated associations among various tumor-infiltrated immune cells, it is capable to promote or inhibit the establishment of a permissive tumor microenvironment, depending on the type of cancer.

P.10-022-Mon
Inducibly decreased MITF levels do not markedly change proliferation and invasion but reduce differentiation of melanoma cells

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Melanocytes residing mostly in the skin of the adult organism may be transformed into melanoma. Epithelial-mesenchymal transition (EMT) is a tumorigenic program through which cells acquire mesenchymal, more prooncogenic properties. The EMT features and increased invasiveness are known to be associated with lower levels of the crucial melanoma-specific transcription factor MITF (microphthalmia-associated transcription factor), whereas increased proliferation is associated to higher MITF levels. To exclude the changes which occur upstream of MITF during the phenotype switching, we employ a model in which MITF expression is inducibly regulated by shRNA in melanoma cell lines. We find that the decrease of MITF causes only moderate drop of proliferation in the whole cell line population. Proliferation was found to be decreased in five of 15 clones expanded to cell lines, in three of them profoundly. Reduction of MITF levels alone does not generally produce EMT-like phenotype. The stem cell marker levels also do not change dramatically, only a sharp increase of SOX2 accompanies MITF knockdown. The MITF downstream differentiation markers and its targets melastatin and tyrosinase are profoundly decreased, as well as its target livin. Surprisingly, after the MITF decrease, invasiveness is not appreciably increased, irrespective of proliferation. Altogether, the results suggest that low levels of MITF may still reasonably maintain proliferation and might reflect, rather than cause, the EMT-like changes in melanoma. The work was supported by grant GAUK 172214 and Progres Q25 from Charles University Prague.

P.10-023-Tue
Oxysterols and their importance in breast carcinoma

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Oxysterols are oxygenated derivatives of cholesterol that exert many important roles in the human body, including modulation of membrane fluidity, signaling pathways, or sterol metabolism and transfer. In addition, the oxysterol action is also connected to several pathologies including cancerous disease, and oxysterols were shown to influence cell proliferation, migration or apoptosis. Moreover, oxysterols can modulate the activity of estrogen receptor (ER) and bind antiestrogen binding sites, in a similar way as hormonal therapeutics, e.g., tamoxifen. Thus, oxysterols could potentially interfere with endocrine therapy effect and outcome of hormonally treated patients. In our study, we aimed to determine whether there are changes in plasma levels of oxysterols in breast carcinoma patients after surgical removal of tumor and initiation of the therapy. Paired plasma samples were collected from patients before and 12–24 months after surgery, and the levels of four major oxysterols was measured by LC-MS. Of the oxysterols we analyzed, 7-ketocholesterol (7-KC) was shown to be increased after tumor removal. Therefore, we initiated a study of the role of 7-KC *in vitro* and how its presence influences the growth and other events, e.g., cell migration in an ER positive breast cancer model, an MCF7 cell line. We find that 7-KC has a very similar potential for growth inhibition as tamoxifen. 7-KC levels were deregulated after tumor removal and 7-KC was identified as an interesting candidate for further functional *in vitro* analyses. The study was supported by the Czech Science Foundation (grant no. P303/12/G163), the Ministry of Health of the Czech Republic, (project no. 17-28470A), the National Sustainability Program I (NPU I) provided by the Ministry of Education Youth and Sports of the Czech Republic (grant no. LO1503), the Center of clinical and experimental liver surgery project no. UNCE/MED/006, and United States NIH R01 GM118122.

P.10-024-Wed
Circulating oxysterols in breast cancer patients

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Despite their low tissue levels, oxygenated metabolites of cholesterol – oxysterols, play important roles in the organism and, e.g., through interaction with liver X receptors, oxysterol-binding proteins, or drug transporters, can influence both carcinogenesis and cancer progression. The present study aimed to introduce method for detection of selected oxysterols in plasma of breast cancer patients and analyze paired samples before and after surgical removal of their tumors. Blood samples were collected from 24 histologically diagnosed breast cancer patients one day before surgical tumor removal (first sampling) and then from the same patient in the period 12–24 months after surgery (second sampling). 25-Hydroxycholesterol, 27-hydroxycholesterol, 7α-hydroxycholesterol, and 7-ketocholesterol were quantified in plasma extracts by the positive-ion APCI LC/MS/MS with deuterated standards for all analyzed oxysterols. The results demonstrate that the assessment of main oxysterols in plasma of breast cancer patients is feasible. Most importantly, we have observed that 7-ketocholesterol levels rise in circulation of patients after tumor removal. Furthermore, we have shown that personal characteristics of patients as menopausal status, body mass index, and history of steroid homeostasis-related conditions may influence results of such analyses. Follow up and mechanistic studies are currently being performed to assess the reproducibility of these observations and their clinical utility, e.g., estimation of disease risk, monitoring of disease recurrence, or employment of

therapeutic interventions. The study was supported by the Czech Science Foundation grant no. P303/12/G163, the Czech Ministry of Health project no. 17-28470A, National Sustainability Program I (NPU I) provided by the Ministry of Education Youth and Sports of the Czech Republic (grant no. LO1503), the Center of clinical and experimental liver surgery project no. UNCE/MED/006, and United States NIH R01 GM118122.

P.10-025-Mon
Downregulation of miR-195 and miR-497 in luminal breast carcinoma is associated with high grade of tumors, expression of HER2, and luminal B subtype but not with presence of LN metastasis in patients

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Estrogen receptor positive breast carcinoma (luminal subtype) is the most common type of breast cancer in women. The presence of regional lymph node metastasis (LN+) has been linked with worse prognosis in both luminal subtypes, A and B. MicroRNAs (miRNAs) are short noncoding RNAs involved in cancer development and progression including processes associated with metastatic spread. This study focused on search for miRNA expression profile distinguishing patients with pathologically proven regional lymph node metastasis from those without such tumor spread. Levels of 2006 miRNAs were assessed in 53 LN+ and 48 LN- primary luminal breast carcinomas by SurePrint G3 Human miRNA Microarrays (8×60k, Release 19.0, Design ID 046064; Agilent Technologies). The obtained expression profiles were analyzed in context with clinico-pathological data of patients by Genespring GX program (Agilent Technologies). We have not identified any miRNA differently expressed in LN+ in comparison to LN- luminal breast carcinomas (all $P > 0.05$, fold change <1.5). MiR-127-3p, miR-195-5p, miR-199a-5p, miR-199b-5p, miR-214-3p, miR-376a-3p, miR-455-3p, miR-497-5p, and miR-99a-5p negatively correlated with tumor grade and were significantly downregulated in luminal B subtype. Low levels of two miRNAs, miR-195-5p and miR-497-5p, significantly associated with high grade, positive expression of HER2 receptor and luminal B subtype, thus, generally with a worse prognosis. Our data suggests the lack of specific miRNA profile in luminal breast carcinoma patients with regional lymph node metastasis enabling their separation from patients without such spread. However, miR-195-5p and miR-497-5p seem to be main miRNAs associated with aggressive behavior in luminal breast carcinoma patients. This study was supported by projects of Ministry of Health of the Czech Republic, (no. 15-25618A), Czech Science Foundation (no. P303/12/G163), and National Sustainability Program I (NPUI, no. LO1503).

P.10-026-Tue
Crosstalk of breast tumors and tumor-associated stromal tissues: its association with the oxidative stress index, tissue factor and aromatase activity

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Tumor microenvironment has a heterogeneous edifice, including nearby endogenous stromal cells recruited by the tumor. The crosstalk between cancer cells and stroma are known to regulate breast cancer pathways, but many underlying molecular mechanisms are yet to be discovered. We investigated the oxidative stress index (OSi), tissue factor (TF) and aromatase activity alterations within the tumor tissue (T) and matched tumor-associated stromal tissue (TAST) in postmenopausal invasive ductal breast cancer (IDC) cases (n = 11). Three tumor-free breast tissue samples (N) were obtained from premenopausal women with no history of breast cancer who underwent reduction mammoplasty surgery as the control group. The highest OSi was found in TASTs where a 6-fold increase was observed compared to tumor tissues ($P = 0.018$). Interestingly, tumor and healthy stroma exhibited approximately the same levels of oxidative stress, a possible strategy of avoiding apoptosis and senescence mechanisms. TF activity, which is known to synergize with the estrogen receptor pathway in promoting breast cancer progression from previous studies, was slightly increased in tumor tissues compared to TASTs but the difference did not reach statistical significance ($P = 0.083$). The conversion of testosterone to 17ß estradiol (E2) was determined via LC-MS/MS and aromatase activity of microsomal fractions were calculated. Aromatase activity levels were in the order of TAST>N>T. More than 20 fold higher aromatase activity was observed in TASTs compared to Ts ($P = 0.003$), which may be one of the sources of reactive oxygen species within the tumor microenvironment. Our results suggest that high TF activity in tumors together with high levels of oxidative stress and aromatase activity in TAST may facilitate an alternative way of crosstalk between tumor and its microenvironment for further IDC progression in addition to the well-known estrogen driven mechanism.

P.10-027-Wed
SUV39H1/DNMT3A-dependent methylation of the RB1 promoter in melanoma development

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Melanoma is among the most aggressive and treatment-resistant human cancers. Aberrant histone H3 methylation at Lys 9 (H3K9) correlates with carcinogenic gene silencing but the significance of suppressor of variegation 3–9 homolog 1 (SUV39H1), a H3K9-specific methyltransferase, in melanoma initiation and progression remains unclear. Here we show that SUV39H1-mediated H3K9 trimethylation (H3K9me3) facilitates retinoblastoma (*RB1*) promoter CpG island methylation by interacting with DNMT3A and decreasing RB mRNA and protein in melanoma cells. Reduced RB abundance, in turn, impairs E2F1

transcriptional inhibition leading to increased peptidyl-prolyl cis-trans isomerase NIMA interacting 1 (PIN1) levels, human keratinocyte neoplastic cell transformation, and melanoma tumorigenesis via enhanced RAF1/MEKs/ERKs signaling pathway activation. In a synergistic model with B16-F1 murine melanoma cells, SUV39H1 and PIN1 overexpression increased melanoma growth, which was abrogated by their inhibition in SUV39H1-overexpressing B16-F1 mice. SUV39H1 also positively correlated with PIN1 expression in human melanoma. Our studies establish SUV39H1 as an oncogene in melanoma and underscore the role of chromatin factors in regulating tumorigenesis.

P.10-028-Mon
Tumor suppressor miRNAs deregulated by methylation and its potential targets in breast cancer

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DNA methylation and miRNA are the most dynamic mechanisms of gene regulation in cancer. Although intensively studied, they are still poorly understood. In this work, we aimed to identify novel miRNA genes deregulated by DNA methylation and their potential target genes involved in breast cancer (BC). We used the representative set of 58 paired (tumor/normal) BC samples to assess expression levels and methylation profiles of twenty previously selected miRNAs with methylation-specific PCR and RT-qPCR. Of these, twelve miRNA genes were hypermethylated: MIR-124-1/-3, 125b-1, 127, 129-2, 132, 148a, 193a, 34b/c, 9-1/-3, 1258, and one, MIR-191, was hypomethylated. The correlation between methylation and expression alterations was strong for all of these miRNA genes. Next, we compared the expression profiles of these miRNAs with their 15 protein-coding cancer-associated targets predicted with CrossHub tool. We revealed significant negative correlations for 10 miRNA-mRNA pairs: miR-124-3p – BCL2, miR-124-3p – RASSF1A, miR-127-5p – DAPK1, miR-127-5p – BCL2, miR-137 – CHL1, miR-193a-5p – RHOA, miR-34a-5p – CCND1, miR-34c-3p – CCND1, miR-375 – RASSF1A, miR-9 – DAPK1. Then, we transfected breast cancer MCF7 cells with miRNA mimics and confirmed the interaction of miR-124-3p with BCL2 mRNA, as well as miR-34c-3p and -34a-5p with CCND1 mRNA. Among the studied miRNAs, the maximum decrease in the CCND1 mRNA level was observed for miR-34c-3p and was 1.7-fold; it was also accompanied by the most noticeable effect on the cell cycle. Thus, we revealed 12 novel miRNA genes deregulated by methylation in BC. Eight miRNAs (miR-124-3p, 127-5p, 137, 193a-5p, 34a-5p, 34c-3p, 375) could play a role in the downregulation of 6 cancer-associated protein-coding genes. Of these, miRNA interactions with BCL2 and CCND1 were confirmed using miRNA mimics, and functional consequences of that were shown for CCND1. This work was financially supported by the Russian Science Foundation grant 14-15-00654.

P.10-029-Tue
Novel miRNA genes deregulated by hypermethylation in epithelial ovarian cancer

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The methylation of promoter CpG islands can inhibit expression of miRNAs and, consequently, their suppressive effect. This study is dedicated to analyze the role of promoter methylation in altering the expression of 12 miRNAs that are associated with epithelial ovarian cancer (EOC): miR-124-3p, -125b-5p, -127-5p, -129-5p, -132-3p, -137, -148a-3p, -191-5p, -193a-5p, -203a, -339-3p, and -375. Seventy-six paired (tumor/normal) EOC samples, additional sample set of 13 primary ovarian tumors and matched peritoneal metastases, methylation-specific PCR, and quantitative RT-PCR were used. We demonstrated significant aberrations in the methylation patterns of 11 miRNA genes and identified 8 novel hypermethylated miRNA genes (MIR-124-1, -124-2, -124-3, -127, -132, -137, -193A, and -339) and one hypomethylated (MIR-191). The subset of 29 EOC samples allowed us to reveal a strong correlation between methylation aberrations and expression level alterations for all 12 miRNAs studied. Moreover, we showed significant association of hypermethylation of 10 miRNA genes (MIR-124-2, -124-3, -125B-1, -127, -129-2, -137, -193A, -203A, -339, -375) with EOC metastasis (to lymph nodes, peritoneum, and distant organs). Hypermethylation of 10 miRNA genes in EOC metastases was validated in additional sample set of 13 primary tumors and matched peritoneal metastases. Combinations of miRNA genes MIR-124-2, -127, -129-2, -137, and -193A might be suggested as potential diagnostic marker panel: AUC = 0.86–0.90. Combinations of MIR-124-2, -137, -203A, and -375 might be suggested as potential biomarker panel for prediction of EOC dissemination: AUC = 0.78–0.86. Together, our results revealed 12 miRNAs to be hypermethylated and deregulated in EOC, the involvement of 10 hypermethylated miRNA genes in the formation of metastases including peritoneal macro-metastases, and suggested novel potential biomarkers. This work was financially supported by the Russian Science Foundation grant 14-15-00654.

P.10-030-Wed
Hypermethylated tumor suppressor microRNAs as novel markers of clear cell renal cell carcinoma

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MiRNAs and methylation of miRNA genes play a key role in epigenetic deregulation in malignant tumors. The aim of this work was to define the role of methylation of novel tumor suppressor miRNA genes in clear cell renal cell carcinoma (ccRCC) pathogenesis and identify markers for diagnostics and metastasis prediction. The set of 70 paired (tumor/normal) ccRCC samples, 19 post-mortal renal tissues from individuals without cancer history, methylation-specific PCR, and RT-qPCR were applied. The 14 miRNA genes (MIR-124-1/-2/-3, -125b-1, -129-2, -132, -137, -193a, -34b/c, -9-1, -9-3, -107, -130b, -1258) among 19 analyzed were significantly hypermethylated and 2 miRNA genes (MIR-191, -212) were hypomethylated. Using the subset of 14 ccRCC samples, 11 miRNA genes were found to be downregulated, and significant correlation was revealed between miRNA level alterations and methylation aberrations for 7 genes: MIR-124-3, -125b-1, -129-2, -137, -34b/c, -375, -9-3. Moreover, hypermethylation of most miRNA genes studied was associated with ccRCC progression (advanced clinical stage, tumor size, differentiation grade). In particular, hypermethylation of 6 miRNA genes (MIR-125b-1, -129-2, -203a, -375, -107, -1258) significantly correlated with metastasis presence. Of interest, MIR-203a and MIR-375 were hypermethylated only in primary tumors from patients with metastases. Novel marker systems were suggested for ccRCC diagnostics (MIR-125b-1, -375, -137, -193a) and metastasis prediction (MIR-125b-1, -375, -107, -1258, -203). Both systems were characterized by high enough clinical sensitivity (86%) and specificity (95%) on examined sample set (AUC 0.93). In conclusion, hypermethylation of 14 miRNA genes and association of 6 miRNAs with metastasis were revealed in ccRCC, as well as novel marker systems for diagnostics and metastasis prediction were suggested. This work was supported by the Program of fundamental research for state academies for 2013-2020 years (№ 0520-2014-0030).

P.10-031-Mon
Novel inhibitors of lysine (K)-specific demethylase 4A with anticancer activity

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Lysine (K)-specific demethylase 4A (KDM4A) is a histone demethylase that removes methyl residues from trimethylated or dimethylated histone 3 at lysines 9 and 36. Overexpression of KDM4A is found in various cancer types. To identify KDM4A inhibitors with anti-tumor functions, screening with an *in vitro* KDM4A enzyme activity assay was carried out. The benzylidene-hydrazine analogue LDD2269 was selected, with an IC_{50} of 6.56 μM of KDM4A enzyme inhibition, and the binding mode was investigated using *in silico* molecular docking. Demethylation inhibition by LDD2269 was confirmed with a cell-based assay using antibodies against methylated histone at lysines 9 and 36. HCT-116 colon cancer cell line proliferation was suppressed by LDD2269, which also interfered with soft-agar growth and migration of HCT-116 cells. Annexin V staining and PARP cleavage experiments showed apoptosis induction by LDD2269. Derivatives of LDD2269 were synthesized and the structure–activity relationship was explored. LDD2269 is reported here as a strong inhibitor of KDM4A in *in vitro* and cell-based systems, with anti-tumor functions.

P.10-033-Wed
The investigation of the effects of *Cinnamomum cassia* extracts on Type 1 diabetes in vitro model

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There are a large number of studies suggesting that increased oxidative stress plays a pivotal role in the development of complications of diabetes in patients with Type 1 diabetes mellitus (T1DM). It is alleged that increased oxidative stress induces oxidative DNA damage and that it may contribute the development of cancer due to insufficient DNA repair capacity. Therefore, studies focused on the investigation of the protective drugs and herbal products against to oxidative stress and DNA damage in diabetic patients. Cinnamon is a herbal product that its diverse biological activities including antidiabetic and anti-tumor properties has been proved by scientific studies. *Cinnamomum cassia* (*C. cassia*) is a cinnamon species commonly presents in commercial cinnamon preparations. The purposes of our study were; 1- To investigate the effects of *C. cassia* bark extracts on cell viability and the apoptotic activities of the extracts on type 1 diabetes *in vitro* model; 2- To investigate of the effects of the extract(s) with no apoptotic activity on endogenous and H_2O_2-induced oxidative DNA damage; 3- To investigate the implication of reactive oxygen species (ROS) generation on these effects. In the present study, IC_{50} values of the extracts were determined by WST-1 test. Annexin V/PI and TUNEL tests were performed for determination of apoptotic activities, by using flow cytometry. The effects of butanol and water extracts with non-apoptotic activities on endogenous and H_2O_2-induced oxidative DNA damage were determined by alkali comet assay and confirmed by TUNEL test. The effects on the production of ROS were investigated by DCF-DA test using flow cytometry. Our results showed that the *C. cassia* water extract has protective effect against to H_2O_2-induced oxidative DNA damage and that this effect might be related to its reducing effect on ROS production in T1DM *in vitro* cell model.

P.10-034-Mon
Arctigenin induces cell death through oxidative mitochondrial damage against acidity-tolerant prostate carcinoma PC-3 cells

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Extracellular acidity in the tumor microenvironment contributes to the chemoresistance of malignant tumors. Herein, the chemosensitizing effects of arctigenin, a novel anti-inflammatory lignan from the seeds of *Arctium lappa*, on the acid-tolerant prostate cancer PC-3AcT cells and their parental acid-sensitive PC-3 cells were studied. The PC-3AcT cells manifested increased tolerance to low-pH media together with enhanced percent cell viability, and an increase in the resistance to docetaxel, as compared with their parental PC-3 cells. A preferential antiproliferative activity of arctigenin against PC-3AcT cells was associated with a non-necroptotic, necrotic type of cell death, which was accompanied by an increase in the cell fraction with a sub-G_0/G_1 peak, an increase in the levels of ROS production and depolarization of mitochondrial membrane potential, and an increase in viable cells exhibiting intense LysoTracker Red staining. A series of changes caused by arctigenin were significantly recovered by

pretreatment with the radical scavenger N-acetylcysteine, thus positioning ROS upstream of arctigenin-driven cytotoxicity. Collectively, evidences that arctigenin enhanced oxidative stress-mediated cytotoxicity toward the acid-tolerable PC-3AcT cells underlines the possible role of arctigenin as a potential therapeutic candidate to overcome the acidic-microenvironment-associated chemotherapeutic resistance in prostate cancer.

P.10-035-Tue
Photodynamic therapy with N, N-difluoroboryl-N-[3-(4-tert-butylphenyl)-2H-isoindol-1-yl)]-N-[3-(4-tert-butylphenyl)-1H-isoindol-1-ylidene]amine against S-37 murine sarcoma *in vivo*

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The sensitivity of the solid form of S-37 sarcoma in the CBA mice to photodynamic action with N,N-difluoroboryl-N-[3-(4-tert-butylphenyl)-2H-isoindol-1-yl)]-N-[3-(4-tert-butylphenyl)-1H-isoindol-1-ylidene]amine was evaluated in vivo. This photosensitizer (PS) has a strong absorption band at λ_{max} = 725 nm, where the penetration of the exciting radiation into the tissue is greatest. The studies have shown that both irradiation without preliminary sensitization of the tumor (light dose – 120 J/cm2), and administration of PS (5.0 mg/kg) without subsequent irradiation did not affect the dynamics of tumor growth in comparison with the control group of untreated mice. At the irradiation session at 24 h after the administration of PS at a dose of 5.0 mg/kg, a significant inhibition of growth of the tumor was observed (tumor growth inhibition values 86–98%). The time of doubling the tumor volume was 11–27 days. For comparison, in the control group, the tumor volume increased by 2 times on average for 1–5 days. At 20 days after irradiation with PS at a dose of 5.0 mg/kg, complete tumor regression was obtained in 67% of the animals. With the decrease in the PS dose to 1.0 mg/kg, the significant effect of inhibition of tumor growth persisted, with mean tumor growth inhibition values in the experimental group being 64–81%, and the tumor doubling time 6–18 days. Complete tumor regression was obtained in 50% of the animals. Differences in the values of tumor volumes in both test groups during irradiation within 24 h after the administration of PS at doses of 5.0 and 1.0 mg/kg compared to the control were statistically significant. Further reduction of the PS dose to 0.1 mg/kg led to a decrease in antitumor efficacy: mean values of tumor growth inhibition were 14–37%, and the tumor doubling time was 2–6 days. Differences in tumor volume compared with controls were statistically unreliable. The publication was prepared with the support of the "RUDN University Program 5-100"

P.10-036-Wed
Detection of blood-circulating androgen receptor variants in castration-resistant prostate cancer

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Despite the latest progress in prostate cancer (PCa) treatment, castration-resistant prostate cancer (CRPC) remains incurable and is attributed to the highest mortality rates in PCa. Treatment resistance mechanisms are inseparable from androgen receptor (AR) signaling axis, which remains active in CRPC. One of the reactivation mechanisms might be AR splice variants (AR-Vs) that lack ligand binding domain and are constitutively active. Residing not only in tumors but also in blood-circulating tumor cells, PCa-specific AR-Vs might reflect tumor development, disease progression and response to treatment, and therefore could serve as the non-invasive PCa monitoring tools. The aim of our study was to develop novel AR-Vs-based molecular tool to non-invasively diagnose PCa, predict its progression and response to treatment directly from the blood sample. 127 PAXgene RNA blood samples were collected from 92 CRPC patients (33 cases have serial samples) during 2016–2017. The response to treatment was rated as positive (*Pos*, N = 80), progression (*Progr*, N = 14) or death (*Death*, N = 12). For the detection in patients' blood, custom made TaqMan assays for AR-V1, V3, and V7 were used for target-specific reverse transcription, pre-amplification and real-time PCR. AR-V1, -V3 and -V7 were detected in 17 (14%), 59 (46%), and 86 (68%) samples, respectively. 25 samples were AR-Vs-free, and 12 contained all variants tested. After the cases were stratified according to response to treatment, AR-V3 was detected more frequently in *Progr* vs *Pos* (FC = 5.4, $P = 0.034$), whereas V1 – in both, *Death* vs *Pos* (FC = 60.0, $P = 0.001$) and *Death* vs *Progr* (FC = 69.4, $P = 0.040$) comparisons. Furthermore, V1 and V3 were independent prognostic markers for disease progression separately ($P = 0.003$ and $P = 0.007$, respectively) and in combination ($P = 0.028$). Blood-circulating androgen receptor variants can serve as the non-invasive biomarkers for reliable prediction of PCa recurrence and response to treatment.

P.10-037-Mon
Investigation of the role of BAG-1 in ubiquitin/proteasome system in both healthy & tumor tissues as well as cell lines in breast cancer

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The expression level of three major receptors; estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) are important for diagnosis and treatment in breast cancer. Bcl-2-associated athanogene-1 (BAG-1) is an anti-apoptotic protein and frequently overexpressed in breast carcinoma. BAG-1 and its interaction partners such as heat shock proteins, Raf-1 kinase, nuclear hormone receptors, the anti-apoptotic Bcl-2 protein and ubiquitin/proteasome machinery components play key role in the regulation of various cellular pathways including proliferation, transcription, cellular mobility,

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apoptosis and cell survival. Here, to ultimately understand the adaptor role of BAG-1 in breast cancer, we aimed to determine relative expression levels of BAG-1 and its interaction partners involving in the ubiquitin/proteasome system. For this purpose, we first isolated proteins from tissues, which are classified according to the expression of ER, PR, HER2 receptors in normal and tumor tissues and their corresponding cell lines. We transfected C-terminal tandem affinity purification (TAP)-tagged *BAG-1* and small-interfering RNA (siRNA) targeting BAG-1 into breast cancer cell lines for the upregulation and downregulation of BAG-1, respectively. We performed immunoprecipitation after protein extraction and subsequent immunoblotting experiments to investigate the association of BAG-1 with p97/valosin-containing protein (VCP), UV excision repair protein RAD23 homolog B (Rad23B), 70 kDa heat shock protein (HSP70), E3 ubiquitin-protein ligase (CHIP), which are essential components of proteasome machinery. Results highlighted the involvement of BAG-1 in ubiquitin/proteasome system, which includes the action of endoplasmic reticulum-associated degradation (ERAD) mechanism to maintain intracellular homeostasis of cellular proteins.

P.10-038-Tue
Preoperative and postoperative plasma levels of circulating microRNAs in patients with colorectal cancer

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Colorectal cancer (CRC) ranks among the most common cancers worldwide. Surgical removal remains the best strategy of treatment for resectable tumours. One of the ways how to more accurately estimate prognosis of surgically treated patients with CRC is the use of a combination of already proven tumour markers (*e.g.* carcinoembryonic antigen, CEA) with new promising markers. MicroRNAs have the potential to become valuable biomarkers for this purpose. From the point of view of analytical features, microRNA molecules exhibit high stability in body fluids and tissues, and can be assessed from blood plasma samples. The aim of the study was to identify microRNAs whose plasma levels reflect the course of the disease. The estimation of selected microRNAs was performed in 88 paired (preoperative and postoperative) blood plasma samples of CRC patients by RT real-time PCR (TaqMan Advanced miRNA Assays) after isolation of total RNA from 200 µl of blood plasma with cel-miR-39 as spike-in control. We have revealed statistically significant decrease in plasma levels for miR-20a-5p, miR-23a-3p and miR-223a-3p ($P = 0.0069$, $P = 0.0010$, $P = 0.0088$, respectively) after surgical removal of the tumour tissue. In line with our results, the effect of these microRNAs has been described as oncogenic. We will continue to monitor the relationship of these microRNAs to prognosis (disease-free interval and overall survival) and we will also perform multivariate analysis along with classical markers. Study was supported by the grant of Ministry of Health of the Czech Republic – Conceptual Development of Research Organization (Faculty Hospital in Pilsen—FNPl, 00669806).

P.10-039-Wed
Polyamine catabolism activating agents show antiproliferative activity

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Polyamines (PA) are small positively charged molecules involved in the regulation of cell proliferation, differentiation and death. Many cancer types overproduce polyamines due to abnormal regulation of PA metabolism, which results in rapid cancer progression. The products of PA oxidative deamination (hydrogen peroxide, acrolein, ammonia) are cytotoxic and can cause apoptosis. Hence, tissue specific drug-induced depletion of intracellular polyamine level through the activated PA catabolism could be a useful tool for the control of neoplastic growth. We recently reported the high cytotoxic activity for activator of PA catabolism – copper complex of 3-(1-phenyl-2-fluoroanilino)-propanone-1. Currently we perform an extended study of effects of copper complexes with diverse ligands on PA exchange and their cytotoxicity. We synthesized and described 20 copper complexes. The following methods helped us to prove their structure: IR-spectrometry, X-ray diffraction, ultimate and thermogravimetrical analyses. We determined the PA levels and activities of key PA metabolic enzymes – ornithine decarboxylase and polyamine oxidase. Membrane permeability was also measured. Flow cytometry evaluation of CD95 expression was added to all previously applied methods. We screened 20 copper complexes and compared their biological effects with parent ligands. Complexation with copper ions improved significantly the membrane permeability and enhanced cytotoxicity though slightly decreased PA catabolism activating influence and inhibitory effect on ODC as well. A correlation was shown between cytotoxicity of the compounds and their influence on CD95 expression which may indicate the start of preparation of cells for apoptosis. Further investigations are recommended for this group of compounds as potential anticancer agents. The publication was prepared with the support of the "RUDN University Program 5-100" and Ministry of Education and Science of Russian Federation (Agreement No. 02.A03.21.0008)

P.10-040-Mon
Role of TGFbeta-1 in the regulation of fibroblast activation protein expression in human glioblastoma

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Our previous work showed increased expression of a proline-specific protease fibroblast activation protein (FAP) in glioblastomas (GBMs). FAP was present in both glioma cells and stromal cells. Since it was demonstrated that *FAP* gene is a transcriptional target for TGF-beta signaling, we addressed the question whether TGF-beta1 could contribute to the upregulation of FAP in GBM by affecting various subpopulations of transformed and stromal cells present in the GBM microenvironment. Using a kinetic assay with a specific FAP substrate N-quinoline-4-carbonyl-D-Ala-Pro-AMC, we found that glioblastoma non-stem cell cultures and permanent glioma cell lines express substantially higher levels of FAP activity than glioblastoma stem-like cells (GSC). Recombinant TGF-beta1 increased the activity of FAP several-fold in permanent glioma cell lines whereas GSC showed no or

negligible increase. The TGF-beta1-induced increase of FAP activity could be prevented by the TGFR inhibitors A8301 and A7701. These inhibitors also decreased the basal FAP activity in permanent glioma cell lines, which were shown to secrete their own TGF-beta1. In contrast to endothelial cells which had very low level of FAP activity that was not increased by TGF-beta1 treatment, human brain vascular pericytes displayed a high basal FAP activity which could be further upregulated by TGF-beta1. We also analysed the levels of TGF-beta isoforms 1, 2 and 3 (using specific ELISAs) and FAP activity in a set of GBMs. We found that the levels of TGF-beta isoforms significantly decreased in the order 1 > 2 > 3. In addition, we revealed a significant positive correlation between the level of TGF-beta1 and FAP activity in GBMs. In conclusion, our results provide evidence that TGF-beta1 can upregulate the level of FAP activity in GBMs through its effect on both non-stem glioma cells and vascular pericytes. Acknowledgement: Ministry of Health of CR grant 15-31379A, Progres Q28/1LFUK and grant LM2015064 of the EATRIS-CZ.

P.10-041-Tue
Bone morphogenic protein receptor type 1a (BMPR1A) and Caveolin-1 contribute to trastuzumab resistance of breast cancer cells

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Trastuzumab is a monoclonal antibody used in the treatment of human epidermal growth factor receptor 2 (HER-2) -positive metastatic breast cancer. However, resistance to trastuzumab hampers its clinical benefits. During the past decade, many attempts have been made in order to understand the mechanisms and factors involved in the trastuzumab resistance. More recent evidence highlights that bone morphogenic protein (BMP) signaling may represent a pathway in cancer that can sensitize cells to chemotherapy. Moreover, recent work also indicates that Caveolin-1, required for caveolae formation and endocytic membrane transport, may be a molecule mediating cancer drug resistance and metastasis. The aim of the present study was to determine and compare the expression of BMP receptor, type 1A (*BMPR1A*) and Caveolin-1 (*CAV1*) genes in trastuzumab sensitive or resistance BT-474 cells. Trastuzumab-resistant BT-474 cells were generated through extended exposure to trastuzumab for a total of six months and after which, an MTT assay was performed to verify the resistance. Following RNA extraction and cDNA synthesis, the expression levels of *BMPR1A* and *CAV1* were evaluated using real-time PCR. The results of MTT assay confirmed that trastuzumab resistance was well generated in BT-474 cells. Compared to the parental cells, *CAV1* expression markedly up-regulated (2.4 fold, $P < 0.05$) whereas *BMPR1A* expression significantly down-regulated (8.26 fold, $P < 0.05$) in trastuzumab-resistant cells. The evidence from this study suggests points toward the idea that *BMPR1A* and *CAV1* regulation participate in BT-474 trastuzumab resistance. Future studies on the current topic are therefore required to verify the role/s of these genes in trastuzumab resistance.

P.10-042-Wed
Role of genetic variants of membrane transporters in breast cancer

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Breast cancer is the most common cancer in women. One of the major obstacles to successful treatment is the multidrug resistance. Among other factors as activity of metabolic enzymes, variability of targets of cytostatics, impaired DNA repair or apoptosis; the expression of membrane transporters, e.g. P-glycoprotein, can cause such resistance. Gene expression in tumor cells of such transporters was previously associated with prognosis and response to neoadjuvant chemotherapy (ABCD2 and SLCO1A2). We aimed to investigate the germline genetic background of solute carrier (SLC) and ATP-binding cassette (ABC) transporters in breast cancer patients. We analyzed DNA samples from blood (n = 105 patients) using next-generation sequencing (NGS) with target enrichment of 49 ABCs and 45 SLCs. The functional effects of genetic variants, coding and non-coding ones, were predicted by *in silico* tools and associated phenotypes were searched in public databases. Altogether, we found 1683 variants (15% coding) in SLCs; 20 missense variants were predicted as deleterious *in silico* and four were frameshift deletions. In ABCs, 2864 variants were found (24% coding – 139 deleterious, 7 frameshifts). About 10% of variants was novel. Selected variants were compared with response to therapy and prognostic clinical features of the patients. Five single nucleotide polymorphisms (SNPs) in *SLC46A1* and *SLCO1A2* were assessed in a historical cohort of breast cancer patients with clinical follow-up (n = 819). In conclusion, SLC and ABC transporters contain clinically relevant genetic alterations and represent putative candidate genes of breast cancer prognosis or response to chemotherapy. Moreover, NGS is a novel and promising method for assessment of candidate biomarkers with potential for development of new therapeutics. Supported by grants no. AZV 15-25618A, NPUI no. LO1503, and Conceptual Development of Research Organization program of MH CR (FNPl, 00669806).

P.10-043-Mon
The role of transcriptional regulation in lipid oxidation in breast cancer

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Cancer cells have been known to have specific metabolism. We have hypothesized that oxidative phosphorylation plays important role in providing ATP in cancer cells and this process is supported by the oxidation of alternative substrates instead of glucose. These alternative energy sources are lipid oxidation and oxidation of branched chain amino acids, such as valine, leucine and isoleucine. Genome scale metabolic model was used in order to quantify the potential role of these amino acids as alternative sources on the total ATP supply. We used gene silencing in lipid synthesis and oxidation pathways as well as in the degradation of

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branched chain amino acid pathway to show the contribution of the mentioned metabolic reactions to proliferation and viability of breast cancer cell lines. For this purpose we used isotopic labelling, where citric acid pathway metabolites have been verified for fully labelled C-13 atoms when lipid and branched chain amino acids degradation pathways were blocked by appropriate siRNAs. We demonstrated that lipid synthesis and oxidation can be simultaneous in breast cancer cells.

P.10-044-Tue
Trace elements deficiency and integrin beta3a Leu33Pro polymorphism and cancer

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The genetic variations alone do not explain the observed differences in incidence of prostate cancer (PC) and bladder cancer (BC) in the populations, indicating that environmental and dietary factors are of importance. The glycoprotein alpha (IIb) beta3a (GPIIb/IIIa) is the main fibrinogen receptor on platelets. Using participants (n = 150) from the Moscow population, we assessed the risk of PC and BC in individuals with the beta3a Leu33Pro polymorphism (allelic variants *PLA1* and *PLA2)* and zinc (Zn) and iron (Fe) deficiency. A study group of 90 patients with PC, 30 patients with BC and 30 age-matched controls healthy volunteers were measured for Leu33Pro polymorphism with the PCR-RFLP assay. Detection of trace elements in the hairs of the patients with PC and BC and healthy adults was done by using atomic absorption spectrophotometer. Relative risks of cancer and 95% confidence intervals were calculated. The frequency of PLA2 carriers (heterozygous and homozygous) was significantly higher in patients with PC and BC, comparing to population frequency (42.2% vs 24.0%). For the patients with *PLA2* the tumor progression has a greater rate of local invasion and metastases. Concerning the metastases group for BC the frequency of Leu33Pro polymorphism carriers was higher than in non-invasive group (41.7% vs 23.3%). For the carriers of Leu33-Pro polymorphism (*PLA1*/*PLA2*) the frequency of local PC and of metastases PC is 2.8 and 2.4 times higher than that for non-carriers patients respectively. The concentration of Zn for the patients with invasive forms of PC and BC were significantly lower than for patients with non-invasive cancers. The tumor progression, local invasion and metastases have a greater rate for the patients with Zn deficiency and for the carriers of Leu33Pro polymorphism (*PLA1*/*PLA2*) than that for non-carriers. The publication was supported by "RUDN University Program 5-100" and the Ministry of Education and Science of Russia (the Agreement No. 02.A03.21.0008)

P.10-045-Wed
Triton-X based micellar coordination clusters loaded by a novel peptidyl platform drug conjugates and their activity against HeLa cells

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Among natural receptor-binding peptides, the neuroendocrine hormone somatostatin (SST) and its analogs received much attention because of their high affinity to five human receptors (SSTR1–SSTR5) overexpressing in cancer cells. However, the main limit of native SST for anticancer therapy is the very short half-life, relatively low receptor subtype selectivity and low aqueous solubility. For these purposes, a novel synthetic analog of SST with higher stability to enzymatic degradation in means of including *D*-amino acids with higher selectivity for specific SST subtypes was synthesized and characterized. A peptide drug conjugate represents analog of the somatostatin hormone receptor platform, linked to two known hydrophobic anticancer drugs, camptothecin and chlorambucil, acting through different oncogenic mechanisms. To solve the solubility problem and increase potential activity of the drug, a novel approach for encapsulation was applied and the anticancer activity against HeLa cells has been checked. The approach to Triton X-114 micellar coordination clusters (MCCs) formation is based on partial entrapment and stabilization by hydrophobic forces of chelate ligands found on the non-ionic surfactant micelles interface of the Triton X-114 with further "cross-linking" of the formed structures by various metal ions. An incubation of the HeLa cells culture with drug-loaded MCCs showed that during the the process, the MCCs are captured by the immobilized cells without intrusion into them and stayed on their surface. After 16 h of incubation, the total amount of living cells didn't exceed 5–10%. MCCs are unable to penetrate into the cells, but could attach to the cells surface and release the drug. The incubation of the drug with the culture has only a slight cytostatic effect. MCCs themselves without a drug do not influence on the cells. The work was supported by the Grant of the President of the Russian Federation No. MK-2124.2017.3 (2017–2018).

P.10-046-Mon
Combined epigenetic therapy with HMT and HDAC inhibitors caused acute promyelocytic leukemia cell differentiation and apoptosis *in vitro* and *ex vivo*

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Acute promyelocytic leukemia (APL) is a subgroup of acute myeloid leukemia, possessing a PML-RARA translocation. APL patients are conventionally treated with combinations of Retinoic acid and chemotherapeutics. Although, prognosis after such treatment is quite satisfactory, relapses and patients resistant to retinoic acid occur. Thus, we investigated the effect of epigenetic agents' 3-Deazaneplanocin A (histone methyl transferase inhibitor, HMTi) and Belinostat (histone deacetylase inhibitor, HDACi) in combination with Retinoic acid on APL cells in vitro and ex vivo. NB4 cell line was used for in vitro experiments. Samples of APL patient bone marrow cells were collected at diagnosis stage and used for ex vivo experiments. Both NB4 cells and patient bone marrow cells possess PML-RARA translocation. Both cell types were treated with 3-Deazaneplanocin A, Belinostat, and Retinoic acid combination. We showed that such treatment induced granulocytic cell differentiation both in vitro and ex vivo. Also, we observed increase of differentiation (CEBPE, PPARG), DNA damage (ATM), and cell cycle arrest (p53, p21) associated gene expression, meanwhile, oncogene (MYC, WT1) expression significantly decreased. After the treatment, pro-apoptotic protein BAX level increased, while, anti-apoptotic protein Bcl-2 level decreased showing triggered apoptosis process both in vitro and ex vivo. Elevated phospho-H2AX histone modification level suggests that apoptosis might be

triggered by DNA damage response. Analysis of H3K4me3, H3K14Ac, and Hyper-Ac-H4 histone modifications showed that the levels of these active chromatin markers increased after treatment. In conclusion, treatment with 3-Deazaneplanocin A, Belinostat and Retinoic acid combination induced acute promyelocytic leukemia cell differentiation, apoptosis, and chromatin remodeling in vitro and ex vivo. Therefore, we suggest that this combination might be a promising acute promyelocytic leukemia treatment approach.

P.10-047-Tue
Autocrine cytokine stimulation of MPM SCs might be responsible for chemoresistance in MPM

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Standard therapy of malignant pleural mesothelioma (MPM) is based on cisplatin and pemetrexed which is so far considered as "golden standard". Only partial response is obtained with existing therapy due to the intrinsic chemoresistance of MPM. Tumor-derived stem cells (SCs) are responsible for MPM dissemination and progression, but it is not known if they can contribute to its chemoresistant phenotype. The aim of this work was to investigate the mechanisms by which MPM SCs could exert chemoresistant properties of this tumor. From biopsies and pleural effusions of MPM patients we collected and stabilized MPM cell lines. The SC component was isolated by sorting the $SOX2^+$ $Oct4^+$ $Nanog^+$ $ALDH^{br1ght}$ cells and then confirming their clonogenicity and self-renewal potential. High-throughput PCR array was used to examine interleukins genes expression. Interleukins production were measured using Elisa. By using CRISPR/CAS system we created KO SCs subclones which we tested for cytotoxicity and cell viability. MPM SCs showed higher resistance to cisplatin and pemetrexed compared to non SCs. High-throughput PCR array showed higher expression of a vast number of cytokines in MPM SCs, particularly of IL1β and IL8. Higher production of these two cytokines were confirmed by ELISA. KO IL1β and IL8 MPM SCs sub-clones showed restored chemosensitivity to cisplatin and pemetrexed. Our study showed that MPM SC are resistant to a broad spectrum of first-line drugs such as cisplatin and pemetrexed. Knocking out experiments suggested that IL1-β and IL-8 autocrine stimulation might play a significant role in MPM SC resistance to cytotoxic effects induced by chemotherapeutic drugs.

P.10-048-Wed
The role of S100A4 in response to anti-neoplastic drugs and lymphocyte anticancer cytotoxicity

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Mts1/S100A4 is a calcium binding protein involved in metastasis. S100A4 is present in cytoplasm of many types of cancer cells and is secreted in the surrounding medium. Interestingly S100A4 is also present on the surface of CD^{4+} CD^{25+} $PGRPs^+$ $S100A4^+$ lymphocytes as a component of complex involved in their cytotoxicity. Here we investigated the relation between S100A4 expression in cancer cells and CD^{4+} CD^{25+} $PGRPs^+$ $S100A4^+$ lymphocytes and the cytotoxicity of lymphocytes to cancer cells. We show that the decrease of extracellular S100A4 led to increase of lymphocyte cytotoxicity against CSML100 and M3 melanoma cells. The decrease in S100A4 level in CD^{4+} CD^{25+} $PGRPs^+$ $S100A4^+$ lymphocytes inhibits their cytotoxic activity. Thus, the resistance to CD^{4+} CD^{25+} $PGRPs^+$ $S100A4^+$ lymphocyte cytotoxicity depends on S100A4 expression level in target cells and in lymphocytes. The effect of several anti-cancer drugs on cancer cells survival on the background of decreased S100A4 level was also studied. The decreased intracellular S100A4 level declines the resistance of malignant lymphoid Namalwa cells to dexamethasone. In Namalwa cells, dexamethasone decreased the effect of camptothecin but the decrease of intracellular S100A4 level retained their antitumor effect to the level of combined effect of dexamethasone and camptothecin. Studying the sensitivity of MDA MB 231 breast cancer cells having the decreased level of S100A4 to different combinations of anti-cancer drugs revealed that combination of Dexamethasone and Docetaxel highly increased their cumulative effect. Dexamethasone and Doxorubicin had a lower joint effect which was negated by the Docetaxel. Studying the mechanisms of these pharmaceutical combinations has determined the potential role of S100A4 in MDA MD 231 cell anticancer sensitivity. This work was supported by Russian Science Foundation grant 14–15–01032 and the Program of fundamental research for state academies for 2013–2020 years (№ 01201363822).

P.10-049-Mon
MiR-106b mediates sorafenib refractory hepatocellular carcinoma progression

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Hepatocellular carcinoma (HCC) is the leading cause of cancer-related death in Taiwan. Curative options for HCC are limited and exclusively available for 15% patients carrying an early stage HCC. Systemic target therapy sorafenib which could inhibit angiogenesis and Raf-1 protein is the only target therapy drug proved in advanced HCC patient's treatment. However, the sorafenib had only marginal effects and prolonged 3–4 months survival times in advanced HCC patients. The resistance to sorafenib caused the rapid disease progression and poor clinical outcome. Thus, it is emergent to understand the sorafenib resistant mechanisms and develop a new strategy to improve the effective treatment. In this study, we want to explore the roles of miR-106b in sorafenib refractory HCC progression. Sorafenib treatment inhibited RAS signaling and proliferation in HCC cells. Meanwhile, reduced expression of E2F1, MCM7 mRNA, and miR-106b was significantly observed in sorafenib-treated HCC cells. Our previous studies were showed that high expression of miR-106b with poor prognosis in HCC. Moreover, we found that overexpression of miR-106b resulted in downregulation of PTEN and upregulation of PD-L1. HCC cells which were long-term exposed with sorafenib led to a greatly decreased level in PTEN. Furthermore, PD-L1 levels were elevated in these cells. These findings suggested that miR-106b mediated sorafenib resistance by regulation of PD-L1 in HCC. The mechanisms of miR-106b and its regulatory axis in sorafenib-refractory HCC patients might apply to develop a new drug therapy in advanced HCC patients.

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P.10-050-Tue
Investigating the effects of boric acid and disodium octaborate tetrahydrate on cell death in glioblastoma cell lines

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The aim of this study was to investigate the cytotoxic, apoptotic and autophagic effects of boric acid (BA) and disodium octaborate tetrahydrate (Etidot-67, EDT-67) in glioblastoma cell lines (U-87 MG and T98G). Cytotoxic effect of BA and EDT-67 in U-87 MG and T98G cell lines was evaluated by using the MTT method. Besides, apoptotic and autophagic effects of BA and EDT-67 were determined by Annexin V and LC-3 antibody based methods, respectively. As a result, IC_{50} values obtained after 72 h of incubation were 4.8 mM (U-87 MG, BA), 4.9 mM (U-87 MG, EDT-67), 3.5 mM (T98G, BA) and 2.4 mM (T98G, EDT-67). According to the flow cytometry analysis, apoptosis rate increased by 2.4- fold (4.8 mM BA), 3.25- fold (9.6 mM BA), 5.6- fold (4.9 mM EDT-67) and 9.8- fold (9.8 mM EDT-67) in U-87 MG cell line compared to the untreated control group ($P < 0.001$). Furthermore, apoptosis rate also increased approximately by 1.7- fold (3.5 mM BA and 7 mM BA) and 2.6- fold (2.4 mM EDT-67 and 4.8 mM EDT-67) in T98G cell line ($P < 0.001$). On the other hand, no autophagy induction rate was determined in both cell lines for both agents. In conclusion, our preliminary results showed that although both agents induced apoptosis rather than autophagy, EDT-67 was found to be more effective than BA. As a conclusion both agents might be considered as potential agents in the treatment of glioblastoma.

P.10-051-Wed
Trace metal analysis & *in-silico* prediction: promising tools in environmental research and cancer risk prediction

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Environmental toxicity and its adverse health effect is an important issue of global concern. Chronic exposure to arsenic causes drastic patho-physiological outcomes ranging from cancerous to non-cancerous health effects. Arsenic-exposed populations are not only exposed to arsenic, rather exposed to a spectrum of other heavy metals and trace elements. Synergistic toxicity of these trace elements and heavy metals are less known. Long term Arsenic exposure along with other heavy metals may aggravate the risk of toxicity and carcinogenicity. Herein, EDXRF and ICP-OES are the analytical tools used for the analysis of biological samples like urine, blood and hair and the role of elements like Fe, Zn, Mn, Rb etc. are evaluated. The another important tool is "in silico analysis" used here for the prediction of Cancer risk. The exposure of Arsenic and other heavy metals cause adverse health effect, but the impact on health varies largely in population despite of exposure at similar level due to inter-individual genetic variation. *MC1R* gene regulates human pigmentation and variation in the *MC1R* is associated with pigmentary phenotypes and risk of skin cancer types like Squamous Cell Carcinoma, Basal Cell Carcinoma and Melanoma. There are several polymorphisms in *MC1R* gene, but it is indeed important to identify the functional ones. Through in-silico study, we have predicted the most significant missense variants of *MC1R* to be associated with different skin cancer types. R151C, R160W and D294H are found to be the most potential variants associated with skin cancer risk. Among 247 unreported SNPs of *MC1R* gene, P256S, C273Y, R306H, C289R, R67W and L206P are likely to have potential association with cancerous outcome.

P.10-052-Mon
Extracellular vesicles containing Caveolin-1 from metastatic breast cancer cells are enriched in adhesion proteins and enhance migration and invasion of recipient cells

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Breast cancer is one of the most frequently diagnosed cancers and the leading cause of cancer-related deaths in women worldwide. Moreover, most breast cancer-induced deaths are due to high invasiveness and the development of distant metastasis. In this respect, Caveolin-1 (CAV1), a multifunctional membrane protein that is typically upregulated in the final stages of cancer disease, promotes migration and invasion of tumor cells. Elevated levels of this protein have also been detected in extracellular vesicles (EVs) from advanced cancer patients. Thus, an intriguing possibility we explored is that the presence of CAV1 in EVs may enhance migration and invasion of recipient tumor cells. To that end, EVs were purified from supernatants of metastatic MDA-MB-231 wild-type (wt), MDA-MB-231(shCAV1) (possessing the plasmid pLKO.1 encoding a "small hairpin" directed against CAV1), as well as MDA-MB-231(shControl) breast cancer cells. Nanoparticle Tracking Analysis revealed an average particle size between 130 and 200 nm for all preparations. As anticipated, CAV1 was detected in MDA-MB-231 WT and shControl EVs, but not in MDA-MB-231(shCAV1) EVs. Mass spectrometry analysis revealed the presence of cell adhesion-related proteins only in EVs from WT and shC, but not shCAV1 cells. Importantly, EVs containing CAV1 were found to promote the migration and invasion of cells lacking CAV1. We conclude that the presence of CAV1 in EVs from breast cancer cells is associated with enhanced migration and invasiveness *in vitro*, suggesting that intercellular communication promoted by EVs containing CAV1 could favor metastasis of cancer cells. FONDECYT 11140204 (LLG), BASAL PFB-16 (LLG) CONICYT Student Fellowship (AC); CONICYT-FONDAP 15130011 and FONDECYT 1130250, 1170925 (AFGQ).

P.10-053-Tue
Fabrication of andrographolide analogue-loaded nanocarriers for cancer treatment

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Semi-synthetic andrographolide analogue namely 19-tert-butyldiphenylsilyl-8,17-epoxy andrographolide or 3A.1 is a C-19 substituted andrographolide which is the major compound from Andrographis Paniculata Nees (Acanthaceae). This analogue has been shown to be highly cytotoxic against several human cancer cell lines. However, the major problem of 3A.1 is poor water solubility hindering the preparation of a delivery system for clinical applications. Nanocarriers (liposomes and self-assembled nanoparticles) were fabricated and evaluated for their physicochemical characteristics, such as particle size, surface charge, entrapment efficiency (EE), loading capacity (LC) and in vitro cytotoxicity. The 3A.1-loaded self-assembled nanoparticles were successfully prepared by dropping method using amphiphilic chitosans. These 3A.1-loaded self-assembled nanoparticles were nano-size (66.26 to 102.53 nm) and spherical shape with negative surface charge (−30.50 to −22.23 mV). The 3A.1-loaded self-assembled nanoparticles with 40% drug loading exhibited the maximum values of both %EE (90.84%) and LC (363.25 mg/mg), indicating that high amount of 3A.1 could be entrapped into the self-assembled nanoparticles. In addition, the liposome formulations contained controlled amounts of phosphatidylcholine (PC) and cholesterol (Chol) at molar ratio of 10:2 mM and various amounts of 3A.1 were prepared using thin film hydration and sonication method. The vesicle sizes of all formulations were in the range of 75.37 to 120.40 nm with narrow size distribution. Zeta-potential values of all formulations were not significantly different, and were in the range of −2.02 to −4.32 mV. The maximal %EE and LC were 76.59% and 84.39 μg/mg, respectively. Both 3A.1-loaded liposomes and self-assembled nanoparticles exhibited anticancer activity higher than the free drug. Therefore, these nanocarriers containing 3A.1 may be potential nanocarriers for the treatment of cancer.

P.10-054-Wed
The role of HOTAIRM1 on tamoxifen-resistance in ER+ breast cancer cells

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Breast cancer is one of the most commonly occurring cancers in women worldwide. Approximately 40% of breast cancer patients acquire endocrine-resistance following therapy with tamoxifen. Many explanations for the development of endocrine-resistance have been put forward, one of them being the dysregulation of long non-coding RNAs (lncRNAs), as lncRNAs are the most diverse group of non-coding RNAs that are known to play important functions in various cellular and physiological processes. In fact, a number of lncRNAs have been reported to be involved in endocrine-resistance, as well as breast cancer progression and metastasis. In this study, we show that HOTAIRM1, an intergenic lncRNA located between HOXA1 and HOXA2 is upregulated in tamoxifen-resistant MCF7 (MCF7-TAMR) cells. We demonstrate that this upregulation results from an increase in the enrichment of H3K4me3, and a decrease in the enrichment of H3K27me3 marks in TAMR cells compared to MCF7 cells. The knockdown of HOTAIRM1 in TAMR cells significantly decreased cell proliferation and viability through the re-sensitization of cells to tamoxifen. Further characterization of the role of HOTAIRM1 in controlling gene expression and its interaction with chromatin modifiers may elucidate novel insight into the molecular mechanisms of HOTAIRM1 involved in the pathogenesis of breast cancer.

P.10-055-Mon
An evaluation for antimetastatic role of camalexin on human breast carcinoma cells

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Camalexin is a phytoalexin accumulated in some cruciferous plants upon exposure to environmental stress and plant pathogens. Camalexin has antiproliferative and chemopreventive roles in some cancers, besides its antifungal and antimicrobial activities. This study was designed to evaluate the cytotoxic, apoptotic and antimetastatic effects of camalexin on MCF-7 breast cancer cells through MMP-9 related pathway. Cytotoxic effect of camalexin was measured by MTT test. Annexin V binding assay and fluorescence imaging were performed for evaluation of apoptosis. Anti-metastatic activities were determined by measurement of enzyme activity and mRNA expression of MMP-9 protein which has a potential role in metastasis of cancer. MTT results showed that camalexin inhibited cell growth significantly at 10 μM and higher concentrations ($P < 0.01$). It has also induced an arrest of cell cycle at S phase. Camalexin significantly induced apoptosis in a dose dependent manner ($P < 0.05$) and the apoptotic effect was significantly correlated with the decrease of MMP-9 activity and mRNA expression ($P < 0.001$). This study reveals that camalexin has strong anticancer effects on MCF-7 breast cancer cells and it may be a candidate compound for treating the metastasis of cancer cells.

P.10-056-Tue
Non-genomic contribution of HIF-1α to hypoxic adaptation via formation of a mitochondrial anti-apoptotic complex

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Cells respond to lack of oxygen (hypoxia) by changing their gene expression pattern. Essential to this response, is Hypoxia-Inducible Factor 1 (HIF-1), a heterodimeric transcriptional activator. HIF-1 is a valid target of anticancer therapy because it is exploited by cancer cells in order to adapt to the hypoxic environment of solid tumors. HIF-1 activity is controlled by oxygen levels that regulate the hydroxylation and stability of its alpha subunit. However, oxygen-independent mechanisms that include direct phosphorylation of HIF-1α or protein interactions also govern its function. We have recently reported that lack of HIF-1α phosphorylation by ERK1/2 drives HIF-1α out of the nucleus in a CRM1-dependent manner. While in the cytoplasm, non-phosphorylated HIF-1α interacts with the mitochondrial protein mortalin and localizes to the outer mitochondrial membrane. There, HIF-1α mediates a non-transcriptional anti-apoptotic function that involves the appearance of a truncated VDAC1 form. Aim of the present study is the investigation of the molecular mechanism underlying this non-nuclear pro-survival function

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of HIF-1α under hypoxia. Following HIF-1α or mortalin immunoprecipitation from extracts of HeLa cells grown under normoxia or hypoxia in conjunction with ERK-pathway inactivation, we were able to detect association of HIF-1α with VDAC1 and hexokinase II under conditions that promoted HIF-1α localization to the mitochondria. Biochemical fractionation and immunofluorescence microscopy experiments could further show that formation of this complex protected cells from induced apoptosis by inhibiting translocation of the pro-apoptotic factor BAX to the mitochondria. Our results demonstrate a novel non-genomic function of HIF-1α that involves formation of a protein complex between HIF-1α and mitochondrial proteins which, ultimately, helps cells evade apoptosis under hypoxia.

P.10-057-Wed
Serine phosphatases of the CTDSP/SCP family suppress the proliferation of lung tumor cells by reduction of the Rb phosphorylation level

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Small serine phosphatases CTDSP1, CTDSP2 and CTDSPL (CTDSP1/2/L) belong to the CTDSP/SCP family and can exhibit multiple functions in a variety of cellular and biological processes. Their protein sequences demonstrate high homology. The dysfunction of the proteins may result in carcinogenesis. Despite the great interest to the phosphatases, there are a few data about their role in lung cancer. We used original CrossHub tool to evaluate expression alterations of *CTDSP1/2/L* in non-small cell lung cancer (NSCLC) and to reveal possible mechanisms of their regulation. Then we evaluated relative expression level of *CTDSP1/2/L* genes in primary samples comparatively adjacent normal tissues using qPCR. The significant (5-fold) and simultaneous decrease of these mRNA levels was revealed in the majority of NSCLC samples (84%, 39/46). Moreover, *CTDSP1/2/L* genes showed statistically significant down-regulation frequency (up to 90%) in metastatic samples, comparing with non- metastatic ($P < 0.05$). To elucidate the mechanism of suppression, we performed experiments on the electrotransfection of *CTDSP1/2/L* genes into the lung adenocarcinoma cells (A549). To introduce genes we used Sleeping Beauty transposase. Exogenous expression of *CTDSP1/2/L* inhibited growth of A549 cells. This was accompanied by increasing of the active dephosphorylated form of Rb protein in the nuclei. The level decrease of the phosphorylated form of pRb at Ser-807/811, Ser-780 and Ser-795 sites ($P \leq 0.05$) was observed for *CTDSPL* and *CTDSP2*. In addition, preliminary data showed a similar action of *CTDSP1*. Our data expand knowledge about the potential suppressor properties of CTDSP1/2/L associated with NSCLC. Study of cell growth suppression was supported by the Program of fundamental research for state academies for 2013–2020 years (№ 01201363823, № 01201363819) and gene expression analysis was supported by grant 14-50-00060 of the Russian Science Foundation.

P.10-058-Mon
Antibiotics as repurposed drugs for anticancer therapy

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Resistance of cancer to current treatments and metastatic relapse are the main causes of the poor prognosis of this disease. Such resistance can be acquired by cancer cells due to prolonged chemotherapeutic treatment as well as by intrinsic qualities of so-called cancer stem cells. Since these resistant populations are thought to be responsible for tumor progression and growth maintenance, several approaches have been recently undertaken to eradicate them effectively. Recently, it has been suggested that "de-energizing" cancer cells through the induction of mitochondrial dysfunction (MDF) may represent an effective way to target cancer metabolism and progression. Interestingly, due to evolutionary similarities between bacteria and mitochondria, antibiotics have been proposed as MDF inducing drugs. In this project we elucidate the possibility that antibiotics affecting mitochondrial function could target chemoresistant cancer cells through the induction of MDF. Through shotgun proteomic profiling we observed an upregulation of metabolic pathways involving mitochondria in chemoresistant populations. Upon screening a panel of antibiotics we identified two promising candidates that we proceeded to validate *in vitro by* measuring several metabolic functions in breast cancer lines (mitochondrial membrane potential, ROS production, oxygen consumption, ATP production). We also found that autophagy seem to be involved upon antibiotic treatment, which was revealed through western blot and fluorescent confocal microscopy studies. Additionally, *in vivo* studies revealed an inhibition of tumor growth in antibiotic-treated mice. Finally we have also explored the possibility that combinatorial treatment with antibiotic and an autophagy inhibitor may act in a synergistic manner. Overall, our results represent new findings which can benefit both fundamental research and translational medicine and will propose repurposing some existing drugs to be used as anticancer therapeutic agents.

P.10-059-Tue
Toxic effects of binase towards cancer cells are enhanced at physiological level of oxygen and hypoxia

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As a rule, cells are grown in an *in vitro* system in atmosphere containing 21% O_2 and 5% CO_2 (standard conditions). Under these conditions, cells exist in a situation of chronic oxidative stress, since in different tissues the oxygen partial pressure (pO_2) is 3–8 kPa, which corresponds to 3–8% O_2 (normoxia). In the growth tumor occurs oxygen starvation – hypoxia ($[O_2]< 2\%$), which is a key factor in the development and spread of the tumor. One of the main problems of antitumor therapy is that many drugs effective under standard conditions lose their antitumor activity when the redox status of cells changes in conditions of tissue normoxia and hypoxia. Thus, it is necessary to investigate the effect of therapeutic agents precisely under conditions of

normoxia and hypoxia. Ribonuclease binase from *Bacillus pumilus* effectively eliminates various malignant cells under standard conditions. It does not contain cysteine and methionine residues and, accordingly, is insensitive to changes of redox conditions. We tested the effect of binase on the acute myelogenous leukemia Kasumi-1 cells, and cervical cancer SiHa cells growing at different oxygen content. A decrease in $[O_2]$ from 21 to 5 and 1% resulted in an almost twofold increase in the proportion of apoptotic cells in the Kasumi-1 and SiHa populations compared to standard conditions. Under normoxia and hypoxia, the effect of binase is characterized by other changes in intracellular parameters (glutathione, calcium, pH) than under standard conditions. In particular, in malignant cells treated by binase the pH level, increased initially due to active glycolysis, decreases significantly more in normoxia and hypoxia than under standard conditions. Thus, in normoxia and hypoxia the antineoplastic properties of binase are enhanced, which indicates the promise of its use for the therapy of tumors, including those resistant to other chemotherapeutics. Supported by RFBR #17-00-00061 and PRFSA #01201363823.

P.10-060-Wed
Targeting of hypoxia-inducible factor 1α in cancer cells by prodrugs based on quinoxaline 1,4-dioxide scaffold

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Hypoxia mediates chemo- and radioresistance in a majority of solid tumors. So far as hypoxia is a main character of solid cancers and determines aggressive phenotype, tumor cell invasion, metastasis, and relapse of the disease, so it is one of the most challenging therapeutic targets. There are several treatments for targeting hypoxic tumors among which hypoxia-activated prodrugs, specific targeting of hypoxia-inducible factor 1α (HIF-1α) or/and VEGF-A/VEGFRs, or targeting other pathways important in hypoxic cells. Based on these researches our group synthesized and evaluated series of new bioreductively activated derivatives of quinoxaline 1,4-dioxide (QdNOs). So, 3-arylquinoxaline-2-carbonitrile 1,4-dioxides with halogens in aryl or/and quinoxaline rings were assayed for cytotoxicity against of human breast cancer cell lines (MCF-7 and MDA-MB-231) under hypoxic and normoxic conditions. Results showed that majority of derivatives have higher hypoxic cytotoxicity and selectivity than reference agent tirapazamine. In contrast to doxorubicin, all tested QdNO's have similar potencies for human colon cancer cell line (HCT-116) and its isogenic drug resistant counterpart (HCT-116p53KO ($p53^{-/-}$)) with deletion of tumor suppressor p53. The activities of HIF-1α and p53 were assessed by reporter analysis. The most hypoxia-specific derivatives were discovered as potent HIF-1α signaling pathway inhibitors. Analyze participation in p53 signaling of hit-compound by p53-dependant reporter assay confirm that it has p53-independent mechanism of action. Tumor cell death was associated with a growth of QdNOs-induced ROS independently of p53 which caused DNA damage, decrease in HIF-1α expression, and downregulation of VEGF-A transcription. These results support that quinoxaline derivatives might be a promising class for further development as hypoxia-selective anti-tumor agent. The biology experiments of the research were supported by RSF 14-15-00362.

P.10-061-Mon
DNA methylation pattern to identify trastuzumab resistance in HER2-positive breast cancer models

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Introduction: Approximately 15% to 20% of patients with breast cancer overexpress the human epidermal growth factor receptor 2 (HER2). Despite the development of anti-HER2 treatments has significantly improved outcome in breast cancer, up to 62% of patients develop resistance within a year. Several efforts have been focused on finding and understanding the molecular and cellular mechanisms involved in treatment resistance. It was believed that these changes were produced by abnormal alterations in the genome, but this concept was modified with the discovery of epigenetic regulation. The DNA methylation status of promoter regions of genes has been described as a common epigenetic alteration for transcriptional repression in human malignancies such as breast cancer. The purpose of this study was to identify a potential biomarker to predict trastuzumab resistance in HER2-positive breast cancer. Results: We analyzed the promoter DNA methylation status and expression pattern of trastuzumab-sensitive (SK) and –resistant cells (SKTR) by Infinitum Human Methylation 450K array and RNA-seq, respectively. We found six genes with a significant correlation between their methylation and expression pattern. Furthermore, their epigenetic and expression status was validated by pyrosequencing and MSP as well as by DNA demethylating agent treatment (5-AZA) in SKTR cells. The most noteworthy gene as a potential biomarker was the gene-1. Our functional assays revealed that gene-1 overexpression in SKTR cells produces a significant increase in trastuzumab sensitivity compared to its control. Furthermore, it has been observed some HER family receptors and downstream protein with activation levels similar to sensitive cells (SK). Conclusions: These results provide a basis for further studies to validate the hypermethylation status of this candidate gene as predictive or monitoring biomarkers of trastuzumab resistance in HER2 breast cancer patients.

P.10-062-Tue
Thy-1/CD90 induces metastatic breast cancer cell migration involves a Ca^{2+}/hemichannel/ATP/P2X7 receptor-signaling axis

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Cancer cell adhesion to the vascular endothelium is an important step in tumor metastasis. Thy-1/CD90, a cell adhesion molecule expressed in activated endothelial cells, has been implicated in melanoma metastasis by binding to integrins present in cancer cells. Ca^{2+}-dependent hemichannel opening, as well as the P2X7 receptor are reportedly key players in the Thy-1-integrin-induced astrocyte migration. However, the signaling pathway triggered downstream of integrins after Thy-1 stimulation of breast cancer cells remains to be explored. We hypothesize that a

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Ca^{2+}/hemichannel/ATP/P2X7 receptor-signaling axis is involved in breast cancer cell migration stimulated by Thy-1. MDA-MB-231 metastatic breast cancer cells were stimulated with Thy-1. Intracellular calcium concentrations ($[Ca^{2+}]i$), ATP release, and cell migration were monitored in the presence or absence of Apyrase, hemichannel inhibitors or P2X7 receptor silencing. We observed that Thy-1 induced a rapid increase in $[Ca^{2+}]i$, ATP release, and cell migration. The connexin/pannexin inhibitors decreased cell migration, indicating that opening of these hemichannels is required for migration. In addition, cell migration was precluded when the P2X7 receptor was silenced. We also assessed the *in vitro* transendothelial cell migration (TEM) capacity of MDA-MB-231 cells. Importantly, the ability of breast cancer cells to transmigrate through an endothelial monolayer was significantly decreased when the P2X7 receptor was silenced in cancer cells. Thus, our results suggest that ATP signaling via the P2X7 receptor is a conserved signaling pathway involved in cell migration that is not restricted to astrocytes, but also relevant in cancer cells. These findings open the possibility of utilizing hemichannel or inhibitors of Thy-1/integrin binding as therapeutic agents to prevent breast cancer metastasis. Acknowledgements: FONDECYT #3160349 (MB); #1170925 (AFGQ); #1150744 (LL). CONICYT-FONDAP #15130011 (AFGQ, LL).

P.10-063-Wed
Investigation of non-canonical signal molecules alteration dependent on leptin in MCF-7 breast cancer cell lines

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Obesity is a global disease characterized by high levels of body fat mass and cytokines derived from adipocytes. Research shows that body fat and leptin (Lep) levels are linked to obesity and cancer. In this study, it was aimed to investigate the pro-angiogenic effects of different doses of Lep on breast cancer cells and to determine the levels of canonical and non-canonical signaling molecules in the mitogen activated protein kinase (MAPK) pathway of leptin. Different concentrations of Lep (0, 10, 20, 100 and 1000 ng/ml) were applied on MCF-7 cell lines and MAPK multi ELISA kit was used for p44 MAPK, p38 MAPK, MAPK/ERK kinase (MEK1), pMEK1, stress activated protein kinase (SAPK)/Janus kinase (JNK) and Psapk/JNK molecules have been determined. Total protein was determined using bicinchoninic acid (BCA) protein assay kit on MCF-7 cell media. The quantities of the molecules were calculated as ng/mg protein and the statistical analyzes were performed with Kruskal Walls and Tamheinn test. According to the findings obtained, it was found that, at low doses of Lep (10 ng/ml and 20 ng/ml) there was a statistically significant increase difference of all signal molecules studied compared to the control group which was not receiving leptin. In all signal molecules at the pharmacological Lep dose (1000 ng/ml) were likely to decrease, while obesity Lep dose (100 ng/ml) was found to be effective in all signaling molecules except p38 MAPK and pMEK1. In our study, leptin was found to have a maximal inducing effect on the molecules in the MAPK signaling pathway in all signal molecules where 20 ng/ml leptin dose was applied. It has been determined that the increase in the signal molecule of Psapk/JNK is the highest. This was revealed by our study as a first demonstration of a marked increase trend of the Psapk/JNK molecule among leptin-induced molecules. The fact that there isn't any literature study about relationship between leptin and these molecules suggests that this study is unique.

P.10-064-Mon
Helicobacter pylori gamma-glutamyl transpeptidase inhibits autophagy in gastric cancer cells favouring survival of internalised bacteria

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Helicobacter pylori (*H. pylori*) is the major risk factor associated with the development of gastric cancer. Multiple virulence factors contribute to its pathogenesis, and recently, the secreted gamma-glutamyl transpeptidase (GGT) has become of increasing interest. Autophagy, a conserved response by which eukaryotic cells seek to maintain cellular homeostasis is also recognised as a host defence mechanism that challenges pathogenic bacteria after internalisation. Although, *H. pylori* is typically considered an extracellular bacterium, several studies have reported that it is internalised possibly as a strategy to avoid exposure to antibiotics. The precise mechanisms by which *H. pylori* manipulates host cell autophagic processes remain unclear and, importantly, none of the available studies consider the possibility that GGT might be involved. Here, we evaluated whether GGT modulates autophagy in AGS and NCI-N87 gastric cells. Following infection for 6 h by the wild type *H. pylori* strain 26695 or the respective isogenic Δggt mutant strain, autophagic flux was determined by LC3 processing in the absence or presence of chloroquine. The ability of *H. pylori* cells to survive inside AGS cells was assessed by the gentamycin assay and reactive oxygen species (ROS) production was measured by flow cytometry. Our results revealed that deletion of GGT increases autophagic flux in gastric cells compared with wild type bacteria. Moreover, this increase in the autophagic flux upon infection with the Δggt mutant strain correlated with a reduction in the ability of *H. pylori* to survive inside gastric cells and with lower ROS levels. These results suggest that inhibition of autophagy by GGT contributes to *H. pylori* survival which is expected to favour the progression of gastric cancer precursor lesions. Fondecyt 3160384 (JB); 1130250, 1170925 (AFGQ); 1151411 (AHC), CONICYT-FONDAP 15130011 (AFGQ,AHC).

P.10-065-Tue
The link between mechanosensitive potassium channel and epithelial-mesenchymal transition in cancer cells

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The two-pore domain potassium channels (K2P channels) play an important role in stabilizing the membrane voltage and are not inhibited by the classical potassium channel blocker. Among these K2P channels, mechanically sensitive K2P channels

(MK2P) are expected to be located near the local adhesion molecules associated with the cell's stress fibers, cytoskeleton and adhesion molecules. Thus, epithelial mesenchymal transition (EMT) can affect gating of channel proteins that can interfere with cell homeostasis by altering cell membrane potential. The phenomenon of EMT in cancer cells is involved in invasion, metastasis, recurrence, chemotherapy and drug resistance, but it is not known that MK2P is involved in EMT. In this study, we used MK2P small interfering RNA (MK2Psi) and rat MK2P plasmid DNA (rMK2Pp) to determine whether MK2P contribute to EMT regulation. Among the EMT markers, alterations in vimentin, E-cadherin, and N-cadherin were determined using MK2Psi or rMK2Pp alone and MK2P siRNA and MK2P plasmid DNA simultaneously (MK2Psi plus rMK2Pp) in prostate cancer cell line. The mRNA levels of cells treated with MK2Psi or rMK2Pp alone and cells treated with negative control siRNA (NCsi) were observed using real-time RT-PCR. Cells treated with MK2Psi twice for 6 days showed an increase in vimentin and N-cadherin compared to NCsi, whereas rMK2Pp-overexpressing cells showed a decrease in mesenchymal marker vimentin compared to non-transfected control cells. The epithelial marker E-cadherin was slightly decreased in MK2Psi treated cells. Cells transfected with MK2Psi and rMK2Pp restored increased vimentin and decreased E-cadherin compared to cells transfected with MK2Psi alone. Immunocytochemistry or real-time RT-PCR analysis showed that MK2Psi increased the binding homeobox transcription factor 1 (Zeb1) and Slug compared to NCsi, and the factors were restored by rMK2Pp. These results suggest that the MK2P is involved in the EMT process of cancer cells.

P.10-066-Wed
An adhesion molecule, ARHGEF7, regulates the cell cycle progression in colon cancer

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ARHGEF7 is a guanine nucleotide exchange factor for Rac/Cdc42 that regulates the important cellular processes such as cell migration, differentiation and proliferation. However, little is known about how plays a role in colon cancer. In this study, we investigated whether the expression level of ARHGEF7 was increased, and the cell cycle by ARHGEF7 was regulated on colon cancer. First, we analyzed the ARHGEF7 expression in colon cancer tissues. ARHGEF7 were overexpressed in cancer tissues compared to paired normal tissues. Knockdown of ARHGEF7 using specific siRNAs inhibited the kinase activity of p21-activated kinase 1 (PAK1) in HCT116, colon cancer cells. Also, overexpression of GFP ARHGEF7-wild type increased the activity of endogenous PAK1 in dose dependent manner. Overexpression of GEF-negative ARHGEF7 couldn't induce the PAK1 activation as well as Rac1. These results implicate that overexpression of ARHGEF7 regulates PAK1 activity through Rac1 activation in colon cancer. Furthermore, we observed that the proliferation of colon cancer cells and cell cycle progression were attenuated compared with control. These results suggest that overexpressed ARHGEF7 in colon cancer regulates cell proliferation by promoting the cell cycle progression.

P.10-067-Mon
Role of PDLIM4 in triple-negative breast cancer

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PDLIM4 (PDZ and LIM domain protein 4) is an adaptor protein belonging to ALP subfamily of PDZ and LIM domain proteins. PDLIM4 is located on human chromosome region 5q31.1 which is frequently deleted in tumor cells of patients with lymphoproliferative disorders. According to several reports, PDLIM4 gene methylation is frequently observed in some cancers; and subsequent loss of expression may serve as an adverse prognostic factor of disease. As a result, PDLIM4 is thought to act as a tumor suppressor protein in some cancers, particularly in colon cancer and prostate cancer. We conducted a systematic analysis of PDLIM4 status and function in the panel of triple-negative breast cancer cell lines (TNBC) and found an ambiguous role of the protein. TNBC cell lines were characterised by wide range of PDLIM4 expression levels varying from higher-than-normal to complete loss. PDLIM4 knock-in/knock-out in lines with respective low and high PDLIM4 expression levels had a distinct impact on cell migration activity and morphology. Assessment of functional interaction between PDLIM4 expression levels and c-Src activity – a previously described possible mechanism of PDLIM4-mediated malignization, revealed no evident correlation. Instead, the alternative theory of PDLIM4 function via CD74/Scribble/E-cadherin axis was proposed and confirmed for TNBC cell lines. Moreover, for the first time PDLIM4 was detected in nuclei of several breast cancer cell lines. To establish the role of nuclear PDLIM4, the protein was immunoprecipitated and the possible new binding partners were studied by LC-MS/MS. The results suggest a new mechanism of PDLIM4 function in breast cancer.

P.10-068-Tue
NNMT knockdown enhances autophagy for resistance to nutrient starvation in liver cancer

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Nicotinamide N-methyl transferase (NNMT) transfers a methyl group from S-adenosyl-L-methionine (SAM) to nicotinamide (NAM), producing 1-methylnicotinamide (1MNA). NNMT levels are variably dependent on tumor types and the underlying roles of NNMT in liver cancer have not been fully investigated. In this study, we found that NNMT expression levels were downregulated in liver cancer cell lines and NNMT knockdown promoted tumor cell survival under nutrient deprivation condition by enhancing autophagy. Consistently, NNMT knockdown enhanced tumor growth by decreasing the regions of cell death in a xenograft model. Increased autophagy flux in liver cancer cells with NNMT knockdown was dependent on ULK1 activation. NNMT knockdown increased both methylation and phosphatase activity of PP2A, resulting in down-regulation of p-ULK1 (S638). Accordingly, NNMT downregulation rescued tumor cells under nutrient deficiency *in vivo*, which was alleviated by ULK1 inhibitor treatment. Taken together, our findings suggest a novel function of NNMT in liver cancer and provide insight into ULK1 inhibition as its effective therapeutic strategy.

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P.10-069-Wed
New synergic biomaterials for anti-cancer therapy

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Recent anti-cancer research has focused on finding novel compound/product combinations with non-overlapping mechanisms of action, due to limited toxicity in normal tissues, harmful side effects and the development of drug resistance. The first objective of this study was to determine the mechanism of action of *Anemone nemorosa*, *Artemisia afra*, N-[[3-(4-bromophenyl)-1H-pyrazol-5-yl]-carbamothioyl]-4-chloro-benzamide (BC-7) and cisplatin through cell cycle arrest, phosphatidylserine translocation, caspase activation and mitochondrial membrane depolarization. The second objective was to investigate mixtures of these extracts and compounds for their synergistic cytotoxic activity and thus formulating the mixtures as potential anti-cancer agents. Thirty combination mixtures were prepared using the IC_{50} values of each extract or compound at ratios of 1:3, 1:2, 1:1, 2:1 and 3:1, respectively. The cytotoxic/anti-proliferative activity of each mixture was determined by the bis-Benzamide H 33342 trihydrochloride/propidium iodide (Hoechst/PI) dual staining method on HeLa cervical cancer cells. The combination index (CI) values, at inhibition of 50% for each mixture was determined by means of the Chou and Talalay method. The combined effect can then be indicated as CI < 1, synergism; CI = 1, additive effect or CI > 1, antagonism, respectively. Most combination treatments showed to have an antagonistic effect except for cisplatin:BC-7 and cisplatin: *A. afra* combinations that showed synergism. The 1:2 ratio of cisplatin:BC-7 and 1:1 and 3:1 ratios of cisplatin:*A. afra* were additive. The current finding is that BC-7 and *A. afra* could lower the dose of cisplatin in combination to achieve a similar anti-cancer efficacy compared to the higher cisplatin dose when used alone. The lower dosage in combination could result in reduced drug resistance as well as limit the toxicity on normal cells associated with cisplatin treatment.

P.10-070-Mon
Role of SCF(FBXW7) on cell proliferation recovery after UV treatment

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SKP1-CUL1-F-box protein (SCF) complex is probably the best-characterized Cullin-RING ubiquitin ligase. The E3 ligases are one of the three key enzymes involved in protein ubiquitination and degradation. The F-box protein is the variable substrate-recognition subunit, being FBXW7 one of the most well studied F-box proteins. FBXW7 is a tumor suppressor that targets multiple oncoproteins for ubiquitination-mediated proteolysis, including cyclin E, c-MYC, c-JUN or MCL1. For this reason, approximately six percent of all human cancers harbor *FBXW7* mutations. In addition to his role in cancer, FBXW7 also plays a key role in metabolism and other processes. We have previously demonstrated that SCF(FBXW7) modulates the intra-S-phase DNA-damage checkpoint by regulating PLK1 stability. We showed that PLK1 degradation contribute to avoid the cell proliferation after DNA damage in the S phase of the cell cycle. In the present study, we continue analyzing the role of FBXW7 on cell proliferation after UV radiation. We compared proliferation of wild type and $FBXW7^{-/-}$ HCT116 cells after UV treatment, and found that FBXW7 enabled a faster recovery of cell proliferation. The analysis of the amount of TP53 in both cell lines determined a faster decrease of TP53 protein level in the wild type compared with knock out cells. These results led us to study TP53 as a new potential substrate of SCF(FBXW7). The results obtained will be shown. In summary, we suggest that FBXW7 has a double function in regulation of cell proliferation. On the one hand, it regulates the cell cycle arrest when DNA damage occurs and, on the other, it allows the cell proliferation when the DNA damage has been repaired.

P.10-071-Tue
Molecular mechanism of action of classical and next-generation taxanes for their potential effects in resistant ovarian tumors

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Taxanes are widely used anticancer drugs. However, drug resistance of tumor cells to classical taxanes presents one of the major obstacles in successful therapy. At present, new anticancer drugs on the basis of taxanes are developed as potential drugs effective in resistant type of tumors. We explored efficacy of classical (paclitaxel) and next-generation Stony Brook taxanes (SB-taxanes) *in vitro* in sensitive and resistant models of cancer cells. Twelve examined SB-taxanes (SB-T-1214 and SB-T-1216 derivatives and fluoro-derivatives) demonstrated significantly higher cytotoxicity and accumulation than paclitaxel in resistant tumor cells. SB-T-1214, SB-T-1216 and SB-T-12854 were also efficient *in vivo* in rat lymphomas and mouse xenograft models of pancreatic carcinoma. Accordingly, SB-taxanes seem to be potential candidates for treatment of classical taxane-resistant tumors. In the frame of molecular mechanism of action, we have measured mRNA and miRNA profile changes using Affymetrix arrays in resistant ovarian carcinoma cells (NCI/ADR-RES) after the treatment with paclitaxel and compared data with one of the most efficient taxanes, SB-T-1216. We have found significant differences in mRNA and miRNA profiles linked to the action of taxanes and identified key pathways and genes for molecular mechanism of taxane action (e.g., SLC transporters, NOTCH and AhR signaling pathways, Hedgehog pathway). Subsequently, NOTCH signaling pathway genes were selected for estimation of their genetic profile in ovarian carcinoma patients. High inter-individual variability of gene expression from NOTCH pathway was found and particular associations of expression with prognosis of ovarian carcinoma were also identified. NOTCH signaling pathway was suggested as potential therapeutic target in ovarian carcinoma therapy suitable for further research. Supported by the research grants CSF no. P303/12/G163, AZV no. 15-25618A and NIH/CA103314.

P.10-072-Wed
The biological role of LYVE-1 in lung cancer

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Lung cancer, is a malignant lung tumor characterized by uncontrolled cell growth in tissues of the lung. Primary lung cancers, are carcinomas that derive from epithelial cells. The closely follow-up of patients having the predisposing disorders can yield an increase in the rates of early diagnosis and curative treatment modalities. LYVE-1, a receptor molecule for hyaluronan, is expressed in the lymphatic endothelium, blood sinus endothelium, and certain macrophage lineages. immunohistochemical study revealed a broader distribution of LYVE-1 in vascular endothelial cells of the murine lung, adrenal gland, and heart as well as the liver and spleen. In the current study, we aim to determine the serum levels of LYVE-1 mRNA levels of this parameter by PCR and levels of LYVE-1 protein verified lung cancer. LYVE-1 protein assay employs ELISA. The colored reaction product was measured using an automated ELISA microplate reader at 450 nm. The results are compared with the controls by using statistical tests. The serum samples of the 60 consecutive patients with lung cancer who referred to Istanbul University Institute of Oncology from 2017 to 2018 were obtained. The healthy control group consisted of 20. Serum LYVE-1 ($P = 0.02$) protein levels were significantly higher in patients with lung cancer than the healthy controls. 30% of serum LYVE-1 mRNA levels were high verified lung cancer. However, known clinical variables including response to adjuvant chemotherapy were not found to be correlated with serum LYVE-1 concentrations($P > 0.05$).Significant relationship between other clinicopathologic variables including localization of lung ($P = 0.04$), presence of metastasis ($P = 0.01$), vascular invasion ($P = 0.02$).We think this parameter will be important in serum samples of patients with lung cancer diagnosis and disease follow-up. LYVE-1 is important in lung cancer targeted therapy.

P.10-073-Mon
Targeting NHL B-cell receptors for personalized CART therapy

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Non-Hodgkin lymphomas (NHLs) represent a heterogeneous group of malignancies that arise from the lymphoid system. At the present time exist a lot of drugs for the NHLs therapy, but mostly all of them are unsafe and there is no consensus regarding the best treatment protocol. To increase the efficacy and safety of therapeutic B-lymphocyte depletion in lymphomas and leukemia's it would be preferable to induce the death of pathological B cells without affecting normal B cells to prevent side effects. Similar to other types of cancer, NHLs arise by a multistep accumulation of genetic aberrations that induce a selective growth advantage of the malignant clone. All B-cells of organism have a unique cell surface marker – antigen B-cell receptor (BCR). We generate novel approach for personalized NHL therapy based on peptide specific to malignant cells surface receptor. For this purpose we designed new lentiviral peptide library screening technique based on fluorescent reporter cells system. Herein 7aa peptide library was used for screening of FL malignant receptor agonist. Variable domain of the lymphoma BCR was used for chimeric receptor generation, where BCR VH part responsible for agonist recognition and bottom part of receptor was retranslate signal to the reporter gene. In this embodiment of the method, very large numbers of candidate 7aa peptides expressing lentivirus and eukaryotic reporter cells are packaged together in a format where each is capable of replication, thereby forging a direct link between genotype and phenotype. After four rounds of screening we discover peptide specifically interacted with malignant BCR. Selected peptide ligand was fused with chimeric antigen receptor III (CAR-III) generation for expression on $CD8^+$ T-cells. Modified T-cells selectively eliminate lymphoma cells *ex vivo* and *in vivo*. This work was supported by a grant from the Russian Science Foundation No. 17-74-30019.

P.10-074-Tue
Can DCLK1 be a biomarker for head and neck cancer?

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Head and neck cancer (HNC) includes epithelial malignancies of the upper aerodigestive tract, including the paranasal sinuses, nasal cavity, oral cavity, pharynx, and larynx; and, as the sixth most common cancer worldwide. HNC represents about 6% of solid tumors. The adjacency to the central nervous system, cranial nerves and eye, constrains the treatment options and approaches for HNC. Doublecortin-like kinase type 1 (DCLK1) is a serine–threonine kinase expressed in the central nervous system, belongs to the family of calmodulin-dependent kinases. Its function was first described in neuronal migration and development. In preinvasive pancreatic cancer, cells with stem cell-like characteristics were positive for DCLK1. An expression of DCLK1 is observed in clear renal-cell, colorectal and esophageal adenocarcinoma. Recent studies have considered the DCLK1 gene methylation as a marker of colorectal cancer stem cells. Also, hypermethylation of the DCLK1 gene promoter has been found in tissues of colorectal cancer and cholangiocarcinoma samples. DCLK1 expression associated with poor survival in head and neck squamous cell and minor salivary gland carcinoma. We aimed to research serum DCLK1 protein and mRNA levels in patients with HNC, who were not received any treatment. The results were compared with the healthy controls to investigate DCLK1 as a useful marker for HNC patients. Pretreatment serum DCLK1 protein and mRNA levels will determine by the sandwich ELISA and qRT-PCR, respectively in Istanbul University Oncology Institute Cancer Biochemistry Laboratory. We planned to collect 80 HNC patients and 20 healthy controls. When we reach the sufficient number of samples to perform the tests, the results will be evaluated using SPSS 21. The statistically significant differences between the patient with HNC and the control group will be examined. The obtained data and conclusions of this study will be presented in the poster during the FEBS 2018 Prague.

P.10-075-Wed
Novel minor isoform of securin lacks D-box and exhibits distinct expression pattern and transcriptional activity

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Securin (PTTG1) is a separase inhibitor, which is ubiquitinated by the anaphase-promoting complex (APC) and degraded in

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mitosis. Timely release of separase is essential for the proper sister chromatids separation. Securin also acts as a transcriptional regulator for more than a thousand genes mainly involved in proliferation, cell cycle progression and DNA repair. It is chiefly expressed in the G2 phase of cell cycle. We discovered a novel minor isoform of securin, which lacks exons 3 and 4. These exons contain the main APC recognition site (D-box), which is necessary for the swiftness of degradation. The presence of the new short isoform mRNA is confirmed in multiple cancer cell lines and primary cells by qRT-PCR at levels 0.4–2% of the major isoform. Preliminary results obtained on synchronized cells suggest that the short isoform has higher mRNA level in some phases of the cell cycle as compared to the full-length isoform. We have shown that overexpression of the short isoform activates transcription of several growth factors, for instance FGF2 and VEGFA and cyclins such as CCND3. However, the absence of the DNA-binding domain in the short isoform of securin leads to its inability to affect expression of some critical cell cycle regulators, for example MYC and TP53. Our preliminary data suggest that short isoform has transcriptional effect on a distinct set of genes. We propose that this functional dissimilarity from the major isoform mainly stems from the difference in their expression throughout the cell cycle. This work is supported by the Program of fundamental research for state academies for 2013–2020, research topic 01201363823.

P.10-076-Mon
Effect of *Acorus calamus and Apium graveolens* extracts on Egfr and Erbb2 in LNCaP cells

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Prostate cancer is one of the most commonly diagnosed cancers in men of developed Western countries. Several treatments are available for treatment of prostate cancer. These include surgery, radiation, radioactive implants and hormonal therapy. However, the treatment often impacts the quality of life due to side-effects or complications. Thus, numerous investigators have focused on discovering novel drugs or treatments. Among all the agents tested, natural products derived from medicinal plants are among the most favorable. Migration and invasion of cancer cells is regulated by multiple pathways that employ various growth factor and their receptors, integrins and cytoskeletal elements. A key role is played by the EGF receptor (EGFR), which, following interaction with the integrin a6b4, promotes cell migration through activation of PI3K and other downstream pathways. The androgen receptor can be activated indirectly by growth factor receptors, mainly the transmembrane tyrosine kinases EGFR and ERBB2, members of the epidermal growth factor receptor (EGFR) family, which are activated inappropriately in many human cancer types. It has been reported that the EGF receptors EGFR (HER-1) and ERBB2 (HER-2/neu) are overexpressed in metastatic prostate tumors. In this study, we investigated the effect of A. Calamus and A. Graveolens extracts on Egfr and Erbb2 on the human prostatic carcinoma cell line LNCaP. LNCaP cells were treated with increasing concentrations of an ethanolic extract of A. graveolens ranging from 1000 to 3000 μg/ml, A. Calamus 250 to 750 μg/ml and viability was determined after 24 and 48 h using the XTT cell proliferation assay. The levels of EGFR and ERBB2 were analyzed. Finally, quantitative gene expression analysis of EGFR and ERBB2, was performed using real-time reverse transcription– polymerase chain reaction. As a result, we observed that A. calamus and A. graveolens extracts affected EGFR and ERBB2 levels in LNCaP cells.

P.10-077-Tue
Apium graveolens extract induces apoptosis via Bax and p-53 proteins in the LNCaP human prostate cancer cell line

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Apium graveolens (A. graveolens) have shown significant antiproliferative and apoptotic properties in various types of cancer cells, including prostate cancer cells. However, the mechanism by which *A. graveolens* induces apoptosis is not completely understood. In this study, LNCaP cells were treated with increasing concentrations of an ethanolic extract of *A. graveolens* (1500 μg/ml, 2000 μg/ml and 2500 μg/ml) and viability was determined after 24 and 48 hr using the XTT cell proliferation assay. The levels of cleaved poly (ADP-ribose) polymerase (PARP), one of the best biomarkers of apoptosis, were analyzed. Finally, protein content of Bcl-2, Bax and p53 were measured. We found that *A. graveolens* extract inhibited the proliferation and viability of human prostate cancer LNCaP cells via induction of apoptosis. LNCaP cells showed clear apoptosis within 48 h after *A. graveolens* (2000 and 2500 μg/ml) treatment. We observed no changes in Bcl-2 protein levels when increased Bax and p53 protein levels 48 h after *A. graveolens* (2000 and 2500 μg/ml) treatment. These results indicate that the induction of apoptosis by *A. graveolens* may be mediated by induction of proapoptotic mechanism rather than suppression of antiapoptotic mechanisms.

P.10-078-Wed
Characterization of the ubiquitin ligase for S6K2 may overcome apoptotic barrier for breast and lung cancer cells

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The younger member of S6 Kinase family, S6 Kinase 2 (S6K2) was displayed to have anti-apoptotic roles in breast and lung cancer cells. S6K2 was shown to complex with anti-apoptotic proteins BRAF and protein kinase C-epsilon (PKCε) in Fibroblast Growth Factor (FGF-2)-stimulated non-small cell lung cancer cells (NSCLC). This complex helps NSCLC cells to evade apoptosis [1]. In another study, S6K2 was displayed to use an indirect way to escape from apoptosis in HEK 293T cells. S6K2 phosphorylates and therefore degrades PDCD4, a tumor suppressor protein. The disappearance of PDCD4 yields to the translation of anti-apoptotic proteins Bcl-XL and XIAP [2]. Two studies by Basu and Sridharan uncovered the role of S6K2 in breast cancer cell survival. S6K2 downregulation inactivates Akt in MCF-7 cells and decreases the level of Mcl-1 in T47D cells. Therefore, apoptosis of MCF-7 and T47D cells induced by TNF and TRAIL, respectively, is stimulated. In the former study, S6K2 was displayed to inactivate Bid, a proapoptotic protein as well [3,4]. All the aforementioned studies point out the importance of regulation of steady-state level of S6K2 in a cell. The acetylation of S6K2 by

the acetyltransferase p300 contributed to the stability of S6K2 protein [5]. Although ROC1 is known to be the ubiquitin ligase for S6K1 [6], the ubiquitin ligase for S6K2 still remains to be discovered. Therefore, characterization of the ubiquitin ligase responsible for S6K2 degradation promises a very important potential in induction of apoptosis in breast and lung cancer cells. A large-scale interactome analysis of S6K2 by Pavan et al. [7] reveals that TRIM25 might be a candidate for the ubiquitin ligase of S6K2. We aim to search and find out the ubiquitin ligase of S6K2 to overcome the apoptotic barrier for breast and lung cancer cells.

P.10-079-Mon
Specific effectors of topoisomerase II poisons contribute to cytotoxicity in AML cell lines

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The use and efficacy of topoisomerase II (TOP2) poisons, such as anthracyclines and epipodophyllotoxins, is constrained by indiscriminate toxicity towards normal cells and by the resistance of cancer cells. There is a need for drugs with similar efficacy but higher specificity. To identify targets of such drugs, we first investigated the relevance of pre-treatment basal gene expression levels (GEL) in response to the epipodophyllotoxin etoposide in 11 AML (Acute Myeloid Leukemia) cell lines. We identified both genes positively correlating (assisting) and negatively correlating (impeding) with the etoposide IC_{50}. The impeding genes BIRC7, ESRRB and PARP9 exerted effects synergistic with etoposide, as demonstrated using inhibitors. Furthermore, gene induction predominated among etoposide-evoked gene expression changes (GEC) in these AML cell lines. By integrating public resources Project Achilles and Connectivity Map, we predicted 111 GEC essential for AML cell survival. Out of the five essential GEC investigated in more depth, BCL2A1, IGF1R and PLK1 exerted cytotoxic effects when targeted with inhibitors. We also identified genes, modulation of which results in GEC either similar (TOP2-like targets) or opposite (TOP2-contrary targets) to those evoked by etoposide. Functional validation using inhibitors revealed effects synergistic with etoposide for TOP2-contrary targets ROCK1 and HDAC9. Altogether, these results demonstrate that pre-treatment GEL and drug-evoked GEC of some genes contribute to the cytotoxic effects of etoposide. These genes may serve as targets for etoposide dose reduction, or for overcoming resistance to this drug. This approach is applicable to other anticancer drugs interfering with DNA integrity and gene expression.

P.10-080-Tue
Serine phosphatases of the CTDSP/SCP family show tumor-suppressing activity for kidney tumors: bioinformatic approach, study of clinical samples and experiments in vitro

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Proteins of the CTDSP/SCP (Small C-terminal domain Phosphatase) family, which include CTDSP1, CTDSP2, and CTDSPL (CTDSP1/2/L), are involved in a variety of cellular and biological processes including cell proliferation, differentiation, migration and apoptosis. They play biological roles that are related both to tumor suppression and progression. Bioinformatic analysis of high-throughput sequencing data generated by The Cancer Genome Atlas project (TCGA) using original CrossHub software revealed that expression of *CTDSP1/2/L* genes is often reduced in tumors as compared to normal tissues. This data was confirmed in the study of clinical specimens of clear cell renal cell carcinoma (N = 32, paired samples) using qPCR, which showed frequent and significant down-regulation of *CTDSP1/2/L* genes. For further analysis, protein-coding DNA sequences of the genes *CTDSP1/2/L* were cloned into the pT2/HB vector and transfected into Caki cells by electroporation together with the plasmid pSB100 encoding the Sleeping Beauty transposase. Caki is a human clear cell renal cell carcinoma line that displays epithelial morphology and grows as an adherent culture. These cells are a useful preclinical model to study renal cancer. Exogenous expression of *CTDSP1/2/L* inhibited ($P \leq 0.01$) growth of renal cancer cells (Caki) *in vitro*. Proportion of cells in the G1 phase of cell cycle was increased. The size of the transfected cell colonies was decreased in most cases, the cells became enlarged and less mobile. A partial mesenchymal-epithelial transition was observed. These findings demonstrate that *CTDSP1/2/L* genes can play the role of tumor-suppressors in renal cancer. Study of cell growth suppression was supported by the Program of fundamental research for state academies for 2013–2020 years (№ 01201363823, № 01201363819) and gene expression analysis was supported by grant 14-50-00060 of the Russian Science Foundation.

P.10-081-Wed
Salivary squamous cell carcinoma antigen 1 and 2 in oral cancer patients: a pilot study

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According to the World Health Organization, oral cancer (OC) is the eighth most common cancer in the world, with a five-year survival rate of 50%. Squamous cell carcinoma antigens (SCCA) are part of the family of inhibitory serine protease inhibitors (serpins), involved in inflammation, apoptosis, cell migration and invasiveness and expressed in the squamous cell epithelium. SCCA2 protein is 92% homologous to SCCA1, however these antigens are not expressed concurrently and play different roles in the human body. Apart from inactivation of certain enzymes, their role in the healthy and malignant tissues is not clearly understood. The origin of SCCA antigens in serum and body fluids remains unclear. Unstimulated whole saliva (UWS) and stimulated whole saliva (SWS) was sampled from 10 patients with OC (2 female, 8 male) and 15 control subjects (6 female, 9 male). Only patients with histologically diagnosed oral squamous cell carcinoma were included in the study. Sandwich human SCCA1 and SCCA2 ELISA Kits, My BioSource, San Diego, USA, were used. Respondents' alcohol consumption, papilla bleeding index (PBI), smoking, drug consumption and medical condition was registered. This research was funded by the Croatian Science Foundation (IP-2014-3796). Salivary SCCA1 was significantly higher in OC patients, both in UWS and SWS (Mann Whitney U test, $U = 14$, $P = 0.0004$). Although no statistically significant difference in SCCA2 levels in UWS and SWS between the OC and control group was found ($U = 43$, $P = 0.081$), the average value in the control group was 895.92 pg/ml, while in OC patients

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319,45 pg/ml. Due to the small sample size, smoking, PBI, medical conditions, drug and alcohol consumption had no statistical significance. Salivary SCCA1 could serve as a satisfactory biomarker for OC. However, a greater sample is needed to establish the diagnostic value of the investigated biomarkers. To our knowledge, SCCA1 and SCCA2 have not yet been measured in the saliva of OC patients.

P.10-082-Mon
Oncogenic potential of Wip1 and its role in modulation of DNA damage response in human non-hodgkin lymphoma (hNHL)

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Wild-type p53-induced phosphatase 1 (Wip1/PPM1D), is a serin/threonine phosphatase induced upon DNA damage in a p53-dependent manner. Upon induction, Wip1 dampens the cellular stress responses by dephosphorylating multiple proteins including p53, Chk1, CHK2, H2AX, ATM/ATR. Wip1/PPM1D is overexpressed, amplified and mutated in human solid tumors hence displaying typical oncogenic properties. However, in hematological cancers, so far no data has shown whether Wip1 has an oncogenic potential. Thus, we evaluated the role of Wip1 in modulation of cellular stress responses as well as its oncogenic potential in human NHL cell lines and specimens, respectively. Therefore, human FFPE NHL and non-cancer lymphoid tissue as well as cell lines were assessed for mRNA and protein levels of Wip1 by qRT-PCR, WB and immunohistochemistry analysis. PPM1D locus was analysed by Taqman gene copy number assay. PPM1D exons were amplified by PCR and subjected to sequencing analysis. Apoptosis was measured by Annexin V and Caspase3/7 assays. RNA interference was used to target Wip1. Here we show for the first time Wip1 overexpressing NHL cell lines maintain resistance to cell cycle arrest and apoptosis through decreased phosphorylation of ATM, ATR, Chk1, Chk2 and p53 in response to etoposide or doxorubicin treatment. Specific knock down of Wip1 significantly induced phosphorylation of Chk1, Chk2, p53, ATM and increased apoptosis in cell lines expressing wt p53. Moreover, Wip1 is overexpressed and amplified in human NHL specimens. Overexpression of Wip1 was independent of p53 status of tumors. Further, heterozygous mutations were detected within exon 6 of Wip1 gene in particular in diffuse large B cell lymphoma. Hence, Wip1 may have an oncogenic potential in hematological cancers in particular in NHL which may play an important role in the initiation, progression or therapeutic outcome of the disease. (This work is supported by TUBITAK Gr. No. 214S200 to MKE).

P.10-084-Wed
A link between alternative splicing regulation and the efficacy of cancer treatment

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Dysregulation of pre-mRNA splicing often occurs in cancer cells and many studies report about an interplay between alternative splicing and cancer resistance to chemotherapy. However, most of these studies were focused on the functions of specific isoforms rather than global changes in the transcriptome. In our study, we revealed that different types of chemotherapy lead to similar changes in alternative splicing. Moreover, the most pronounced alternative splicing differences after exposure to various types of chemotherapy were observed for intron retention of splicing-related genes. To reveal the mechanism underlying this effect, we analyzed changes in gene expression, abundance of proteins, phosphorylation level after exposure of γ-irradiation, hypoxia and 10 different types of chemotherapeutic drugs. After treatment of cancer cells with the various type of chemotherapies, we demonstrated a decrease the number of spliceosomal proteins by multiple mechanisms, such as intron retention, reduced gene expression, phosphorylation, and extracellular export. We conclude that a decrease of active spliceosomal protein levels in response to stress is aimed at cell cycle arrest. We demonstrated a strong interplay between splicing- and cell cycle-related genes in stressed cancer cells at the expression and processing levels. Disturbance of the splicing components by these processes can benefit the survival of cancer cells after chemotherapy. We showed that this mechanism can be inhibited by Pladienolide B, which significantly increased the sensitivity of cancer cells to cisplatin treatment. We suggest that Pladienolide B is a potential therapeutic drug improving the efficiency of cancer therapy. Also, we found that dysregulation of pre-mRNA splicing in splicing-related genes is evolutionary conserved and could be observed in other organisms. This work was supported by the Russian Science Foundation 17-75-20205.

P.10-085-Mon
Hsp70-GAPDH complex is a potential target for rat glioma therapy

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Hsp70 protein carries a protective function in the cell. It increases the resistance of cells to stress of different types, including suppressing toxic effects of anticancer drugs. In cancer cells, Hsp70 is able to bind damaged proteins and peptides and take part in ubiquitination processes. Its other function – binding proteins involved in apoptotic signaling. One of the proteins sensitive to oxidative stress in the cell is the enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH); this protein is capable of forming insoluble intracellular aggregates and induce apoptosis. According to our earlier data Hsp70 is able to bind GAPDH. We hypothesized that in oxidative stress conditions Hsp70 can increase cell survival by binding to oxidized GAPDH. Work was carried out on the rat glioma cell line C6. For the analysis of GAPDH aggregates we used confocal microscopy and filter trap assay. The interaction of Hsp70 and GAPDH was monitored by enzyme linked immune sorbent assay and immunoprecipitation. Toxic effects of oxidative stress was evaluated using the CytoTox

96 kit (Promega). Using rat glioma cell line C6, we have found that in response to oxidative stress intracellular GAPDH denatures and cells die. Using tests developed in our laboratory, we found that Hsp70 can bind to oxidized GAPDH in ATP-dependent manner. Varying the level of *hsp70* expression by triptolide (expression inhibitor) or U133 (a derivative of echinochrome, expression inducer) demonstrated that the number of GAPDH aggregates and cellular resistance to oxidative stress depends on intracellular Hsp70 level: the greater the Hsp70, the smaller aggregates of GAPDH and less mortality. These results suggest that to reduce the resistance of cancer cells to oxidative stress it is advisable to use chemical preparations – inhibitors of Hsp70 synthesis. This work was supported by grant of Russian Science Foundation № 14-50-00068.

P.10-086-Tue
Construction of an apoferritin nanoparticle bearing ellipticine and a study of release of this drug from the nanocarrier

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Ellipticine (Elli) is an anticancer agent exhibiting high efficiencies in antineoplastic action. There are, however, several phenomena that can cause a limited usage of ellipticine and/or its limited anticancer efficiencies. Because of its adverse effects, novel methods of targeting the drug to cancer cells are being investigated. Nanocarriers are a suitable platform for this specific delivery. Apoferritins (APO), which are responsible for the storage and transfer of iron, can provide these much needed properties. APO protein subunits assemble to form a hollow cage into which diverse substances, such as drugs, can be placed. It was shown that while disassembled, APO can be mixed with drug molecules and they are encapsulated within APO cavity once reassembled. In this study, the simple-to-use encapsulation protocol to construct naturally found protein nanocarrier apoferritin encapsulating ellipticine (creating a nanocarrier construct ApoElli) was developed and the prepared nanocarrier was characterized. The visualization of nanocarrier prior to removal of released ellipticine was performed using transmission electron microscopy (TEM). The nanocarrier exhibits narrow size distribution suggesting to be suitable for entrapping of the hydrophobic molecule of ellipticine. Ellipticine is gradually released from its ApoElli form into the water environment under acidic pH; more than 80% ellipticine was released after 48 hrs incubation at pH 6.5 at 37°C. In contrast, ApoElli is stable after its storage at physiological pH (7.4) up to 1 month at 4°C; less than 5% ellipticine was released after this storage. Furthermore, the prepared ApoElli nanocarrier is capable of the interaction with microsomal subcellular particles resulting in its release and subsequent oxidation by microsomal cytochromes P450 to its hydroxylated metabolites. This nanoparticle form of ellipticine is considered to be promising tool to be used in cancer treatment. Supported by GACR 17-12816S.

P.10-087-Wed
The dose-dependent effects of brassinin on apoptosis in SW480 colorectal cancer cells through MMP-9 related pathway

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Brassinin (BSN) is an essential indole phytoalexin first identified as a constituent of Chinese cabbage. Studies have reported significant roles of brassinin including chemopreventive, antiproliferative, antifungal and anticarcinogenic activities against lung carcinoma. Furthermore, brassinin showed strong antiproliferative effects on several human cancer cell lines. The present study aimed to evaluate the cytotoxic and apoptotic effects of brassinin in SW480 human colorectal cancer cells through MMP-9 related pathway. Cytotoxicity of the compound was determined by MTT test and apoptotic effects were evaluated by annexin V binding and fluorescence imaging methods. The alteration in mRNA expression of MMP-9 in brassinin treated cells was measured by real-time PCR method. Results showed that brassinin significantly induces cell death at concentrations of 200 μM and 50 μM following 24 and 48 h of treatment, respectively. According to cell cycle analysis, brassinin induced a cell cycle arrest at G0/G1 level. Apoptosis detection assays also showed that brassinin displays a potential apoptotic effect on SW480 cells and it correlates with the decrease of enzyme activity and mRNA expression of MMP-9 protein, which has an important role on metastasis of cancer cells. Taken together, these results confirm that brassinin has a significant apoptotic and anti-metastatic activity against SW480 colorectal cancer cells and could be considered as a potential therapeutic candidate.

P.10-088-Mon
Cobalt(II) complexes with various ligands express different cytotoxic activity

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Demand for effective new anti-tumor agents is one of the major challenges facing modern biomedicine and medical chemistry. Particulary promising in this respect are the compounds of essential metals that act as biological response modifiers, such as cobalt. The aim of our study was to evaluate the cytotoxic activity of ten Co(II) complexes with various ligands: i) Mannich bases N,N'-bis (4-antipyrylmethyl)-piperazine (BAMP) and N,N'-tetra-(antipyryl-l-methyl)-1,2-diaminoethane (TAMEN) – Group A; ii) Schiff bases derived from condensation reactions between o-vanillin and S-tyrosine, L-threonine, DL-tryptophan or L-serine – Group B; monensic acid – Group C. The following cell lines served as model systems in our investigations: human MCF-7 (luminal A type breast cancer), HepG2 (hepatocellular carcinoma), 8MGBA (glioblastoma multiforme); rat – LSR-SF-SR (sarcoma induced by SR-RSV) and chicken – LSCC-SF-Mc29 (hepatoma induced by the myelocytomatosis virus Mc29). The compounds were applied

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at a concentration range of 1–200 μg/ml (Group A), 1–400 μg/ml (Group B) and 1–25 μg/ml (Group C). Their influence on cell viability and proliferation was determined in short-term (24–72 h, with monolayer cell cultures) and long-term (20–40 days, with 3D cancer cell colonies) experiments by MTT test, neutral red uptake cytotoxicity assay, double staining with acridine orange and propidium iodide, Comet assay and 3D colony forming technique. The results obtained revealed that: i) the investigated compounds reduce to varying degrees the survival and proliferative activity of the treated cells in a time- and concentration- dependent manner; ii) Co(II) complexes with BAMP express higher cytotoxicity than Co(II) complexes with TAMEN. Tested independently BAMP and TAMEN do not significantly inhibit the growth of the treated cells as compared to the control; iii) the most promising cytotoxic/cytostatic agent has been found to be the Co(II) complex of monensic acid $[Co(Mon)_2(H_2O)_2]$.

P.10-089-Tue
A functional approach for searching biomarkers of glioblastoma multiform

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Trends in developing biomarker for tumors, including gliomas, are bifurcated into following directions. Firstly, studying differential expression of genes in tumors versus corresponding normal tissues. Secondly, expert studying of expression of particular genes selected according to presumed function in glioblastoma multiform (GBM) development and malignancy. Neural cancer stem cells (CSCs) or neural/glial cancer progenitor cells (CPCs) are developing into different glioma grades: diffuse astrocytoma (II), anaplastic astrocytoma (III), primary and secondary glioblastomas (IV). Series of surgical specimens with different clinical cases were analyzed by RT-qPCR. GBM is built by hierarchies developed by tumorigenic GSCs, which are control by Notch, Wnt, BMP, TGFb, RTK pathways. We have studied expression of some of these genes in progression of astrocytomas and GBM; in particular, growth factor receptors EGFR, PDGFR, FGFR, Notch; members of signaling pathways, including cytoplasmic and nuclear intermediates (MELK, MAP, TUBB3, GFAP and CDK4, CDK6, MDM2, GAPDH); and transcription factors (OLIG2, SOX2, OCT4, NANOG). Some marker of SCs and neural differentiation were also included (CD133). The level of expression of egfr was increased in the majority of GBM compared to other grades. The rate of expression of pdgfr was fairly similar; though pdgfr expression level was lower. Both genes could be developed to diagnostic markers. Expression level of transcription factors (sox2, oct4, nanog) involved in maintenance of stemness was increased in all samples, suggesting relationship between stemness and GBM progression. The initial steps for aptamer applications to study EGFR functioning has been taken. This research has been supported by grants 17-00-00157/17-00-00160/17-00-00162 (K) from RFBR.

P.10-090-Wed
Flavin mononucleotide as a photosensitizer for melanoma treatment

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Melanoma is one of the most aggressive types of tumors, often associated with high metastatic ability, resistance to chemotherapy, and poor prognosis. Since melanoma is a skin cancer which is easy to irradiate, photodynamic therapy (PDT) is a promising strategy to cure melanoma with minimally invasive therapeutic procedure. In the current research, we propose water-soluble flavin mononucleotide (FMN), which is an active form of riboflavin, as a photosensitizer capable to treat melanoma. For this purpose, a panel of melanoma cell lines was evaluated in terms of FMN accumulation and phototoxicity both *in vitro* and *in vivo* conditions. It was found, that FMN uptake by metastatic human melanoma cell lines Mel MTP, Mel IL, and Mel Z was 2- and 5-fold higher than that of the control human fibroblasts Bj-5ta and keratinocytes HaCaT, respectively. Human melanoma A-375 and murine melanoma M-3 cells demonstrated moderate FMN uptake (3-fold higher than that of HaCaT). The FMN phototoxicity *in vitro* was induced by 450-nm light exposure at the dose 5 J/cm^2 followed by 24 h incubation. It was found, that phototoxicity was in a good correlation with FMN uptake, and IC_{50} level was 28.5, 24.5, and 103.0 μM for Mel MTP, Mel IL, and HaCaT, respectively. Additionally, the ROS level (100 μM FMN, 450-nm light exposure, 5 J/cm^2) in Mel IL cells was 3.5-fold higher compared to that of M-3 cells. The in vivo FMN-induced PDT efficacy was assessed by measuring the tumor sizes in bearing mice. Melanoma xenografts were treated intravenously with 2 mM FMN and incubated for 1 h, followed by irradiation with a 450 nm of the dose 5 J/cm^2 for 15 min. The tumor growth inhibition was estimated as 85% of volume for A-375 and 89% of volume for Mel IL on the day 50 after PDT treatment. In summary, FMN can be proposed as a promising agent for melanoma PDT treatment. This scientific work has been supported partly by Grant RSF No. 16-13-10528 and partly by Grant RFBR No. 17-00-00122 (K) (17-00-00118).

P.10-091-Mon
High-impact mutations in carotid paragangliomas

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Carotid paragangliomas are rare neuroendocrine neoplasms of head and neck. Molecular genetic study is essential to better understand the mechanisms of their initiation and progression. In

this work, the exome sequencing of 52 carotid paragangliomas was performed. Exome library preparation was carried out using the Nextera Rapid Capture Exome Kit (Illumina, USA). Sequencing was performed on NextSeq 500 System (Illumina) with 2 × 75 bp paired-end reads at EIMB RAS "Genome" center (http://www.eimb.ru/rus/ckp/ccu_genome_c.php). Mutations were called using freebayes software including filtration of identified variants with vcffilter from vcflib. SnpSift of snpEff program package was used for annotation of variants. Online resources such as ClinVar, SIFT, PolyPhen-2, dbSNP, COSMIC, 1000 Genomes Project, ExAC, and others were applied for variant assessment. We identified 43 high-impact mutations in eight genes (*BRAF, IDH1, MEN1, RET, SDHAP2, SDHB, SDHC*, and *SDHD*) in carotid paragangliomas. These mutations have a major negative impact on protein function. We found splice site mutations in *SDHAP2* and *SDHB*, frameshift variants in *SDHC* and *SDHD*, stop-gain mutations in *SDHB*, *SDHC*, and *SDHD*, mutations affecting protein structure and function in *BRAF*, *IDH1*, *MEN1*, and *RET* genes. Two mutations in *SDHC* (NM_003001: c.409delT, p.(Trp137 fs) (chr1:161332121)) and *SDHD* (NM_003002: c.205G>T p.(Glu69*) (chr11: 111959626)) genes were found for the first time; they have not been described in any databases. All of mutations detected are indicated in different online resources as pathogenic/probably pathogenic or predicted the same by predictor tools. Thus, we identified potential causal genes and variants that are associated with carotid paragangliomas. This work was funded by the Russian Science Foundation, grant 17-75-20105.

P.10-092-Tue
Withdrawn

P.10-093-Wed
Epigenetic effect of vincristine on miR-9-3 and miR-335 genes in gastric cancer cell lines

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The aberrant DNA methylation of a promoter region is an important epigenetic mechanism for gene silencing in cancer. The DNA methylation is reversible; gene expression can be restored by demethylation agents such as decitabine (DAC). The hypermethylation of miRNA genes leads to downregulation of tumor suppressor miRNAs during the pathogenesis of gastric cancer. It has been reported that miR-335 and miR-9-3 have low expression and aberrant methylation status in gastric cancer. Vincristine (VCR), which is used therapeutically in many types of cancer, functionally blocks tumor growth by blocking microtubules in mitotic spindles. Therapeutic use of VCR may suggest that this agent activates tumor suppressor genes or inhibits proto-oncogenes due to the gene methylation. VCR has been shown to increase methylation in lung adenocarcinomas, but there is no study about the epigenetic effect of VCR on DNA methylation in gastric cancer cells. Furthermore, it is not known whether the effect of VCR on DNA methylation is gene specific in any cancer. Our methylation-specific PCR and qRT-PCR results showed that VCR increases methylation of miR-335 gene and consequently decreases the expression level of pri-miR-335 in AGS cell line. On the other hand, VCR decreases methylation of miR-335 gene and hereby increases the expression level of pri-miR-335 in MKN45 cell line. VCR has been demonstrated to increase demethylation in the miR-9-3 gene and enhance the expression of pri-miR-9-3 in both cell lines. The expression level of the both pri-miRNAs and its methylation status are varies depending on the time. Our findings showed that the effect of VCR on methylation is miRNA gene-specific which was related to pri-miRNA expression levels in gastric cancer. Therefore, epigenetic-based therapeutic approach using VCR can be attractive and, our results may provide critical information for the development of sensitive drug therapies for gastric cancer.

P.10-094-Mon
POU2F1(Oct-1) isoforms contribute to hematopoiesis in human and the variation in the structure of their N-terminal parts results in different patterns of genes regulated by them

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The multiplicity of functions fulfilled by the transcription factor POU2F1(Oct-1) may be accounted for by the existence of isoforms which result from the transcription from alternative promoters and alternative splicing. We have studied the expression of the Oct-1 isoforms differing by their N-terminal parts: Oct-1A

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(GenBank: NM_002697), Oct-1L (AY113189), Oct-1X (KT438684.1), Oct-1Z (AK091438.1) during the hematopoiesis using human hematopoietic progenitor cells (HPCs) ($CD34^+$), B-cells ($CD19^+$) and T-cells ($CD3^+$), and monocytes ($CD14^+$). Using RT-PCR, we revealed high levels of Oct-1A,L mRNAs, and low level of Oct-1X mRNAs in the HPCs. The differentiation of HPCs to B and T cells and monocytes led to an increase in Oct-1X and Z levels. The dramatic decrease in the Oct-1L mRNA levels was observed in T cells and monocytes. Oct-1 isoform expression levels also changed during the *in vitro* differentiation of the HL-60 cells to granulocytes induced by DMSO. The differentiation of HL-60 caused a decrease in the mRNA levels for the Oct-1A,X,Z, as well as for the surface antigen $CD14^+$, while it led to a considerable increase in the Oct-1L expression level. In such a way, both *in vivo* and *in vitro* differentiation of cells is associated with the dynamic changes in the Oct-1 isoform composition and relative ratio. We have shown that the variation in the structure of N-terminal parts of the Oct-1 isoforms results in the difference in the patterns of genes regulated by them. Our findings indicate that during hematopoiesis, Oct-1 isoforms show cell type-specific expression, and the regulatory interplay between the Oct-1 isoforms contributes to hematopoietic cell differentiation. The work was supported by the Fundamental Research Program for State Academies for the years 2013–2020 (№ 01201363822), the Russian Science Foundation (№ 14-15-01032-P) (Oct-1 expression in hematopoiesis and microarrays).

P.10-095-Tue
Novel photosensitive conjugates as a tool for multimodal therapy

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Currently, there are many methods to treat cancer, the most utilized of which is chirurgical dissection of a tumor tissue, chemotherapy, and radiotherapy. It has been shown that the most effective treatment method is a combination of several approaches, so-called multimodal therapy. In this study, we have employed this principle of dual/multimodal effect in the development of a cytotoxic agent combined with photosensitizer (PS) for photodynamic therapy (PDT) of cancer. PDT belongs among noninvasive methods of cancer treatment. Its principle lies in photoactivation of PS in the presence of molecular oxygen. After that, reactive oxygen species are generated, which leads to the cell death. in order to reach this goal, we prepared a series of conjugates of iodo-BODIPY will colchicine, which is the inhibitor of microtubule polymerization leading to cell cycle arrest in the G2/M phase followed by apoptosis. Using live-cell fluorescent microscopy, we found that the newly synthesized conjugates localized predominantly in endoplasmic reticulum od MCF-7 and PC-3 cells. Further, we determined cyto- and phototoxicity of the conjugates as well as of pure colchicine and pure BODIPY/iodo-BODIPY in a panel of human cell lines. Any of the conjugates did not exhibit significant toxicity (without illumination) in cancer cell lines of LNCaP, PC-3, U-2 OS, HeLa, MiaPaCa-2, and MCF-7 up to 1 μM concentration. An iodo-BODIPY conjugate was very potent in terms of phototoxicity upon illumination with the light of 450–800 nm (the light dose of $4 J \cdot cm^2$). After photoactivation, the iodo-BODIPY conjugate resulted in a 50% decrease in cell proliferation already at 5 nM concentration for HeLa cells and 10 nM for U-2 OS and PC-3 cells. This derivative was also able to arrest the cell cycle in G2/M phase in HeLa cells. The high phototoxicity and low dark toxicity make the newly prepared colchicine-iodo-BODIPY conjugate a suitable candidate for use in PDT and an effective tool for multimodal therapy.

P.10-096-Wed
The investigation of antimetastatic effects of colchicine through MMP-9 downregulation in MCF-7 breast cancer cells

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Colchicine is an alkaloid agent and it's been clinically used for a long time because of its microtubule destabilizer effect. Beside its strong binding capacity to tubulin to perturb the assembly dynamics of microtubules, colchicine may also increase cellular free tubulin and limit mitochondrial metabolism in cancer cells. Since the use of colchicine is limited because of its toxicity, the studies targeting to demonstrate the significant effects of the drug at lower doses gain importance in cancer investigations. This study is planned to evaluate the effects of colchicine on proliferation, apoptosis and metastasis of MCF-7 human breast carcinoma cells. In this study, proliferation of MCF-7 cells was determined by MTT assay. Annexin V binding, fluorescence imaging studies and multi-caspase assay were performed to evaluate apoptosis. The expression levels of MMP-9 as a metastatic enzyme were measured by real-time PCR. We found colchicine significantly inhibited cell proliferation at 10 μg/ml and higher concentrations when compared to nontreated control ($P < 0.001$) and it has induced cell cycle arrest at G2/M phase. Colchicine has also induced apoptosis of MCF-7 cells by increasing Annexin V binding and caspase levels in a dose dependent manner. There was a significant decrease in IκBα levels in colchicine treated group, whereas we didn't find any difference in the expression of NF-κB protein among treated and nontreated groups. Colchicine induced the downregulation of MMP-9 protein in a dose dependent manner. This study indicates that colchicine has promising effects on MCF-7 cancer cells at lower concentrations and may be a candidate compound for the treatment of metastatic breast cancer.

P.10-097-Mon
Requirement of the IRE1-XBP1 axis for long-term hypoglycemia-induced reversible resistance to anticancer therapies

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Cancer cells survive in harsh microenvironments that are influenced by various factors including pH, hypoxia and nutrient deprivation. Emerging evidence suggests that hypoxia is a cause of resistance of cancer cells to various anticancer therapies. Despite the accumulation of new knowledge, it is still unclear whether and how nutrient deficiency makes resistance to anticancer therapies. In this study, human lung adenocarcinoma cells (A549 and PC-9) were cultured in the medium containing different low concentrations of glucose. The cells which were cultured under hypoglycemic condition for more than 7 weeks became resistant to cisplatin and taxol. Interestingly, it was also observed

that the resistant cells were resistant to immunotherapy, and their migration capacity was remarkably enhanced compared with those of control group maintained under normal condition. Cisplatin-induced apoptosis was diminished in hypoglycemic condition, but the reversion to normal glucose condition only for short term periods made the cells susceptible to cisplatin-induced apoptotic cell death. Among ER stress responses, BiP-XBP1 axis was increased by long-term hypoglycemia, whereas PERK axis was decreased. Inhibition of XBP1 splicing rendered the cells susceptible to cisplatin and immunotherapy. This study demonstrated that hypoglycemia-induced cisplatin resistance is reversible and dependent upon IRE1-XBP1 pathway. This research was supported by the National Research Foundation of Korea (2016R1 D1A1A0293, 2017R1D1A1B03036008 and 2013M3A9D3045881).

P.10-098-Tue
Effect of cisplatin treatment on protein expression of vitamin D metabolizing CYP24A1 in PC3 cell line

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Vitamin D is a sterol derivative, composed of four rings and found in two common forms; ergocalciferol (D2) and cholecalciferol (D3). Vitamin D3 is produced in the skin and metabolized in liver and kidney by cytochrome P450 enzymes to its active form (1,25-dihydroxycholecalciferol). This active metabolite is then inactivated to calcitroic acid by hydroxylation with CYP24A1 enzyme. 1,25-dihydroxycholecalciferol binds to its receptor and controls expression of several genes that have roles in differentiation, proliferation, and apoptosis of cancer cells. Because of this, vitamin D deficiency is considered as risk factor for cancer development and high rates of mortality. Cisplatin is a chemotherapeutic drug used to treat wide variety of cancer types. The aim of this study is to examine the effect of cisplatin on protein expression of vitamin D metabolizing CYP24A1 on prostate cancer cell line PC3. Cytotoxicity of cisplatin was determined with Alamar Blue Assay and IC50 value was calculated. The effect of cisplatin on CYP24A1 protein expression was determined by western immunoblotting technique. Results were normalized with b-actin as an internal standard. The intensity of each band was analyzed by Image J software. IC50 value of cisplatin on PC3 cell line was found as 30 μM. CYP24A1 protein expression was significantly decreased (0.66 fold) in cisplatin treated PC3 cells with respect to control groups. The decreased protein expression of CYP24A1 in cisplatin treated PC3 cells may result in increased active vitamin D metabolite. In conclusion, due to downregulation of CYP24A1, cisplatin may have potential to prevent rapid inactivation of active vitamin D and this may improve beneficial effects of vitamin D for cancer patients.

P.10-099-Wed
European HPV DNA test external quality assurance scheme (EHEQAS)

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EHEQAS is a program of external quality control used to assess and improve the quality of laboratories in HPV detection and typing. Batches of 5–7 samples are sent from the coordinator to participants 1–2 times per year. Samples are either real patient samples or prepared from international standards. Samples that are not international standards are pre-tested by reference laboratories. To test for reproducibility, samples are used in duplicate in the same and in different rounds. Linearity is evaluated by different dilutions of the same sample in the same and in different rounds. Results are evaluated and consensus results are issued and announced to participants in a confidential way. Marks are awarded to participants based on defined rules that reflect the clinical value of the result (e.g. higher penalty for errors regarding types 16 and 18). Certificates of competence that reflect the performance of a laboratory during the past 4 years are issued. During the period 2006–2017 a total of 233 samples have been tested in 24 rounds: 65 negative, 67 single infections, and 101 co-infections. 32 different types (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 66, 68, 70, 73, 81, 82, 83, and 84) were detected. Surprisingly even laboratories using IVD tests made significant errors in HPV detection and typing, depending on the skills of laboratory personnel and on whether they correctly followed manufacturer's instructions. EHEQAS improves quality with the coordinating team providing feedback to participants on how to improve their methodology. EHEQAS assesses the quality of laboratories in: (a) detecting a shift in sensitivity and specificity over time. (b) HPV typing (high- or moderate- or low-resolution). Successful participation in EHEQAS is extremely helpful to high-quality HPV labs that also have to verify or validate their methods: success in an EQA is an absolute prerequisite for granting ISO15189 accreditation.

P.10-100-Mon
STING-related innate immunity pathways, inflammation and mediators of invasive growth: transcriptome-wide profiling of HPV-positive microcarcinoma of the cervix

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Antitumor innate immunity pathways are inherently related with angiogenesis and epithelial-mesenchymal transition (EMT), the key determinants of epithelial tumor invasiveness, via common mechanisms of inflammation and functioning of immunosuppressive cells. We aimed to study which of these mechanisms may undergo the most pronounced changes upon induction of invasive growth in virus-related cervical cancer. By means of RNA-seq, transcriptome-wide profiles of gene expression were obtained for preinvasive and early-stage invasive cervical cancer; selected markers of angiogenesis and EMT and tumor-infiltrating

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regulatory T cells (Treg) were assessed by flow cytometry of dissociated tissue samples. Most significant differences found between early invasive cancer and cancer in situ include cytosolic DNA sensor (particularly, STING) dependent responses, with noticeable up-regulation of interferon-stimulated genes and inflammatory response genes in microcarcinoma. In spite of inflammatory profile, analysis of T/NK cell-response pathways and phenotyping of Treg indicated predomination of suppressive microenvironment. Increased expression of cytokines, which besides proinflammatory or immunosuppressive properties are known to be potent mediators of lymphangiogenesis/EMT, was observed. The expression pattern of genes responsible for cell morphology and cell contacts remodeling corresponded to invasive phenotype, while flow cytometry showed high level of VEGFR3 and HGFR expression on nonimmune cells. Taken together, the triggering of processes mediated by sensors of tumor-derived DNA at early stages of cervical cancer progression, including augmentation of inflammation, may serve one of the driving forces for tumor cell invasion. This may cause the need to combine therapeutic stimulation of STING-related mechanisms with inhibition of adverse effects of inflammation and compensatory recruitment of immunosuppressive cells. The work is supported by the RSF project No. 17-15-01024.

P.10-101-Tue
Rhenium complexes of benzazole derivatives: anticancer potential

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Cancer is a complex, multifactorial disease that affects millions of individuals every year. The adverse side effects of current therapies coupled with the increased incidence of resistance to these therapies make it imperative that we explore novel treatments for the disease. The benzimidazole ring system has shown potential as a scaffold for designing novel anticancer agents. Conjugation of the metal rhenium to novel variants of this ring system open up the possibility of designing novel drugs that serve both a diagnostic and a therapeutic function. We investigated the *in vitro* therapeutic anticancer potential of ten such complexes against selected breast and cervical cancer cell lines. The selectivity of the complexes for cancer cells over normal cells was also investigated while the mechanisms of action of effective complexes were determined by exploring cell cycle arrest, biochemical markers of apoptosis and mitochondrial membrane disruption. All ten complexes were screened against MCF7 breast and HeLa cervical cancer cell lines with four showing cytotoxicity against both cancer cell lines and one showing cell line specific toxicity against MCF7 breast cancer cells. The complexes showed greater cytotoxicity against the MCF7 breast cancer cells with IC_{50} values ranging from 3.2 to 7.0 μM versus a range of 7.6 to 24.4 μM being obtained on the HeLa cervical cancer cells. Cytotoxic complexes were tested against confluent and log phase Vero cells at their respective IC_{50} values to determine their effect on "normal" cells. Confluent Vero cells showed less cell death (9.4%) than those in log phase (60.2%) indicating that the complexes show preference for proliferating cells. The mechanism of action of the complexes was studied on both cancer cell lines via cell cycle analysis and apoptosis assays investigating phosphatidylserine translocation, caspase -3 and -8 activation and mitochondrial membrane potential.

P.10-102-Wed
Downregulation of midkine enhances the efficacy of quercetin on androgen-sensitive prostate cancer stem cell survival through arresting cell cycle and inducing apoptosis

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Prostate cancer (PCa) is the second most common cancer type and is the fifth leading cause of cancer-related deaths among men. Cancer stem cell population may play a role in tumor relapse and progression of the disease, and targeting of stem cells might prevent tumor recurrence following androgen deprivation therapy. Midkine (MK) is a heparin-binding growth factor, which is overexpressed in various cancers. The aim of the study was to investigate the functions of MK and quercetin (QUER), a natural flavonoid, with a potent androgen receptor blocker enzalutamide (ENZ) on cell survival and apoptosis of PCa stem cells (PCSCs). The PCSCs were sorted from human androgen-sensitive LNCaP cells by MACS. Three-dimensional cell culture was used to evaluate the ability of ENZ, QUER or 50 pmol/ml MK siRNA and their combinations. Stem cells were treated with 40 μM of QUER or 5 μM of ENZ for 24 – 72 h. MK expression was knocked down using MK-specific siRNA. The percentage of cell cycle phase distribution, apoptotic and necrotic cell death was determined by image-based cytometer. ENZ and QUER treatment significantly inhibited stem cell proliferation in a time- and dose-dependent manner. ENZ, QUER, MK siRNA and ENZ/QUER/MK siRNA treatments reduced stem cell survival by 46%, 51%, 25% and 65%, respectively. Apoptosis caused by ENZ, QUER, MK siRNA and combinations thereof are 33%, 37%, 5% and 20%, respectively. QUER therapy increased G1 cell cycle arrest to 88% from 63% compared to the control group. However, while silencing of MK kept the cells in phase S, co-treatment of the three caused S and G2/M phase arrest. Taken together, the growth factor MK plays an important role in proliferation of PCSCs, and QUER and MK-silencing therapy can be an important strategy in targeting stem cells that play a role in aggressiveness of cancer and relapse. This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK grant no: 115S356).

P.10-103-Mon
Identification of candidate genes underlying soft tissue sarcoma progression using a progression series of murine fibrosarcoma cell lines

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Soft tissue sarcomas are known for their great variability in clinical behaviour. We established a unique single-background progression series of murine sarcoma cell lines: JUN-2, slowly

proliferating nonmotile and noninvasive; JUN-3, rapidly proliferating, motile and invasive and JUN-2fos-3, with slow proliferation, but pronounced motility and invasiveness. This made us possible to identify two separate groups of genes tentatively involved in sarcoma progression in a single transcriptomic analysis. Proliferation-related genes could be identified by their differential expression in JUN-3 compared to both JUN-2 and JUN-2fos3. Motility and invasiveness-related genes showed common expression pattern in JUN-2fos3 and JUN-3 cells compared to JUN-2. The high-throughput gene expression analysis has been performed using the GeneChip Mouse Genome 430 2.0 Array (ThermoFisher Scientific). We identified 277 up- and 212 down-regulated unique transcripts in JUN-2 and JUN-2fos3 compared to the JUN3 cells, and 29 up- and 112 down-regulated unique transcripts in JUN3 and JUN2fos3 compared to JUN2 cells (adjusted $P < 10^{-4}$). The chemokine Ccl-8 was identified as a possible druggable target responsible for sarcoma motility, as it is overexpressed in both motile cell lines and its pharmacological inhibition significantly downregulated JUN-3 cell motility. Interestingly, the scrutiny of differentially expressed genes in the motile cell lines JUN-2fos3 and JUN-3 revealed a significant downregulation of several stemness genes like Abcg2 (21x reduction) or CTGF (10x reduction). This is reminiscent of the phenomenon "go or grow", known for brain tumours. Our sarcoma cell lines could thus provide a valuable model to identify key genes responsible for sarcoma progression as well as to decipher a relationship between stemness and cancer invasion. Supported by the Czech Grant Agency project No 17-17636S and by the project of Faculty of Medicine in Pilsen (grant no. SVV-2017-260393).

P.10-104-Tue
Determining the effects of ferulic acid on apoptosis, cell cycle, invasion, migration and colony formation in MDAH 2774 ovarian cancer cell line

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Increasing understanding of the molecular biology of ovarian cancer has led to the development of numerous targeted therapies such as cellular signalling pathway inhibitors, immunotherapies, antiangiogenic molecules and new effective novel anticancer agents. Ferulic acid is a common dietary plant phenolic compound abundant in fruits and vegetables and have powerful antioxidant capacity and anti-cancer effects. The aim of this study is to investigate the potential therapeutic effects of FA on cell proliferation, apoptosis, invasion, migration, colony formation and wound-healing in MDAH 2774 human ovarian endometrioid adenocarcinoma cell line. The cytotoxic effects of FA were determined by XTT method. The mRNA expression analysis of BAX, BCL-2, BCL-XL, CASPASE-3, 8, 9, 10, BID, CYLIND1, CDK6, P53, P21, FADD, PUMA, NOXA, MMP-2, MMP-9, TIMP-1, TIMP2 was performed on Real-time PCR (RT-PCR) according to the SYBR Green qPCR Master Mix. Effects of FA on cell invasion, migration and colony formation were detected by Matrigel-chamber assay, wound-healing and colony formation assay, respectively. The statistical analysis of the findings has been made with the ΔΔCT method with RT2 Profiler PCR Array Data Analysis. IC50 value of FA in MDAH 2774 cells was detected as 150 μM at 24th hours. It was determined that FA caused a decrease in the expression of BCL-2, BCL-XL, CYLIND1, CDK6, PUMA. It is also observed that FA caused a significant increase in the expression of BAX, CASPASE-8, 9, 10, BID, P53, NOXA, TIMP-1 and TIMP2. It was also found that FA has effect in MDAH 2774 cells on suppressed invasion, migration and colony formation. In conclusion, we demonstrate that FA significantly affect cell cycle, apoptosis, invasion, migration and colony formation of MDAH 2774 cells. FA may be a potential candidate as chemotherapeutic agent for the treatment of ovarian cancer. More studies have to be performed to profile the mechanisms and genome wide effects of FA to understand its therapeutic potential.

P.10-105-Wed
Determination of metabolites and DNA adducts formed by nitro-reduction of a carcinogen aristolochic acid I *in vitro*

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Balkan endemic nephropathy (BEN) is a chronic tubulointerstitial nephropathy characterized by a gradual progression to end-stage renal disease that is similar to another renal fibrosis, aristolochic acid nephropathy (AAN). A characteristic feature of AAN and BEN is their close association with upper urothelial carcinomas (UUC). Aristolochic acid (AA), a plant alkaloid, has been shown to be the major cause of BEN/AAN/UUC development. Nitro-reduction of AAI, the major component of AA, is required to exert its genotoxicity (AA-DNA adduct formation) and carcinogenicity. Initial reduction of AAI to the corresponding N-hydroxyaristolactam is the activation pathway responsible for this genotoxic effect. During this reaction AAI is enzymatically reduced to the cyclic acylnitrenium ion that is capable of binding to the exocyclic amino groups of adenine and guanine in DNA. The most abundant DNA adduct detected in AAN and BEN patients is 7-(deoxyadenosin-N6-yl)-aristolactam I (dA-AAI) which causes characteristic AT→TA transversion mutations (e.g. in TP53 gene). NAD(P)H:quinone oxidoreductase and cytochromes P450 1A1 and 1A2 are the major enzymes reducing AAI to N-hydroxyaristolactam I, the acylnitrenium ion, the final reductive metabolite aristolactam I and forming also dA-AAI adducts. Here, we investigated formation of the reductive intermediates of AAI, the end reduction product aristolactam I and AAI-DNA adducts. Human NQO1 and hepatic cytosols rich in this enzyme were used. Whereas AA-DNA adducts measured by 32P-postlabeling were formed by both used enzymatic systems, no reductive intermediates of AAI and aristolactam I measured by HPLC have been detected. Two reasons can be responsible for the results; (i) a lower sensitivity of HPLC detecting AAI metabolites than 32P-postlabeling determining AA-DNA adducts and (ii) reactive reductive intermediates of AAI can be trapped by proteins in incubation mixtures, thereby forming AAI-protein adducts. Supported by GACR 17-12816S

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P.10-106-Mon
Investigation of the effect of flavonoid nobiletin on TLR4 signaling pathway in prostate cancer cell lines

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Toll-like receptors (TLRs) are a well-known family of pattern recognition receptors that expressed in epithelial, immune and cancer cells. Nobiletin is an O-methylated flavonoid that possesses anti-cancer properties and has been reported to reduce the risk of prostate cancer. In this study, we investigated the effects of Nobiletin on TLR4 in LNCaP, PC-3 and HUVEC cell lines. Lipopolysaccharide (LPS) was used for TLR4 stimulation. Cell viability was analyzed with the WST-1 assay. Inhibitory concentrations (IC_{50}) of Nobiletin were found 20 μM for LNCaP and 40 μM for PC-3 and HUVEC cell lines ($P < 0.05$). Gelatinase activity and protein expression were examined by zymography and western blotting, respectively. Gelatinase activity of MMP-9 and MMP-2 was found high in PC-3 and LNCaP, although there was high MMP-2 activity in HUVEC, MMP-9 activity was not observed ($P < 0.05$). Also, it was found that Nobiletin reduced TLR4 and IRF-3 protein levels in LNCaP and PC-3 and there was no significant change in the amount of these proteins in HUVEC ($P < 0.05$). TLR4 gene expression was examined by Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR). It was observed that when Nobiletin and LPS were co-treated, TLR4 gene expression decreased in LNCaP ($P < 0.05$). Cytokines (INF-α and INF-β) were analyzed with Enzyme-Linked Immunosorbent Assay (ELISA). It was found that Nobiletin reduced the amount of INF-α and INF-β in LNCaP ($P < 0.05$). It is observed that TLR4 signaling pathway is suppressed by Nobiletin only LNCaP cells. When all the results are evaluated together; the effect of Nobiletin is AR-dependent and shows a reducing effect on TLR4 signaling pathway. Conclusion, Nobiletin may be effective on prostate cancer via TLRs. And also TLR4-dependent signaling pathway with great potential may be important for new therapeutic approaches in prostate cancers.

P.10-107-Tue
Chemotherapy-induced microenvironment of ovarian cancer cells promotes tumor resistance

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Ovarian cancer is characterized by transcoelomic metastasis into the peritoneal cavity. The peritoneal malignant ascites are enriched with ovarian cancer cells and a small amount of tumor-associated immune cells (up to 5%) which create a unique microenvironment actively contributing to progression of the disease. However, it is remain unclear how cancer cells communicate to its local environment under the influence of chemotherapy. To address this issue, we performed LC-MS/MS analyses of ovarian cancer ascites from the same patients before and after chemotherapy. Moreover, we investigated how ascites before and after chemotherapy modulates the behavior of cancer cells. In general, we identified 1775 proteins in tumor ascites. Intriguingly, a large number of known intercellular signaling mediators such as cytokines, chemokines and growth factors were decreased in abundance in ascites samples after chemotherapy. These data were independently confirmed by cytokine profiling assay. Functional annotation of upregulated proteins with the use of KEGG and GO databases revealed that malignant ascites after chemotherapy were enriched with the cluster of spliceosomal proteins. These splicing factors were linked to induction of epithelial-to-mesenchymal transition leading to more aggressive phenotype of cancer cells. We showed that ascites after chemotherapy increases cell mobility, induces the change of surface markers from epithelial (E-cadherin) to mesenchymal (N-cadherin) and finely promoting cancer chemoresistance. In summary, our study uncovers previously unrecognized signaling networks in the ovarian cancer microenvironment that are of potential clinical relevance. This work was supported by the Russian Science Foundation 17-75-20205.

P.10-108-Wed
Kaempferol sensitizes human hepatoma cells to TRAIL-mediated apoptosis through inhibition of Akt activity

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Kaempferol is polyphenolic compounds that are widely distributed in fruits and vegetables. Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a promising cancer therapy that preferentially induces apoptosis in cancer cells. However, many hepatoma cells are resistant to TRAIL by mechanisms that are poorly understood. Here, we show that treatment with tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) in combination with subtoxic doses of kaempferol significantly induces apoptosis in TRAIL-resistant hepatoma cells. Western blot analysis showed that combined treatment with TRAIL and Kaempferol did not change the levels of DR5 or anti-apoptotic proteins (inhibitor of apoptosis, Bcl-2 and Bcl-xL). However, Kaempferol induced potent inhibition of Akt phosphorylation. Taken together, the present studies suggest that kaempferol enhances TRAIL-induced cytotoxicity by activating caspases and inhibiting phosphorylation of Akt. Kaempferol may be a safe strategy for treating resistant hepatomas.

P.10-109-Mon
Mitochondrial 2HG production as a function of IDH2 and HOT in breast cancer cells

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Cancer metabolic alterations result from complex genetic and epigenetic adjustments which include mitochondrial pathways glutaminolysis, reductive carboxylation (RC), 2-hydroxyglutarate

(2HG) production, and NADPH synthesis. 2HG is epigenetically-active oncometabolite which is considered to be a prospective diagnostic marker in serum and urine of the oncological patients. We studied complex mechanisms that promote anabolic function of mitochondrial enzymes isocitrate dehydrogenase 2 (IDH2) and hydroxyacid-oxoacid transhydrogenase (HOT) towards oncometabolite 2HG production in breast cancer cell lines. We demonstrate that IDH2 enzyme produces 2HG in vitro, as assumed by product analysis of enzyme reaction of isolated recombinant wild-type IDH2. Our analysis of metabolic flux shows that mitochondrial production of 2HG by wild-type IDH2 is largely dependent on mitochondrial NADPH balance, because induction of mitochondrial NADPH by dm-S-malate or expression of NADPH-producing enzyme MTHFD2 supports IDH2-dependent 2HG synthesis. In addition, we demonstrate that active interplay and competition between IDH2 and HOT for substrate (2OG) exist; overexpressing superactive mutant of glutaminase 1, which promotes 2OG production, favours HOT reaction at the conditions of low mitochondrial NADPH levels. Our findings impact our understanding mitochondrial metabolism in breast cancer etiology, since breast cancer cell lines exhibit a broad range of IDH2/HOT expression and metabolic phenotypes, including 2HG levels. Supported by Grant Agency of the Czech Republic 16-04788S to P.J. and 15-01897S to K.S.

P.10-110-Tue
Human secreted protein SLURP-1 interacts with nicotinic acetylcholine receptors to control the growth of epithelial cancer cells

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Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels expressed in various tissues and involved in key cellular processes. In tumor cells, nAChRs regulate cell proliferation, migration, invasion, angiogenesis and apoptosis. Therefore, nAChRs are promising targets for development of new anticancer therapies, and nAChR antagonists can become new effective antitumor drugs. Previously, we developed a system for bacterial production of a recombinant human protein SLURP-1, an auto/paracrine regulator of epithelial homeostasis with antagonistic properties towards nAChRs. In the present work we show that recombinant SLURP-1 at nanomolar concentrations effectively inhibits the growth of melanoma A431, lung carcinoma A549 and breast carcinoma SKBR3 cells, while has no cytotoxic effects on normal skin cells (keratinocytes Het-1A and HaCaT). All tested cancer cell lines express α7-nAChRs, and recombinant SLURP-1 colocalizes with the receptor. Inhibition of the cell growth occurs as a result of the SLURP-1 interaction with nAChRs. Exposure of cells to recombinant SLURP-1 down-regulates α7-nAChR expression and induces secretion of endogenous SLURP-1 from intracellular depot, thereby stimulating neighboring cells. The study was supported by the Russian Science Foundation (Project № 17-74-20161).

P.10-111-Wed
Elemental homeostasis in brain tumors

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The role of element homeostasis in neoplastic disease pathogenesis is beyond question. The imbalance of trace elements precisely underlies the initiation and promotion of tumor pathology via nuclear and mitochondrial DNA metabolism modulation and reparation and remodelling of the cell metabolism on the whole. The aim of the study was to investigate blood and tissue macroelements, microelements in brain tissue of healthy persons (control group) and with tumors (experimental group), as well as their intermolecular interactions with other signal proteins and factors. A significant increase (more than 7 times) of calcium concentration in brain tumor tissue and hypercalcemia were detected, compared with control group. magnesium level was also reduced in 1.7 times in comparison with healthy brain tissue. Analysis of trace elements in tumor tissue revealed their significant increase compared with the "normal" brain tissue. Iron, zinc and copper levels were 3.1, 3.2 and 10 times increased respectively. Multidirectional dynamics of changes in the levels of these trace elements were observed in blood plasma. Analysis of microelements level in blood plasma showed a significant decrease in the concentration of Cu (per 49%), Zn (2.5–5 times) and the increase in content of Fe (up to 45%). The increase of calcium concentration leads to activation of inositol-3-phosphate signalling system which is dominant for maintaining the proliferative activity of the embryonic cells. It is also been shown that hypomagnesemia suppresses reactive oxygen species (ROS)-induced HIF-1∝ activity. Detected reduced magnesium content in brain cancer cells can lead to decreasing of HIF-1∝ activation, and, probably, serve as initiating agent for pathologic proliferation development. Thus, mineral metabolism violation exactly is supposed to be the interlink between cells malignant transformation processes and metabolic disturbances in carcinogenesis.

P.10-112-Mon
Does the mitochondrial genome much more stabile in the aggressive papillary thyroid cancers (PTCs)? – The preliminary findings

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It is currently present in the literature that mitochondrial DNA (mtDNA) defects are associated with a great number of diseases including cancers. However, it is reasonably questionable that the mitochondrial genome is a "genetic sanctuary" during the oncogenic process or not. Besides, BRAFV600E mutation is associated with poor prognosis, lymph node metastasis, recurrence and tumor aggressiveness of the PTC in the literature. The aim of this study is to determine the frequency of somatic mutations in mtDNA control region (CR) at benign and malign thyroid tumors. For this purpose, totally 108 hot thyroid nodules (HTNs), 95 cold thyroid nodules (CTNs), 38 PTC samples without BRAFV600E mutation and 10 PTC samples with BRAFV600E mutation with their surrounding tissues were screened for entire

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mtDNA control region (CR) somatic mutations by using Sanger sequencing. The somatic mtDNA mutations were detected either in MtDNA microsatellite sequences or non-microsatellite sequences at 29/108 (26,85%) of the HTNs, 15/95 (13,68%) of the CTNs, 20/38 (52,63%) of the PTCs without BRAFV600E mutation and 2/10 (20,0%) of the PTC samples with BRAFV600E mutation (χ^2 test, $P = 0.0002$). Besides, none of the metastatic BRAFV600E(+) PTCs has somatic mtDNA mutation. When the mutation frequency compared among the PTC cases according to the tumor size, The somatic mtDNA mutations were found either in mtDNA microsatellite sequences or non-microsatellite sequences at 14/21 (66,66%) of the microPTCs without BRAFV600E mutation, 6/18 (33,33%) of the macroPTCs without BRAFV600E mutation and 1/9 (11,11%) of the macroPTCs with BRAFV600E mutation (χ^2 test, $P = 0.01$). These preliminary results indicates that aggressive macroPTCs have more mitochondrial genome stability than the other groups and it can be suggested that stabile mitochondrial genome might give selective advantage to aggressive macroPTCs during oncogenic process.

P.10-113-Tue
Possible regulatory partner of Beclin 1 in apoptosis/autophagy decision: BAG1

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BAG-1 (Bcl-2 associated athanogene-1), a member of BAG family proteins, interacts with various proteins and possess critical functions in apoptosis, transcription, proliferation, cell migration and in other related pathways. Its interactions with Bcl-2 contribute to the formation of homo/heterodimers to regulate cell survival and apoptosis. Major mammalian autophagy regulator protein Beclin 1's direct interaction to Bcl-2 control the autophagic cell survival and apoptotic cell death mechanisms. Here, we aimed to assess the effect of BAG-1 on Bcl-2/Beclin 1 crosstalk and to understand BAG-1's role in cancer progression upon apoptosis/autophagy regulation. Our experiments, consisting both up- and downregulation of BAG-1 showed the association of BAG-1 and Beclin 1 in BAG-1 immuno-precipitates for both wild type and BAG1 overexpressed MCF-7 breast cancer cells. Immunoblotting results further revealed the increase in turnover of LC3-I to LC3-II within 24 h in BAG-1 silenced MCF-7 and BT474 cells, which indicates the existence of autophagy, reflecting that autophagy occurs within this time interval. While the up-regulation of BAG-1 enhanced Beclin 1 phosphorylation resulting in the dissociation of Beclin 1 and Bcl-2, down-regulation of BAG-1 reduced the phosphorylation level of Beclin 1. In this case, Bcl-2 may not be the crosslink between BAG-1 and Beclin 1. Consequentially, BAG-1 might interact directly with Beclin 1 and affect the decision mechanism between cell survival/death mechanism via regulating Bcl-2-Beclin 1 switch.

P.10-114-Wed
The anti-cancer effects of vitamin D in MDAH 2774 ovarian cancer cells

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It is known that vitamin D is important for calcium absorption and bone health. However, recent studies have suggested that Vitamin D modulates cancer cell growth and epidemiologic studies suggest increasingly that vitamin D may be associated with reduced cancer risk. The aim of this study is to determine the anticancer effect of vitamin D on MDAH 2774 human ovarian endometrioid adenocarcinoma cells *in vitro* conditions and understanding therapeutic activity and its underlying molecular mechanism such as cell viability, colony formation capacity, cell migration, cell cycle and apoptosis. For this purpose in the cell culture laboratory MDAH 2774 cells which those treated with various concentrations of vitamin D, dose and time dependent inhibition of proliferation was observed by XTT assay. mRNA expression of cell cycle control, apoptosis and invasion regulated genes such as *PUMA, NOXA, MMP-2, MMP-9, TIMP-1, TIMP2, BAX, BCL-2, BCL-XL, CASPASE-3, 8, 9, 10, BID, CYLIND1, CDK6, P53, P21* were evaluated by Real Time PCR. Effects of vitamin D on cell migration and colony formation were detected by wound-healing and colony formation assay, respectively. The statistical analysis of the findings has been made with the CT method with RT2 Profiler PCR Array Data Analysis. According to RT-PCR results, vitamin D caused a decrease in the expression *of MMP-2, 9, NOXA, PUMA, BCL-2, BCL-XL, CYCLIND1, CDK6*. It is also observed that vitamin D caused a significant increase in the expression of BID, *BAX, CASPASE-3, 8, 9, 10, P53, TIMP-1* and *TIMP2*. It was also found that vitamin D has effect in MDAH 2774 cells on suppressed invasion, migration and colony formation.

P.10-115-Mon
Hypoxia induced transcriptional regulation of the N-Myc down regulated gene 1 (NDRG1) in human brain cancer

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Hypoxia induced HIF-1α-regulated genes are responsible of the tumour response resistance against radiation- and chemo- therapeutic approaches and as a consequence in the life perspective of human cancer patient. These genes are highly overexpressed during the different stages of human brain cancer especially in

Glioblastoma (GBM). One of these genes, namely, *N-myc downstream-regulated gene 1* (*NDRG1*) is a tumor suppressor with the potential to suppress metastasis, invasion and migration of cancer cells. It is regulated under stress conditions such as starvation or hypoxia. NDRG1 regulation is both induced and controlled by HIF-1α-dependent and -independent pathways under hypoxic conditions. However, there are profound differences in the way NDRG1 expression is regulated by HIF-1α and other transcription factors. Our aim was to define the time-dependent and oxygen dependent pattern of NDRG1 mRNA and protein expression in human glioblastoma cell lines in extreme hypoxia and after re-oxygenation as well as under normoxic conditions with description of the NDRG1 regulation by the transcription factors HIF-1α, SP1, CEBPα, YB-1 and Smad7, respectively, in a time-dependent manner. The human malignant glioma cell lines U87-MG, U373 and GaMG were cultured for 1, 6 and 24 h under hypoxic (0.1% O_2) conditions and then they were re-oxygenated. NDRG1, HIF-1α SP1, CEBPα, YB-1 and Smad7 mRNA expression and protein expression were analyzed. Our experiments revealed that long-term (24 h), but not short-term hypoxia led to the induction of NDRG1 expression in human glioma cell lines. NDRG1 expression was found to correlate with the protein expression of HIF-1α, SP1, CEBPα, YB-1 and Smad7. The data of our study suggests for the first time that SP1 regulates NDRG1 expression in glioma cells under hypoxia in a time-dependent manner along with HIF-1α, CEBPα, YB-1 and Smad7 in a cooperative and dynamic manner.

P.10-116-Tue
Proteomics approach reveals a possible way of involvement of exosomal proteins in breast cancer cells promotion

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Interaction between cancer cells and surrounding healthy tissues is a critical factor in regulation of breast cancer progression and metastasis. Extracellular vesicles, especially exosomes, are known to be involved in tumor-associated intercellular communication. By enzyme treatment of exosomal surface we have demonstrated that activation of motility and invasivity of breast cancer cells is mediated through direct surface interaction with plasma exosomes. Mass spectrometry analysis of these exosomes has indicated key proteins associated with exosomes surface and potentially involved in breast cancer cells promotion. Gene ontology analysis revealed that these proteins are involved in FAK signaling pathway. Taken together with observation that inhibition of this signaling pathway in breast cancer cells attenuates stimulatory effect of plasma exosomes, our results show essential role of plasma exosomes in breast cancer progression. Deeper investigation and better understanding of this phenomenon may open a new opportunity to prevention or/and therapy of breast cancer dissemination.

P.10-117-Wed
Potential effects of parietin on apoptosis and cell cycle related genes in SH-SY5Y neuroblastoma cells

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Neuroblastoma (NB) is an embryonal tumor that is derived from neural cells. Parietin has an anthraquinone chemical structure and is isolated from lichens and some plants such as "Iskın" (Rheum ribes L.). It has various pharmacological activities such as antimicrobial, antiinflammatory, hepatoprotective effect, antioxidant and anticancer properties. The aim of this study is to determine the anticancer effect of parietin on SH-SY5Y neuroblastoma cells *in vitro* conditions and elucidate therapeutic activity and its underlying molecular mechanism such as cell proliferation, invasion colony formation, cell cycle and apoptosis. The cytotoxic effects of parietin were determined by XTT method. Total RNA was isolated with Trizol reagent. The mRNA expression analysis of *BID, CYLIND1, CDK6, P53, P21, FADD, TRADD, BAX, BCL-2, BCL-XL, CASPASE-3, 8, 9, 10, PUMA, NOXA, TIMP-1, TIMP2, MMP-2 and MMP-9,* was performed on Real-time PCR (RT-PCR). Effects of parietin on cell invasion, migration and colony formation were detected by Matrigel-chamber assay, wound-healing and colony formation assay, respectively. The statistical analysis of the findings has been made with the ΔΔCT method with RT2 Profiler PCR Array Data Analysis. IC50 value of parietin in SH-SY5Y cells was detected as 35 μM at 48th hours. According to RT-PCR results, BCl-XL, BCL-2, MMP2, MMP9, P21, CYCLIND1 were found downregulated and CASPASE-3 AND 9, BAX, P53, PUMA, NOXA were found upregulated in dose group cells. It was also found that parietin suppressed invasion, migration and colony formation in SH-SY5Y cells. It is considered that parietin can be an alternative, complementary and supporting agent with other drug in neuroblastoma therapy. However, this effect of parietin should be supported by further studies.

P.10-118-Mon
Genome wide screen identifies USP28 and SPINT2 as novel factors mediating cell cycle arrest after whole genome doubling

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Growing body of evidence shows many solid tumors have undergone whole genome doubling (WGD) during their development. Tetraploid cells possessing doubled set of chromosomes are found in all stages of cancer, supporting a role of WGD in cancer evolution and progression. How exactly genome doubling events favour cancer development is not fully understood. Moreover, proliferation of newly arising tetraploids is limited by activation of the p53 tumor suppressor. We observed that less than 1% of cells were able to stably propagate after whole genome doubling. However, how tetraploids escape the p53-mediated cell cycle arrest remains poorly understood. To uncover the mechanisms contributing to the p53-dependent cell cycle arrest after whole genome doubling, we have performed an esiRNA genome wide screen in human cells. We have identified 140 candidate genes that might be involved in regulation of this cell cycle arrest. Further validation confirmed two genes that might regulate the p53 dependent arrest of tetraploid cells: USP28, coding for a

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deubiquitinase that was described to play a role in DNA damage as well as in response to loss of centrosome, and SPINT2, a tumor suppressor frequently inactivated in cancer. Depletion of these two candidates improves proliferation of tetraploid cells by independent mechanisms. Taken together, we describe novel players in regulation of cell cycle arrest upon tetraploidization, whose dysfunction may lead to tolerance of genome duplication.

P.10-119-Tue
Apoptotic effect of essential oils from cultured and wild form of *Origanum acutidens* (Hand.-Mazz.) Ietswaart. (Lamiaceae) on lung cancer cells (A549)

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The aim of the work was to investigate apoptotic effects of essential oils from cultured and wild form of *Origanum acutidens* (Hand.-Mazz.) Ietswaart. (Lamiaceae) on A549 lung cancer cells. The IC_{50} values of wild and cultured forms of essential oil on cancer cells were determined by Cell Titer-Blue^R Cell Viability assay. Lactate dehydrogenase activity were measured by Lactate dehydrogenase Activity Assay Kit. Caspase 3/7 activity in essential oil treated cells were determined by ApoTox-Glo™ Triplex Assay Caspase-3/7 assay kit. IC_{50} values (concentration that kills 50% of cells) of wild and cultured forms of essential oil in A549 cells were calculated as 98.1 μg/ml and 136.2 μg/ml respectively for 24 h. Cytotoxic effect of essential oil increased depend on concentrations. The lactate dehydrogenase (LDH) activities, which is a cytoplasmic enzyme released as a result of the disruption of the cell membrane integrity, were found higher in wild and cultured forms of essential oil (IC_{50}) treatment in A549 cells than control cells. Caspase 3/7 activity, which is one of the key enzymes of apoptotic pathway, increased in A549 cells respectively 2 and 4 times in comparison to control cells after wild and cultured forms of essential oil (IC_{50}) treatment for 24 h. As a result, essential oil from wild and cultured *O. acutidens* caused cell death inducing apoptosis.

P.10-120-Wed
The impact of glucose and mOGT-dependent O-GlcNAcylation on expression of mega-channel proteins in breast cancer cells

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Cancer cells energy metabolism have been reported to be strongly dependent on glucose in comparison to normal cells. This characteristic feature is associated with mitochondrial dysfunction. Mitochondria are described as "the powerhouse of the cell" because they supply most cells with most of their ATP. The rate of ATP production in mitochondria is regulated by activity of mitochondrial mega-channels that are localized to contact sites between the inner and outer mitochondrial membranes. Due to mitochondrial dysfunction cancer cells shift the burden of energy metabolism to cytoplasmic fermentation for ATP synthesis. Within a cell, small fraction of glucose is converted to uridine diphosphate *N*-acetylglucosamine (UDP-Glc*N*Ac) which is substrate for *O*-GlcNAc transferase (OGT). This enzyme is responsible for the attachment of *O*-Glc*N*Ac moieties to cellular polypeptides. The reports related to role of *O*-Glc*N*Acylation in mitochondria are very limited. At the same time, growing body of evidence shows that mitochondrial dysfunction may be caused by hyperglycemia, although mechanism of this regulation is still not fully understood. Therefore, the aim of our study was to determine whether glucose and glucose dependent mitochondrial isoform of *O*-Glc*N*Ac transferase (mOGT) impacts on core mega-channel proteins in breast cancer cell lines (MCF-7, MDA-MB-231, Hs578t). To find out how mOGT affects on mitochondrial mega-channels, cells growing in hypo-, normo- and hyperglycemia was treated with plasmid coding mOGT and its variant without catalytic domain as well as plasmid bearing no insert. We have investigated that the mitochondrial expression of mOGT and some of core mega-channel proteins such as porins VDAC and adenine nucleotide translocases (ANT) are glucose dependent. In addition, upregulation of mOGT impacts on VDAC3 and ANT1 in breast cancer cells. Our results suggests that mOGT-dependent O-GlcNAcylation regulates the expression of mega-channel proteins.

P.10-121-Mon
Implication of protein kinase CK2 in the metabolic rewiring of cancer cells

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Protein kinase CK2 is a Ser/Thr kinase overexpressed in cancer cells. A body of knowledge defines its supporting role in an ample spectrum of signal transduction pathways whose dysregulation leads to malignancy; however, very little is known about its involvement in the metabolic rewiring occurring in cancer. Since CK2 regulates key transcription factors and signal transduction pathways with well-known functions on cancer metabolism (e.g. PI3K/Akt pathway, HIF-1α, c-Myc and p53), we hypothesize that CK2 targeting can counteract aerobic glycolysis and other tumor specific metabolic pathways. The aim of this work is a detailed dissection of CK2 contribution to the establishment of a metabolic onco-phenotype. To this purpose, besides canonical studies of cell treatments with CK2 inhibitors, we exploit neuroblastoma SK-N-BE(2) and osteosarcoma U2OS cell lines, permanently depleted of an individual CK2 catalytic isoform (α or α'), by CRISP/Cas9 technology. Results obtained from cell growth curves, soft agar clonogenic assays and spheroid formation assays suggest that both CK2 α and α' contribute to cell proliferation, survival and tumorigenicity of these cells. Metabolic features are investigated by assessing the expression of several glycolytic enzymes and other regulatory proteins, the mitochondria content by NAO fluorescent probe, and mitochondrial respiration and glycolysis flux using Seahorse XF24 Analyzer. The effects of combined treatments with CK2 inhibitors and metabolic inhibitors are also evaluated on wild type cell viability. Our study represents the first comprehensive analysis on the role of CK2 in tumor metabolism, with the additional advantage of dissecting the separate contribution of each CK2 isoform, whose knowledge will be fundamental for future therapeutic strategies based on CK2 targeting.

P.10-122-Tue
siRNA-directed inhibition of SCN5A increases matrix metalloproteinase-9 expression and activity in MDA-MB-231 metastatic breast cancer cells

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Voltage-gated sodium channels (VGSCs) are heteromeric transmembrane proteins comprised of a single pore-forming α subunit ($Na_v1.1$-1.9) and one or more auxiliary β subunits (β1-4). Alpha subunits (VGSCα) mediate rapid and transient Na^+ influx into cells while beta subunits (VGSCβ) modulate channel gating and function as cell adhesion molecules. VGSCs are shown to be overexpressed in a variety of human cancers and potentiate a number of cellular behaviors associated with metastasis in which the extracellular matrix is degraded and modulated by proteolytic enzymes. Matrix metalloproteinases (MMPs) constitute a family of human zinc dependent endopeptidases and are involved in degradation or shedding of extracellular matrix proteins as well as several other cell modulators. The aim of this study is to determine the functional association between Nav1.5 (gene: SCN5A) activity, the predominant α subunit in human breast cancer, and MMP-9 expression/activity in MDA-MB-231 strongly metastatic human breast cancer cells. MDA-MB-231 cells were transfected with 50 nM SCN5A siRNA and the transfection efficiency was validated with Real-Time PCR and Western Blot. Real Time PCR and Gelatin Zymography were performed to analyze the effects of siRNA-mediated SCN5A inhibition on MMP-9 gene expression and activity level, respectively. Interestingly, MMP-9 gene expression level was significantly higher in transfected cells compared to nontarget-control and control groups. Consistently, SCN5A downregulation resulted in an increase in proMMP-9 activity levels in MDA-MB-231 cells. These findings will contribute to enlighten further role of Nav1.5 in cancer metastasis and pave the way for new therapeutic approaches which target both VGSCs and MMPs. This research was supported by TUBITAK-115S504

P.10-123-Wed
Effects of metformin and dichloroacetate on mitochondrial energy metabolism in oral cavity cancer cells

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Oral cavity cancer (OCC) is the sixth most frequent cancer and increasing rapidly due to limitations of conventional treatment methods. Although chemotherapy is an effective method, the hypoxia which occurs in tumor microenvironment, leads to chemoresistance in OCC cells. Metformin, an antidiabetic agent, inhibits complex I of electron transport chain. Dichloroacetate (DCA), used in the treatment of lactic acidosis, is an inhibitor of pyruvate dehydrogenase kinase (PDK) promoting mitochondrial metabolism. The aim of this study is to determine the functional effects of Metformin and DCA on cell viability, the alterations of expression levels of PDK-1 and HIF-1α, and mitochondrial energy metabolism under normoxia and hypoxia in OCC cell line, UPCI-SCC-131. Cell viability was monitored by xCELLigence RTCA SP system. Gene and protein expressions of HIF-1α and PDK-1 were detected via both RT-PCR and Western blot. Bioenergetic profiles of Metformin and DCA treated cells were analyzed with Seahorse XF Cell Energy Phenotype Test, which measures the extracellular acidification rate (ECAR, a measure of extracellular pH that correlates with glycolysis) and O_2 consumption rate (OCR, a measure of OXPHOS) in real time. The IC_{50} values of Metformin and DCA at 48 h were 1 mM and 25 mM, respectively. Metformin and DCA reduced HIF-1α expression levels under hypoxia and normoxia in UPCI-SCC-131 cells. PDK-1 expression under normoxia was decreased in Metformin/DCA treated cells. According to metabolic flux analysis, DCA treated cells had a significantly higher OCR and ECAR compared to control cells. However, metformin treated cells showed decreased OCR and increased ECAR responses. In conclusion, DCA possibly activated mitochondrial functions via driving metabolism to the energetic pathway whereas Metformin promoted a shift to glycolysis in UPCI-SCC-131 cells. Metformin and DCA may meet the "drug repurposing" criteria as a combination therapy for the OCC treatment.

P.10-124-Mon
Tetracaine suppress metastatic cell behaviors through regulating matrix metalloproteinase-2/-9 and TIMP-2 levels in metastatic breast cancer cells

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Voltage-gated sodium channels (VGSCs) are transmembrane proteins that enable Na^+ flow into cells in order to create action potentials. VGSCs are overexpressed in a variety of human cancers including breast cancer, where expression is associated with metastatic potential. Matrix metalloproteinases (MMPs) play a key role in the modulation of extracellular matrix and signal transduction pathways. Tetracaine is a FDA-approved local anesthetic that inhibits VGSCs and kinesin motor protein function. However, the effect and underlying mechanism of Tetracaine on metastatic cell behaviors in breast cancer cells has remained uncharacterized. The aim of this study is to explore the functional effects of Tetracaine on i) the expression of $Na_v1.5$, β1, TIMP-2, ii) the expression/activity of MMP-2/-9, and iii) metastatic cell behaviors in strongly metastatic and VGSC-expressing human breast cancer cell line, MDA-MB-231. Non-cytotoxic doses of Tetracaine, 50–75 μM, were determined with WST-1 cell viability assay. Real Time PCR, Western blot, and Gelatin Zymography were performed to analyze expression and activity levels of target proteins. Metastatic cell behaviors were evaluated by wound healing, transwell migration, and Matrigel invasion. Tetracaine reduced MMP-2/-9 mRNA expression and activity levels as well as enhanced TIMP-2 expression levels compared to untreated-control group. However, $Na_v1.5$ and β1 expression levels were not affected in Tetracaine-treated MDA-MB-231 cells. As regards metastatic cell behaviors, Tetracaine inhibited both invasion and migration capability of MDA-MB-231 cells. We showed, for the first time, that Tetracaine has a potential inhibitory effect on invasiveness of metastatic breast cancer cells via regulating MMP-2/-9 and TIMP-2 levels. Therefore, we predict that Tetracaine may be used as a novel anti-metastatic agent by identifying further mechanisms in breast cancer metastasis. This research was supported by TUBITAK 115S504.

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P.10-125-Tue
The CRNDEP localization and interactions suggest its role in RNA metabolism, response to stress and cellular component organization

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The *CRNDE* gene was first described as non-protein coding, and growing number of studies confirm its oncogenic functions. Aberrant overexpression of this gene found in various cancers, is correlated with advanced aggressive clinicopathological features and poor prognosis. *CRNDE* was shown to promote migration, invasion, metabolic switch to aerobic glycolysis (the Warburg effect) and inhibit apoptosis. The tumor-promoting capabilities of *CRNDE* are predominantly ascribed to its lncRNA product. We have discovered that one of the *CRNDE* transcripts encodes a peptide, which seems to be involved in cell proliferation, cell cycle regulation and in response to microtubule-targeting agents as a centrosome component. We aimed to investigate the molecular function of this peptide, by identifying its protein partners. CRNDEP-targeted immunoprecipitation studies were conducted in three different variants. Proteins were identified by tandem mass spectrometry and the results were analyzed with MaxQuant and Stat software. The most pronounced GO molecular function found for all CO-IP variants was poly(A) RNA binding. Among GO biological processes, the most significant were: the response to stress and stimulus, cellular component organization and regulation of mitotic cell cycle process. In line with these results, upon treatment with agents affecting RNA metabolism, CRNDEP colocalized with some nucleolar markers and accumulated in nuclear entities. CO-IP and colocalization studies showed that CRNDEP may interact with the DDX6 protein, a marker of P-bodies. In conclusion, we postulate that CRNDEP is involved in RNA metabolism and it possibly regulates the response to stimuli as a component of entities that accumulate RNA-protein (RNP) particles. This work was supported by the grants: 2015/17/N/NZ5/01392 and 2016/23/D/NZ5/01453 from the National Science Centre.

P.10-126-Wed
Compensatory upregulation of neurotrophin signaling rescues neuroblastoma cells from death after KIT knockdown

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Neuroblastoma (NB) is an embryonal tumor arising from neural crest cells and it is one of the most common malignancies of childhood. NBs are highly heterogenic and they account for nearly 15% of all childhood cancer-related deaths. Receptor tyrosine kinase KIT is expressed by considerable number of NB tumors and it is also expressed by several other cancers. KIT positive NB cells characterize an aggressive subset of tumor cells and KIT inhibition results in reduced tumor growth, so it is considered as promising target for NB treatment. Here we report that KIT knockdown by RNA-interference in model NB cells resulted in inhibition of proliferation and induction of apoptosis. Nevertheless analysis of changes in signaling pathways based on microarray-profiled gene expression data revealed that in NB cells simultaneously with apoptosis had happened activation of growth-related pro-survival pathways. Amongst others there was an increase in expression of neurotrophins (NGF and BDNF) and their receptors (TrkA and TrkB respectively). We showed that exogenous NGF and BDNF rescued NB cells from apoptosis and partially restored their proliferation. We identified ERK2 as major component of such compensatory signaling. Inhibition of ERK2 in KIT-downregulated cells resulted in enhanced cell death, blocked compensatory increase in TrkA and TrkB expression, and prevented rescue of NB cells by neurotrophins. Overall we show how neuroblastoma cell can compensate KIT signaling downregulation by employing other pro-survival pathways and that these mechanisms are highly dependent on ERK signaling. The results were obtained within the Program of fundamental research for state academies for 2013–2020 years (№ 01201363823). The results on microarray gene expression profiling and shRNA experiments were obtained within the RSF grant (project No. 14-14-01089-П) and functional cell culture experiments were performed within RFBR grant (project No. 17-04-01697A).

P.10-127-Mon
Epigenetic changes throughout breast cancer treatment: comparison the expression of genes involved in active DNA demethylation

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Every year there is approximately 1,7 million new cases of breast cancer globally. This type of cancer, similarly to the other ones, displays global hypomethylation as a result of genome instability. The experimental evidences have demonstrated that active DNA demethylation process, which involves ten-eleven translocation (TET) family proteins, can affect DNA methylation pattern. TET dependent demethylation residues in DNA hypomethylation by oxidation 5-methylcytosine (5-mC) to its derivatives, which are finally excised by thymine DNA glycosylase (TDG) in base excision repair process (BER). An active DNA demethylation results in unmethylated cytosine attachment, which may be related to silenced genes activation. Given that epigenetic processes may affect cancer initiation and progression, we wanted to determine whether chemotherapy has an influence on DNA demethylation. We applied qRT-PCR with Universal Probe Library probes for measurement of expression of genes involved in active demethylation process, and two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry detection (2D-UPLC-MS/MS) for direct 5–methyl–2′–deoxycytidine (5–mdC) and its derivatives assessment in blood samples. The examination was accomplished in breast cancer patients on various stages of treatment, including neoadjuvant chemotherapy, and approximately one month after surgery. We found differences between samples collected in different phases of treatment process. A reduced expression of TETs and TDG genes was observed in leukocytes isolated after treatment. Additionally,

distinction in 5-mdC level was also observed. Personalized treatment of breast cancer patients may be associated with epigenetic changes in DNA, which could have been a valuable significance in predicting of therapy outcome in the future. The work was supported by the Polish National Science Center [2015/17/B/NZ5/00640].

P.10-128-Tue
Impact of TET3 and OGT on the endometrial cancer progression and metastasis

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Ten-eleven translocation (TET) methylcytosine dioxygenases, especially TET3 can interact with O-GlcNAc transferase (OGT). OGT is an enzyme frequently overexpressed in cancers, responsible for protein modifications by addition of a single N-acetylglucosamine moieties to serine or threonine residues of intracellular proteins. It is known that TET3 can affect the recruitment of OGT to chromatin where OGT can regulate the expression of certain genes. Previous studies conducted by us on clinical samples of endometrial cancer have shown that the high expression of *TET3* is associated with more advanced and aggressive tumors, which may indicate a significant contribution of TET3 to the progression of endometrial cancer. Therefore, the aim of the study was to evaluate the effect of altered *TET3* expression on the expression and cellular location of OGT and on the expression of genes that are key regulators of epithelial-mesenchymal transition (EMT) in two endometrial cancer cell lines. *TET3* expression was down-regulated by the RNA interference, whereas *TET3* overexpression was performed by transfecting cells with expression vector. Although the impaired *TET3* expression had no effect on the OGT gene expression, protein level and cellular location (cytoplasmic/nuclear), differences in the amount of OGT in the chromatin fraction were observed. Moreover, ChIP analyses revealed changes in the amount of OGT in the promoter regions of EMT-regulating genes. Expression of tested genes changed in cells with altered expression of *TET3*. In addition, down- and up-regulation of TET3 in endometrial cancer cells affected their migration and invasion potential. These results suggest that the interactions between TET3 and OGT may have a significant impact on the process of endometrial cancer progression. This work was supported by the grant (2015/19/N/NZ3/01311) from the National Science Center, Poland.

P.10-129-Wed
Anti-cancer activity of a novel flavonoid C-apioglucoside from *Albuca setosa* Jacq. (Asparagaceae)

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Albeit the advantages of natural over synthetic compounds, recent decades have seen a decline in the approval of natural product-based drug candidates due to increased interest in the application of High-Throughput-Screening (HTS) methods to combinatorial synthetic compounds. This challenge to natural products can be circumvented by the merging of conventional natural product drug discovery paradigms with more modern approaches. A good example of such is the screening and bioactivity guided isolation of natural metabolites using High Content Analysis (HCA) equipment. Phytochemical investigation of *Albuca setosa* Jacq., a neglected species from the Asparagaceae family, led to the isolation and chemical characterisation of a novel *C*-glucosylflavonoid-*O*-glucoside with cytotoxic activity against HeLa cervical cancer cells. The *A. setosa* bio-mass underwent soxhlet extraction to obtain a crude extract which was successively partitioned using Hx, EtOAc and n-BuOH. The n-BuOH partition was then chromatographed on Diaion® HP-20 resin with further separation of the most active fraction on Sephadex® LH-20 gel to obtain 12 sub-fractions. Sub-fractions SF-7 to SF-12 were combined to afford 26.7 mg of the novel pure compound. Structural elucidation using various spectroscopic methods led to the characterization of the compound as (2*S*)-6-*C*-[-apio-α-D-furanosyl-(1→6)-β-glucopyranosyl]-4′, 5, 7-trihydroxyflavone. Dual staining with bisBenzamide H33342 trihydrochloride/propidium iodide (Hoechst/PI) was used to determine the anti-proliferative activity of the *A. setosa* extract, fractions and pure compound. At its IC_{50} of 2.5 μM the isolated pure compound was further found to induce cell cycle arrest in M-phase in HeLa cells. Image cytometry confirmed apoptosis induction as evidenced by changes in morphological and biochemical markers of apoptosis.

P.10-130-Mon
Activation of both pro-survival and pro-apoptotic signaling pathways in response to KIT downregulation in leukemia cells

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Receptor tyrosine kinase KIT is found to be expressed in the high percentage of acute myeloid leukemia (AML) cases. Since that KIT is an attractive target for anti-leukemia treatment, but the input of non-mutant KIT up-regulation in AML still remains unclear. We used RNA interference (RNAi) technique to study the mechanisms responsible for survival of leukemia cells following KIT downregulation. We observed significant slowdown of cell growth when KIT was repressed. Also the alteration in expression of genes encoding cell cycle regulatory proteins such as cyclins and cyclin-associated kinases was demonstrated. Further we performed a genome-wide microarray-based screening of gene expression. We showed that KIT downregulation was followed by dramatic changes in signaling pathways activation profile. From the one side, most of them are responsible for cell death and suppression of cell growth. But from the other side, the huge number of activated pathways is associated with the kinases involved in cell survival, avoiding apoptosis and proliferation stimulation. Among them signaling pathways associated with ERK kinases were found to be up-regulated when KIT was suppressed and that could be defined as a compensatory mechanism. We suggest ERK2 as the key regulator, regarding an existence of therapy resistant phenotype when anti-KIT drugs are used. The results were obtained within the Program of fundamental research for state academies for 2013–2020 years (No 01201363823). This work was supported by RSF grant (project No 14-14-01089-П)

P.10-131-Tue
The effect of ERK2 inhibitors on sensitivity of leukemia cells to KIT downregulation

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Cytokines and chemokines are described as major regulators of several malignant diseases progression including AML. Also some of them were suggested to be responsible for the development of drug-resistant leukemia phenotypes. The majority of cytokine pathways are associated with ERK kinases. Early we suggested ERK2 as a key regulator, responsible for malignant blood cells survival. In this study we used ERK2 small-molecular inhibitors to evaluate the response of leukemia cells to anti-cancer therapeutic agents treated with exogenous cytokines and growth factors. Here we demonstrated the increase in sensitivity of KIT-positive cells to anti-KIT small hairpin RNA or anti-KIT drugs when ERK2 inhibitors were added. Also we showed that ERK2 inhibitors may change the sensitivity of leukemia cells to the cytokines and growth factors, when treated with anti-cancer drugs or after KIT downregulation. We suggest the strategy to reduce stress-response mechanisms existing in malignant myeloid cells by that they can avoid apoptosis and survive during the therapy. We provide evidence that targeting KIT in AML cells may be insufficient for successful treatment and other inhibitors targeting possible compensatory mechanisms should be used. The results were obtained within the Program of fundamental research for state academies for 2013–2020 years (No. 01201363823). Flow cytometry experiments were supported by the RFBR grant (project No. 17-04-01555). All viability assays were performed within RSF grant (project No. 14-14-01089-П).

P.10-132-Wed
Tyrosine phosphorylation of vimentin intermediate filaments regulated by Src and SHP2 is important for cell migration

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Vimentin intermediate filaments (VIFs) are expressed in most mesenchymal cells and cancer cells, which undergo dramatic reorganization during cell migration; however, the underlying mechanism remains obscure. In this study, we show that Src directly phosphorylates vimentin at Tyr117 upon growth factor stimulation, which leads to disassembly of VIFs into squiggles and particles at the cell edge when lamellipodia formation is induced. The protein tyrosine phosphatase SHP2 counteracted the effect of Src on tyrosine phosphorylation and organization of VIFs. The VIFs formed by the Y117D mutant were much more soluble and dynamic than those formed by the wild-type vimentin and Y117F mutant. The increased expression of vimentin promoted lamellipodia formation and cell migration upon growth factor stimulation, whereas the Y117D and Y117F mutants suppressed both events. The increased formation of lamellipodia by vimentin was correlated with activation of Rac and Vav2, a guanine nucleotide exchange factor for Rac. Vav2 was largely associated with VIFs and recruited to the plasma membrane upon growth factor stimulation. Taken together, our results unveil a novel mechanism for regulating the dynamics of VIFs through Src and SHP2 upon growth factor stimulation and demonstrate that proper dynamics of VIFs is important for Rac activation and cell migration.

P.10-133-Mon
Peptide parts of Hsp70 chaperone as potential agents of anticancer combination therapy

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Combination therapy based on the use of several drugs with different mechanisms of action is an actively developing area of antitumor treatment. Due to high ability of cancer cells to avoid the effects of antitumor agents, suppression of malignant cell's protective mechanisms is an effective way to enhance the potency of anticancer treatment. Being overexpressed in most cancer cells, molecular chaperone Hsp70 is playing profound role in protection of those cells from stress factors including anticancer agents. Thus, inhibition of protective Hsp70 activity in cancer cells is a promising method of enhancing the efficiency of anticancer treatment. However, the fact that many of Hsp70 inhibitors shown to have anticancer effects are highly cytotoxic is limiting their clinical application. In this study focused on the search of safer Hsp70 inhibitors we performed screening of Hsp70 sequence-based peptides and found modulators of this chaperone. One of the peptides, ICit-2, shown to have the abilities to inhibit substrate-binding and refolding activity of the chaperone. We also demonstrated its ability to bind directly with Hsp70 and calculated the K_d of ICit-2–Hsp70 complex. Using the method of molecular docking, we assumed the most probable sites of Hsp70–ICit-2 binding. ICit-2 exhibit its ability to penetrate cancer cells and enhance the cytotoxic anticancer effect of doxorubicin (Dox) *in vitro*. Using the B16 mouse melanoma model, we confirmed ICit-2 efficiency in combined therapy with Dox, leading to a slowdown in tumor growth rate and increase in life expectancy, in comparison with Dox applied alone. Thus, abilities of ICit-2 to inhibit the activity of Hsp70 and enhance the antitumor effect of Dox, together with its low self-cytotoxicity, make its potential candidate for further trials in therapy of highly resistant tumors. The research is supported by Grant of Russian Science Foundation № 14-50-00068.

P.10-134-Tue
The HSF1 transcription factor as a component of the ERα-dependent signaling pathway in breast cancer cells

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HSF1 is a transcription factor activated by an environmental stress and the main regulator of HSPs (Heat Shock Proteins) proteins. HSPs participate in the folding of other proteins and protect cells from stress-induced apoptosis. HSF1 and HSPs are overexpressed in many types of tumors. HSF1 has been shown to

support neoplastic transformation, as well as to facilitate the survival of cancer-transformed cells by modulating signaling pathways associated with cell growth and proliferation, apoptosis, metabolism, and cell motility. We aimed to explain the molecular mechanisms of HSF1 activation by estrogen (E2). E2 plays an essential role in sexual and reproductive development, but it is also a risk factor for developing breast and endometrial tumors. We found that HSF1 was activated (phosphorylation at Ser326) by E2 via estrogen receptor α (ERα): in ERα-positive breast cancer MCF7 cells, but not in ERα-negative cells (normal breast MCF10A and MCF12A, and ovarian cancer ES2 or OVCAR3). HSF1 activation also occurred in MCF7 cells treated with other ERα agonists (PPT or BPA). Furthermore, silencing of ERα expression by specific siRNA resulted in inhibition of HSF1 activation after E2 treatment. Using specific ERK1/2, PI3K and mTOR inhibitors we showed, that ERK and mTOR-dependent signaling pathways are involved in the phosphorylation of HSF1 by E2. E2-activated HSF1 is transcriptionally potent and binds to the regulatory region of some genes, which affects their expression. To determine the role of HSF1 in MCF7 cells, we derived a cell line with reduced expression of HSF1 (using shRNA) and performed RNA-Seq in search for changes in the expression of E2-induced genes. The analysis showed that among the biological processes influenced by estrogen, the most dependent on HSF1 are processes related to cell adhesion and angiogenesis. This work was supported by Polish National Science Centre, Grants 2014/13/B/NZ7/02341 and 2015/17/B/NZ3/03760.

P.10-135-Wed
Targeted delivery of genes to plasmolipin-expressing cells using lentiviral particles pseudotyped with *Mus caroli* endogenous retrovirus envelope protein

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Development of effective lentiviral-based vector systems for targeted delivery of therapeutic genes in malignant cells is an actual trend in modern anticancer treatment. We constructed lentiviral vector particles pseudotyped with Mus caroli endogenous retrovirus (McERV) envelope protein (McERV env) which can be used for selective transduction of plasmolipin (PLLP) expressing cells (malignant neural cells). These particles were shown to be an effective tool for selective marking of PLLP expressing cells. Furthermore, a gene encoding cytotoxic protein in McERV env-pseudotyped vector particles may be used. We showed significant cytotoxic effect when HSV thymidine kinase/ganciclovir-mediated system or gene encoding phototoxic protein were used (up to 90% of dead cells in population was observed). Here we demonstrated that McERV env-pseudotyped vector particles is a promising tool for targeting of PLLP-expressing cells. The results were obtained within the Program of fundamental research for state academies for 2013–2020 years (No. 01201363823). This work was supported by RSF grant (project No. 14-14-01089-П).

P.10-136-Mon
Assessment of pathway of active DNA demethylation in myelodysplastic syndrome

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The fundamental epigenetic process is DNA methylation, which occurs by 5–methylcytosine (5–mC) formation. 5–mC could be further converted to 5–hydroxymethylcytosine (5–hmC), and the other derivatives (5–formylcytosine (5–fC), and 5–carboxylcytosine (5–caC)) by TET proteins oxidation in active DNA demethylation process. Additionally, TET enzymes could generate 5–hydroxymethyluracil (5–hmU) from thymine. TETs are dioxygenases, which require 2-ketoglutarate (2-KG) as a co-substrate, and iron (II) and ascorbate as co-factors. 2-KG is produced in tricarboxylic acid cycle by isocitrate dehydrogenase (IDH). Mutations of IDH result in 2–hydroxyglutarate (2–HG) production instead of 2-KG, which leads to restraint of TETs function. Recent evidences have demonstrated that equilibrium of cytosine methylation in DNA is a key factor in natural development of hematopoietic cells. We analyzed blood samples from patients who suffer from myelodysplastic syndrome (MDS). Given that, MDS displays genomic aberrations with the highest frequency of TET2 gene mutations, we attempted to evaluate 5–mC and its derivatives levels as products of TET dependent demethylation and mRNA expression of genes involved in active DNA demethylation. We used two–dimensional ultra–performance liquid chromatography with tandem mass spectrometry detection for evaluation of epigenetic modifications. We noticed significant hypo–hydroxymethylation of DNA in MDS in comparison to healthy subjects, and levels of 5–fC and 5–caC were below detection limit in most patients, which suggest impaired activation of active DNA demethylation process, and may be associated with pathogenesis of this hematopoietic disease. Although, we have not observed significant correlations between global level of 5-mC and its derivatives, and factors potentially involved in active DNA demethylation: 2-KG, 2-HG and ascorbic acid. The work was supported by the Polish National Science Center [2015/19/B/NZ5/02208].

P.10-137-Tue
AF4 and MLL-AF4 are novel targets of MiR-27a in t(4;11) leukemia

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The t(4;11)(q21;q23) chromosomal translocation accounts for 40% of infant acute lymphoblastic leukemias (ALL) and is a hallmark of poor prognosis. It results in the *KMT2A/AFF1*

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fusion gene, which encodes the mixed-lineage leukemia (MLL)-AF4 oncogenic chimera that drives transformation of hematopoietic progenitors. In t(4;11) ALL, MLL-AF4 recruits the endogenous co-activator AF4 and aberrantly triggers transcription of MLL target genes, e.g. *HOXA9* and *MEIS1*. We previously demonstrated that the scaffold protein 14-3-3θ is a direct interactor of AF4 and promotes AF4 binding to the *HOXA9* promoter. Notably, 14-3-3θ is a known target of MiR-27a and the expression level of this miRNA correlates with relapse free survival in childhood ALL. In RS4;11 leukemia cells, we proved that MiR-27a transient transfection reduces protein level of 14-3-3θ, AF4 as well as MLL-AF4, and impairs *HOXA9* and *MEIS1* transcription. Here, we demonstrate that *AFF1* and *KMT2A*/*AFF1* mRNAs are novel targets of MiR-27a. Bioinformatic analysis indicated that MiR-27a seed region perfectly matches to a 6-mer sequence within *AFF1* 3′UTR, which is shared with *KMT2A*/*AFF1*. To analyze the interaction between MiR-27a and this region, we created a luciferase construct containing the *AFF1* 3′UTR. The following luciferase reporter assay showed that *AFF1*, and therefore *KMT2A*/*AFF1*, is an actual target of MiR-27a. Consistently, in RS4;11 leukemia cells, transfection of anti-MiR-27a rescued expression of 14-3-3θ, AF4 and MLL-AF4. We found that MiR-27a overexpression reduced viability/proliferation and increased apoptosis of RS4;11 cells. Preliminary analysis of specific cell surface differentiation markers indicated that MiR-27a overexpression induces cell differentiation. We conclude that MiR-27a, by targeting AF4 and MLL-AF4, has a pivotal role in t(4;11) ALL pathogenesis and therefore it is a promising new target for the therapy of this aggressive form of leukemia.

P.10-138-Wed
Dual targeting of glycolysis and linked metabolic pathways increases the efficacy of metabolic intervention in breast cancer cells

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Metabolic aberrations contribute to cancer initiation and progression. Enhanced glycolytic rate, combined with induced activity of glycolysis side branches, namely the pentose phosphate pathway (PPP) and de novo serine synthesis, confers considerable metabolic benefits. This prompted us to investigate the efficacy of dual targeting of glycolysis and one of its side branches in breast cancer (BCa) cell lines of the two major different molecular subtypes: luminal (MCF-7) and basal (MDA-MB-231). Pharmacological activation of the enzymatic activity of the rate-limiting glycolytic enzyme PKM2 (pyruvate kinase M2), in combination with either G6PD (glucose-6-phosphate dehydrogenase) or PHGDH (phosphoglycerate dehydrogenase) inhibition showed enhanced cytotoxic effects in luminal BCa cells but not basal BCa cells. Our results indicate that dual targeting of the main glycolytic pathway and one of its branches could be used as a metabolic intervention to enhance anti-cancer therapy. The differences in the susceptibility of the BCa cell subtypes to the treatment presented can be explained by very clear differences in basal activity levels of key glucose metabolizing enzymes.

P.10-139-Mon
Relationship between the levels of epigenetic DNA modifications, 2-oxoglutarate and 2-hydroxyglutarates in acute leukemias

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Acute leukemia (AL) is a heterogeneous disease characterized by distinct genetic and epigenetic alterations, including aberrant DNA methylation. The most dynamic process which regulates DNA methylation is recently discovered active demethylation involves ten-eleven translocation (TET) enzymes to catalyze oxidation of 5-methylcytosine (5-mCyt) to 5-hydroxymethylcytosine (5-hmCyt) and further oxidation products. TET family proteins are 2-oxoglutarate (2-OG) and iron-dependent dioxygenases. Isocitrate dehydrogenase (IDH) enzymes IDH1 and IDH2 catalyze the oxidative decarboxylation of isocitrate to 2-OG. Mutations in IDH genes are frequently detected in patients with acute myelogenous leukemia. Interestingly, mutant IDH enzymes reduce 2-OG to D-2-hydroxyglutarate (D-2HG) which may act as a competitive inhibitor of TETs. Additionally, it has been suggested that L-2-hydroxyglutarate (L-2HG) also could be a potent inhibitor of TETs. In this study we have examined 3 groups: patients with acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), as well as the healthy control. In all participants we have measured the level of epigenetic DNA modifications in leukocytes using isotope-dilution automated online two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry (2D-UPLC-MS/MS) and plasma/urine concentrations of 2-OG and L-, D-2HG using UPLC- MS/MS method. The study has shown two 5-hmCyt subpopulations in patient cohorts with higher and lower levels of 5-hmCyt compare to healthy controls. Besides, we have observed reverse correlation between global level of 5-hmCyt and 2-hydroxyglutarates. This suggests that 2-HGs are potent inhibitors of TETs activity in vivo, and may play significant role in development of ALs. This work was supported by the Polish National Science Center grant number DEC-2015/19/B/NZ5/02208.

P.10-140-Tue
Is MTT reliable for cell viability analyzes?

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The measurement of cell viability plays a fundamental role in cell culture. The best known method is MTT. A disadvantage of this method many non-mitochondrial dehydrogenases and flavin oxidase can reduce MTT. The ATP assay is also used to determine cell viability. It is the fastest and the most sensitive viability assay. Some plant extracts may give false results in MTT analyzes. In this study, we planned to show that MTT gave incorrect results in plant studies. In this context different concentrations of

idarubicin, bromelain, thymoquinone and Nigella Sativa oil were prepared. MTT and ATP protocol were applied for these substances with non-cell medium. In addition, idarubicin and bromelain were administered at different doses to HL60 and lymphocyte cells. IC_{50} value in the HL60 was 5 μM for idarubicin and >20 mg/ml for bromelain. The IC_{50} value for lymphocytes was 10 μM for idarubicin and >20 mg/ml for bromelain in MTT method. In ATP assays, the IC_{50} value in the HL60 was 2.5 μM for idarubicin and 7.5 mg/ml for bromelain. The IC_{50} value for lymphocytes was 10 μM for idarubicin and 12 mg/ml for bromelain. Studies with medium showed no significant increase in absorbance compared to the control for bromelain (102%) and thymoquinone (107%), while there was a significant increase in absorbance of idarubicin (%150) and Nigella Sativa oil (%121) compared to control. Although bromelain killed the cells in the HL60 and lymphocytes, an effective dose for bromelain was not found even at high doses by the MTT method. When the ATP method was used, it was seen that cells were killed and the IC_{50} value calculated. In the study with idarubicin, IC_{50} value was found by MTT method but lower values were found in ATP method. In particular, plant extracts and active ingredients cause formazan formation in MTT. For this reason, we have concluded that the MTT method is not suitable for the studies performed with plant extracts and that the ATP method should be used.

P.10-141-Wed
The influence of SMAD1 gene polymorphisms on colorectal cancer susceptibility in Bangladeshi population: A case-control study

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Various polymorphisms of SMAD1 gene have been found to be linked with elevated colorectal cancer (CRC) risk worldwide. Genomic analysis has revealed that high incidence of CRC is caused by altered expression of SMAD1 gene. SMAD1 gene is responsible for encoding SMAD1 protein which acts as signal transducers of transforming growth factor- beta (TGF-β) and thus is suggested to be an essential component of the tumor growth inhibitory effect of TGF-β. Again, bone morphogenetic protein (BMP) is a subgroup of the TGF-β superfamily. BMP-SMAD1 pathway functions as tumor suppressor by stabilizing the p53 tumor suppression activity. So we hypothesize that, polymorphisms in SMAD1 could alter its antitumor activities and increase the risk of colorectal cancer. This study aimed to identify the influence of SMAD1 rs11100883 and rs7661162 polymorphisms on colorectal cancer risk in Bangladeshi population. A case-control study was carried out on 188 CRC patients and 180 controls to investigate two allelic variant of SMAD1 gene - rs11100883 and rs7661162 using Polymerase Chain Reaction (PCR) - Restriction Fragment Length Polymorphism (RFLP). An elevated CRC risk was found with heterozygous and heterozygous plus mutant variants of rs11100883 and rs7661162 and the results found were statistically significant. For rs11100883 heterozygote GA and combined GA+AA genotypes (OR = 1.5472, P = 0.0494 and OR = 1.8559, P = 0.0319 respectively) are found to be significantly associated with CRC. Similarly in case of rs7661162, heterozygote AG and combined AG+GG genotypes were also observed to be strongly associated (P = 0.0128, OR = 1.7720; P = 0.0185, OR = 1.6971) with elevated risk of CRC. The present study indicates that SMAD1 is significantly correlated with increased colorectal cancer risk. The result of the investigation point out that SMAD1 could be used as a potential molecular marker for successful identification and treatment for colorectal cancer.

P.10-143-Tue
Synthesis of new hybrid conjugates based on amphiphilic porphyrins and nitrilium derivatives of boron clusters [B10H10]2-

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Photodynamic therapy (PDT) and boron neutron capture therapy (BNCT) are promising alternative methods for treating neoplasms. Boronated porphyrins were suggested as dual sensitizers for both PDT and BNCT because of its tumor affinity of the porphyrin ring; ease of synthesis with a high boron content; low dark cytotoxicity; and desirable photophysical properties, etc. This work is dedicated to the elaboration of synthetic methods for new boron-porphyrin conjugates receiving. Our approaches were based on reactions of nucleophilic addition of [2-B10H9N≡CMe]- nitrilium derivatives by pendant functional amino- or ethynyl groups of substituted meso-tetraphenylporphyrins. In received conjugates we varied amounts of porphyrin moiety (one, two or four). Also, we have investigated interactions of conjugates with DNA under physiological conditions (pH 7.4, and ionic strength 0.15 M NaCl). Complexation of the conjugates with DNA was confirmed by UV-vis, circular dichroism and fluorescence life-time spectroscopy. It was shown the two types of complexes with DNA: intercalation (insertion between DNA bases) and external (attachment to a small groove of DNA). Type of DNA complexation depends on conjugates structure. Further studies of these systems might help to understand the design of anti-cancer drugs with potential biological activity. This research was supported by the Russian Foundation for Basic Research (grants N16-03-01039, N16-33-60182) and by grant "Universitetskiy" (NICH-39).

P.10-144-Wed
Investigation of the effects of histone deacetylase inhibitors (HDACi) on apoptotic pathway in glioblastoma multiforme (GBM)

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Glioblastoma multiforme is an aggressive brain tumour mainly occur in adults. Despite surgery, radiotherapy and chemotherapy, survival rate is 14.6 months. Conventional chemotherapy has limited effect due to blood-brain-barrier, intrinsic heterogeneity of tumor, intrinsic GBM resistance and non-specific toxicity. TRAIL which has no effect for normal cells but targets tumour cells, is a promising agent. It is known that GBM shows resistance to TRAIL-mediated apoptosis. Therefore, combined treatment agents such as HDACi combined with TRAIL is current strategy for GBM therapy. The aim of this study is to determine the best HDACis enhancing the TRAIL-mediated cell death among Vorinostat, MS-275, CBHA, Belinostat and Romidepsin and to investigate the effects of these HDACis upon apoptotic pathway on GBM. Therefore, cytotoxic effects of single and combined treatment of TRAIL and HDACis LN18, T98G, U87MG and U373 was determined with CellTiter-Glo method and IC_{50}

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doses of HDACis were calculated via GraphPad Prism program. Effects of TRAIL combination either Belinostat or Romidepsin to apoptosis were calculated by flow-cytometry. Expression changes of pro-apoptotic and anti-apoptotic genes were analyzed by real-time PCR. Additionally, protein expressions of Bcl-2, Bax, caspase-8 and caspase-9 were analysed by western blot method. According to our results, Romidepsin and Belinostat have more cytotoxic effect on GBM compared with other HDACis. It is found that compared to other HDACis, Belinostat and Romidepsin are more effective for TRAIL-induced cell death. By flow-cytometry analysis, combined treatment of Belinostat and Romidepsin with TRAIL enhanced apoptosis significantly. According to real-time PCR results, Romidepsin and Belinostat increased pro-apoptotic gene expression but decreased anti-apoptotic expression. There isn't any significant changes on protein levels. This results suggest and indicated that Belinostat and Romidepsin is a potential agent for GBM treatment.

P.10-145-Mon
Low-affinity adenosine receptors contribute to chemoresistance in glioblastoma stem-like cells under hypoxia

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Glioblastoma is one of the most common nervous system primary tumors with one of the highest cancer death rates. Glioblastoma stem-like cells (GSCs) have been reported to be responsible for the chemoresistance properties in this neoplasia, and their hypoxic niches enhance the chemoresistant phenotype. We reported previously that adenosine contributes to chemoresistance in GSCs trough MRP1 expression mediated by activation of the A_3 adenosine receptor in normoxia. We observed that under hypoxia, MRP3 transporter expression increases. Currently, the role of adenosine in chemoresistance under hypoxia is poorly understood. Since adenosine levels increase under hypoxia, we propose that lowest-affinity adenosine receptor A_{2B} may be involved in chemoresistance mechanisms through the expression of MRP transporters. In this work, we evaluated the role of low-affinity adenosine receptors on chemoresistance in GSCs under hypoxia. GSCs were cultured under normoxia (21% 0_2) and hypoxia (0.5% 0_2). GSCs were treated with the antagonists of low-affinity adenosine receptors (MRS1220 for A_3 and MRS1754 for A_{2B}) for 24 h under normoxia or hypoxia. Protein levels were evaluated by western blot and MRP activity was determined by CFDA accumulation and quantified by FACS. GSC viability was determined by MTS, cell cycle and apoptosis were determined by FACS. A_3 blockade decreased MRP1 and MRP3 expression under normoxia and hypoxia while A_{2B} blockade decreased MRP3 expression only under hypoxia. Moreover, A_3 blockade decreased MRP activity only under normoxia whereas A_{2B} blockade decreased MRP activity only under hypoxia. MTS and apoptosis assays showed significant chemosensitization after treatment with MRP3 drug substrates plus the A_{2B} antagonist under hypoxia. These results suggest different chemoresistance mechanisms mediated by oxygen-dependent adenosine signaling.

P.10-146-Tue
Potential prognostic markers for localized prostate cancer

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Prostate cancer is the second most common cancer in men. In some cases, prostate cancer develops slowly and overtreated. Molecular markers, which allow for differentiation of aggressive or indolent tumors, can be helpful for therapy optimization. We performed a bioinformatic analysis of The Cancer Genome Atlas (TCGA) project RNA-seq data in EIMB RAS "Genome" center (http://www.eimb.ru/rus/ckp/ccu_genome_c.php), and 96 samples (only Caucasians) of localized prostate cancer without lymphatic dissemination were divided into two groups according to D'Amico classification: intermediate (76 samples) and high-risk (20 samples) groups. In the course of the analysis, the genes previously identified by published data as potential biomarkers of the prognosis were considered. Ten genes were identified, characterized by differential expression as potential prognostic biomarkers. Expression of 4 genes (CYP17A1, FAM72D, UBE2C, ESM1) were increased in the group of intermediate risk, and 6 genes (TCN1, SERPINA3, HP, MMP7, S100A9, FCN1) demonstrated decreased expression. These potential markers are involved in the formation and maintenance of the extracellular matrix (MMP7, S100A9, FCN1, SERPINA3, ESM1), metabolism (CYP17A1), cell cycle (UBE2C), neuronal formation (FAM72D), congenital immune response (FCN1), proteolysis (HP, FCN1, MMP7, S100A9, UBE2C, SERPINA3) and inflammation (SERPINA3, S100A9, HP). Disruption of all these processes is indicated for various types of cancer. After validation on the extended cohort these genes could be used in diagnostic panel for prognosis of localized prostate cancer to select an optimal treatment for an individual patient. This work was funded by the Program of fundamental research for state academies for 2013–2020 years (№ 01201363819) and by RFBR according to the research project no. 17-29-06083.

P.10-147-Wed
Adenosine and its role in stemness of glioblastoma stem-like cells

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Therapy resistance in glioblastoma is mostly attributed to a cellular subpopulation called Glioblastoma stem-like cells (GSCs). The de-differentiated phenotype of these cells makes them extremely resistant to therapy compared to other tumor cells. We previously demonstrated that adenosine (Ado) and its A3 receptor (A3AR) are increased in GSCs in relation to differentiated cancer cells; however, its role in GSC biology is not well understood. The role of Ado in the GSC phenotype has been studied, by our laboratory and others, by targeting PAP (Ado-producing enzyme) and A3AR. In this work we evaluated the expression of stem cell markers such as CD44, CD133, Nestin and ALDH1A3, and differentiation markers such as GFAP and β-tubulin III by western blot. The capacity for self-renewal by limited dilutions and tumorigenicity *in vitro* were evaluated by soft agar colony formation assays and cell viability was measured by MTS assays.

We observed that pharmacological blockade of adenosine receptors decreased the expression of CD44, CD133 and increased GFAP expression. This is correlated with a decrease of 80% self-renewal and 90% colony formation capacity. Finally, antagonism of A3AR and silencing of PAP increased the sensitivity of GSCs to temozolomide, decreasing cell viability by 30% and 40%, respectively. Taken together, these results suggest that targeting the PAP-A3AR axis impairs GSCs stemness, thereby promoting chemosensitization.

P.10-148-Mon
Autonomous switching between therapeutic and imaging modes in a molecular 1:2 demultiplexer

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Molecular logic gate applications, where tangible physical/chemical functions emerge, or digital logic action is not assigned *ex post facto*, are still very rare. Information processing models in therapeutic agents may pave the way for further progress, as one of the likely directions, in the field of molecular logic gates. In view of this, as well as in line with our previous findings, we designed a 1:2 demultiplexer, which can autonomously switch from a therapeutic mode in which singlet oxygen is generated, to a signaling/imaging mode, reporting the fact that targeted cancer cells were killed. Our results demonstrate that the switching process of the molecular automaton is very efficient both in organic solvents with model inputs, or in aqueous solutions of actual cancer cell cultures. *in vitro* cell studies confirmed the function and outcomes of this information processing agent/system, which may indeed prove itself to be a priceless "Killer App" for molecular logic gates.

P.10-149-Tue
Sestrin2 protects against cell death in response to DNA-damaging drugs through regulation of ROS and mitochondrial function

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Sestrin2 is a protein product of the SESN2 gene that belongs to the evolutionary-conserved sestrin gene family. The sestrin family encodes stress responsive proteins responsible for regulation of cell viability, cell growth and metabolism. We have shown previously that Sestrin2 is activated in response to DNA-damage and is a potential regulator of the DNA-damage response (DDR). According to our data, the potential mechanism of regulation of the DDR by Sestrin2 is mediated by the interaction with the GATOR2 protein complex that controls mTORC1 kinase activity and lysosomal function. Sestrin2 is often downregulated in human cancers potentially contributing to tumour progression. We hypothesised that Sestrin2 can modulate the DNA-damage response contributing to the regulation of cell viability. Interestingly we have demonstrated that Sestrin2 protects cells against DNA-damage induced cells death. This mechanism involves regulation of mitochondrial activities and reactive oxygen species through a direct control of mitochondrial respiration and mitophagy. Therefore, Sestrin2 inactivation have an immediate impact on the stimulation of cell death, but facilitates mutagenesis to support genomic instability and carcinogenesis. This work was supported by Russian Science Foundation grant 17-14-01420.

P.10-150-Wed
Association of ATM and PTEN gene polymorphisms with breast cancer risk in Bangladeshi women

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Altered DNA repair and tumor suppression activity can affect an individual's predisposition to cancers due to impaired genomic integrity. Polymorphisms in Ataxia Telangiectasia Mutated (ATM) and Phosphatase and Tensin Homolog (PTEN) genes may result in decreased efficiency in activating DNA repair and triggering tumor suppression respectively. This study was designed to evaluate the effects of functional single nucleotide polymorphisms of ATM (rs3092836; T>C) and PTEN (rs11202607; C>T) genes on breast cancer susceptibility in Bangladeshi women. In this case control study, we included 180 females (92 breast cancer patients and 88 age, sex matched healthy volunteers) and followed polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) procedure for genotyping these single nucleotide polymorphisms. The ATM rs3092836 (T>C) polymorphism was observed to increase the risk of breast cancer, and the CC genotype was found to be significantly ($P = 0.0252$) associated to breast cancer susceptibility when compared to TT genotype. The CT genotype of PTEN rs11202607 (C>T) polymorphism was also found to be significantly ($P = 0.0098$) correlated with increased breast cancer risk when compared to CC genotype. No other clinic-pathological features were found to be linked with elevated breast cancer risk. This study identified ATM rs3092836 and PTEN rs11202607 polymorphism as potential markers for breast carcinogenesis. Further study may reveal more precise information and correlation between ATM and PTEN gene polymorphisms and breast cancer susceptibility.

P.10-151-Mon
Cisplatin treatment induces autophagy and down-regulates E-cadherin in ovarian carcinoma cells in vitro: a preliminary study

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It has been demonstrated both *in vivo* and *in vitro* that macroautophagy (hereafter autophagy) activation may bolster survival of cancer cells during acute cisplatin treatment, as well as development of cisplatin resistance. On the other hand, epithelial-mesenchymal transition (EMT) may be yet another process supporting survival of cancer cells in these conditions. Here we demonstrate that cisplatin treatment leads to induction of expression of autophagy marker LC3B, as well as reduced of E-cadherin expression, the most important marker of EMT, in ovarian carcinoma cells. These preliminary data has been obtained using epithelial ovarian cancer cell line OVCAR3. The cells were incubated in RPMI-1640 medium with addition of fetal bovine serum, insulin, and L-glutamine at 37°C in 5% CO_2 atmosphere. Cells were plated onto 6 well plates (300000 cells/well), incubated for 24 h and treated with cisplatin at concentrations of 18–6 μM/l, or water control. After 24 h of drug treatment, whole cell lysates were collected using RIPA buffer. LC3B and E-cadherin expression were probed using Western blotting. GAPDH expression was used as loading control. The data was quantified using ImageJ software. Cisplatin treatment has led to dose dependent induction of autophagy marker LC3B expression as well as down-regulation of E-cadherin (1.45 and 1.71 folds change

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between highest dose-treated and control cells, respectively). Our preliminary results confirm that both autophagy and EMT induction may occur in ovarian cancer cells in response to cisplatin treatment. Our future work will be dedicated to further understanding the roles autophagy and EMT play in survival of cancer cells in presence of cisplatin, including development of cisplatin resistance, as well as investigating the interaction between autophagy and EMT in context of cisplatin-treated cancer cells. Work supported by Program of Competitive Growth of KFU.

P.10-152-Tue
Investigation of seven european colorectal cancer related SNPs in Turkish sporadic colorectal cancer population

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Colorectal cancer (CRC) is one of the leading causes of cancer-related death in the developed countries. According to 2014 WHO report new incidence rate of CRC in Turkey is %6.5 among other cancer types and %75 of CRC cases are sporadic. CRC is one of the most well studied cancer type. Owing to difficulty of the low allele frequency variations detection genetic association profile of CRC have not been entirely identified. Significance of these variations may change according to population. To date several GWASs (Genome wide association studies) conducted in European CRC population revealed CRC related SNPs (Single Nucleotide Polymorphism). From the findings of these studies, we selected and analyzed 7 GWAS validated SNPs namely rs1035209, rs6691170, rs11169552, rs7136702, rs4925386, rs6983267. rs3217810, with a P value of $<1 \times E^{-8}$ in Turkish sporadic CRC cases. The 7 SNPs which resides at 1q41, 8q24, 10q24.2, 12p13.32, 12q13, 20q13.33 were studied using KASP (Kompetitive Allele Specific PCR) in 1967 cases and healthy controls. Statistical analysis is carried out by Cochran-Armitage chi-square test. According to statistical analysis rs4925386 was found associated with Turkish CRC population in our study ($P = 0.0018$). (This work was supported by TUBİTAK Grant no.: 112S634).

P.10-153-Wed
Evaluation of expression stability of traditional reference genes in different cancer types on the basis of TCGA project data

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Quantitative PCR remains the most widely used technique for gene expression evaluation. To obtain reliable data using this method, stable reference genes are needed. Such gene must have unchanged expression under experimental and control conditions. This issue is especially crucial in cancer studies because each tumor has unique molecular portrait. We evaluated expression stability of traditionally used reference genes on the basis of The Cancer Genome Atlas (TCGA) data. TCGA provides omics data for thousands of samples of dozens of cancer types. We included in our study those cancer types, for which omics data are available for representative sets of primary tumors and matched normal tissues: lung, breast, prostate, kidney, colon, and other cancers. The set of 31 traditionally used reference genes was composed: ACTB, ALAS1, B2M, CDKN1A, G6PD, GAPDH, GUSB, HBB, HMBS, HPRT1, HSP90AB1, IPO8, LDHA, NONO, PGK1, POP4, PPIA, PPIH, PSMC4, PUM1, RPL13A, RPL30, RPLP0, RPS17, RPS18, SDHA, TBP, TFRC, UBC, YWHAZ, TUBB. Besides, we included in analysis *RPN1* that was previously identified by us as a promising reference gene in several cancer types. We developed the scoring that assessed the stability of expression of each gene in each cancer type examined. *PUM1* was one of the most stable genes in most cancer types. While *GAPDH*, which is widely used as a reference gene, was highly variable and inappropriate for expression evaluation by quantitative PCR in more than half of the examined cancer types. *RPN1*, proposed by us earlier as a reference gene, showed high score in lung, kidney, colon, liver, thyroid, and prostate cancers. For each examined cancer type, we suggested pairs of genes which are the most suitable for use as reference genes. These genes are characterized by high scores and absence of correlation between their expression levels. This work was financially supported by the Russian Science Foundation, grant 17-74-20064.

P.10-155-Tue
Insufficient cancer cell elimination by p53-inducer Idasanutlin® (RG7388) leads to the generation of secondary drug-resistance

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The protein p53 is activated in response to DNA damage. The active protein induces cell cycle arrest and DNA repair program and thus protects the organism against carcinogenic events. Inactivation of p53 gives the cancer cell a profit of genetic instability, resulting in enhanced cancer cell microevolution. Overexpression of a negative regulator of p53, Mdm2 protein, is one of the most common methods utilized by cancer to keep p53 inactive. Mdm2 binds and blocks p53 and directs p53 to degradation. Re-activation of p53 protein using Mdm2 antagonists is believed to be a promising therapeutic strategy of the treatment of cancers expressing wild-type p53. Several representatives of this class of molecules, such as Idasanutlin® (RG7388), are currently evaluated in clinical trials. However no data concerning the generation of secondary resistance is available for these modern, optimized compounds. In our study four $p53^{wt}$ human cell lines, SJSA-1, U-2 OS, MCF-7 and HCT116 were treated with Idasanutlin®, followed by the analysis of viability, p53 activation, the induction of apoptosis, cell cycle distribution and cancer cell elimination. The treatment of all four tested $p53^{wt}$ cell lines with Idasanutlin® leads to a strong activation of p53. As a result Idasanutlin® induces tremendous cell cycle arrest. However the apoptosis is induced only in SJSA-1 cells, which are highly sensitive to Mdm2 antagonists. Idasanutlin®-treated U-2 OS cells can be cultured for long time periods in the presence of the drug. This prolonged treatment of U-2 OS cells leads to *de novo* generation of secondary resistance. Although modern Mdm2 antagonists have much improved activities compared to their precursors (i.e. nutlin-3), they suffer from similar weaknesses, which are limited elimination of cancer cells and the generation of p53-mutated drug resistant subpopulations. This research was supported by Sonata grant UMO-2016/21/D/NZ7/00596 from the National Science Centre, Poland.

P.10-156-Wed
Identification of DNA methylation markers of CIMP+ epithelial tumors on the basis of The Cancer Genome Atlas data

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CIMP+ (CpG island methylator phenotype) tumors were separated in a distinct molecular group of colorectal cancer more than two decades ago. Since then, a number of evidences suggested the existence of this phenotype in other cancer types also. CIMP+ tumors are characterized by hypermethylation of promoter regions of many genes simultaneously. Hypermethylation is one of the major mechanism of downregulation of genes including tumor suppressors, which inactivation is necessary for the transformation of a normal epithelial cell into a malignant tumor one. It was shown that determination of CIMP status of tumors (CIMP+ or CIMP−) is important for prognosis of the disease and prescription of an optimal treatment regimen. However, it is hard to implement CIMP status determination in clinical practice because there are no reliable molecular markers of CIMP+ tumors and genome-wide analysis methods are still expensive and complicated enough. The Cancer Genome Atlas (TCGA) database, which store omics data for thousands of tumors, provides methylation level for up to 485 thousand CpG sites through human genome and represents a good start point for identification of DNA methylation markers of CIMP+ epithelial tumors. Our research was aimed at development of scoring that enabled the search for promising CIMP+ markers of epithelial tumors. To reach the goal, we analyzed TCGA methylomic and transcriptomic data as well as ENCODE data on localization of functional elements in human genome with our CrossHub tool. The criteria for promising CIMP+ markers were developed and realized as a scoring. The scoring was applied for more than 10 cancer types, for which methylomic and transcriptomic data were available in TCGA for representative number of primary tumors and matched histologically normal samples, and promising CIMP+ markers were suggested. This work was supported by the Program of fundamental research for state academies for 2013–2020 years (№ 01201363819).

P.10-157-Mon
Effects of IL-2 on cell proliferation and apoptosis in cervical cancer cell lines

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The function and structure of the IL-2 receptor (IL-2R) has been well characterised in normal cells of the immune system but not in the normal cervix. The IL-2R has been found to be expressed on several types of tumour cells. We have shown that cervical cancer cells express this receptor and that low doses of IL-2 induce their proliferation. On the contrary, high doses of IL-2 inhibit the proliferation of cervical cancer cells that could end in cell death. To further characterise the role of the IL-2 on cell death we analysed the expression of cell cycle and apoptosis-related genes in two cervical cancer cell lines, HeLa and INBL. First, we looked for the ideal concentration of IL-2 that could affect cell proliferation, using the crystal violet assay with different concentrations of IL-2. The results show that 100 UI/ml of IL-2 was the optimal concentration to decrease proliferation. Then, we analysed apoptosis by flow cytometry, and we found that only a small fraction of the cell population underwent apoptosis. We determined the expression of p21, p53, pRb, Bcl-2 and Bax by PCR, and the activation of p53 and Rb by flow cytometry. We measured senescence with negative results. The expression of Bax was not affected however the expression of Bcl-2 increased at 96 h. The expression of p53 and p21 increased in HeLa and INBL cells but the expression of Rb did not change. Activation of p53 was observed in INBL cells but not in HeLa cells and activity of Rb was not affected. The effect of IL-2 on the cell cycle does not induce the activation of cellular senescence, does not activate the pro-apoptotic protein Bax and upregulates the anti-apoptotic protein Bcl-2 conferring the cells protection against apoptosis. IL-2 is a pleiotropic cytokine thus a more detailed inspection of IL-2 signalling in cancer cells will aid our understanding of the specific effects of this cytokine on cervical cancer cells.

P.10-158-Tue
Effect of IL-2 on the secretion of lactate and the NADH/NAD+ ratio in cervical carcinoma cell line SiHa

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Cervical Cancer is the second cause of death in women worldwide, in Mexico, the highest incidence in gynaecological tumours is breast cancer followed by cervical cancer. The IL-2R has been found to be expressed on non-haematopoietic cells, especially in several types of solid tumours. However, the function of this receptor on malignant cells has not been defined. We documented the expression of the IL-2R and the production of IL-2 in cervical cancer cells that induces its proliferation. A relevant characteristic of many types of cancer is the ability to reprogram energy metabolism to fuel cell growth and division. The increased uptake of glucose and its conversion into lactate is one hallmark of cancer cells, usually, in these cells, the excess of lactate is secreted to modify tumour microenvironment promoting tumour cell invasion and metastasis formation. To analyse the effect of IL-2 on lactate production and on the $NADH/NAD^+$ redox state we treated cervical carcinoma cell lines with high (100 UI/ml) or low (10 UI/ml) doses of IL-2. The cell line SiHa was treated with IL-2 and the metabolites were measured by using the Lactate Assay Kit or the $NADH/NAD^+$ quantitation kit. Our results show that the treatment with low doses of IL-2 induces an increase in the secretion of lactate, nevertheless, with high doses the production of lactate decreases. The $NADH/NAD^+$ ratio was consistent with a high metabolic flux, typical of transformed cells, where a high $NADH/NAD^+$ redox state is capable of sustaining aerobic glycolysis. The role of IL-2 in the metabolic switch of cancer cells is poorly studied. Here we show that IL-2 induces a change in lactate secretion and the redox state of the cell which is dose-dependent. These results suggest that IL-2 regulates glucose conversion into lactate to increase its concentration for intracellular acidification giving cervical cancer cells an advantage to proliferate and to migrate.

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P.10-159-Wed
Anticancer activity on melanoma cells of new molecular hybrids of tetrahydroquinoline/ isoxazoline

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Melanoma is one of the commonest form of the skin cancer. Melanoma incidence is not strongly correlated with age; however, it is one of the prevalent cancer and cancer deaths in young people. Overall, melanoma is a strong example of how genetics and the environment cooperate to stimulate melanoma-genesis. Despite of there are new molecules to treat several types of cancer, these drugs have side effects, poor selectivity, and in some cases, cancer cells generate resistance to treatment. Based on the above; in this study, we tasted a new family hybrid of tetrahydroquinoline/isoxazoline and its derivatives on murine melanoma cells (B16F10). To achieve this objective the cells were grown in EMEM medium supplemented with 7% FBS and pH 7.4, and cell viability was assessed by the MTT method. Cells were treated with various concentrations (5, 25, 50 and 100 μM) of new molecular hybrids for 48 h. Cytotoxic activity was measured, showing that three of sixteen molecules had a cytotoxic concentration(CC50) lower than 30 μM, the CC50 values for molecular hybrids with tetrahydroquinoline ring substituted at C-6 with hydrogen, methyl and chlorine were 11.37, 21.95 and 25.59 μM respectively. On the other hand, we evaluated these three compounds in lower concentrations with crystal violet method. The compounds were tested at 1, 2.5, 5 and 10 μM for 12, 24, 48 and 72 h, the results showing that hybrid with hydrogen at tetrahydroquinoline ring has a statistically significant difference respect to control cells in 24 h of treatment at 10 μM. meanwhile the other hybrids showed statically differences to 48 h of exposition at the same concentration. In conclusion, according to results obtained, molecular hybrids of tetrahydroquinoline/isoxazoline would be a good alternative to potential treat of melanoma cancer due to reduced cell proliferation at 10 μM from 24 h.

P.10-160-Mon
Comparison of lipid metabolism disruption in various brain tumors

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It is known that the lipid metabolism reprogramming is one of the new hallmarks of cancer. Over the last decade, great progress was made in the understanding of the role of lipid metabolism in the progression of cancer. The main analysis of brain tumor was focused on glioblastoma multiforme (GBM), one of the most aggressive brain cancer. We have collected over hundred untargeted lipidomic profiles from samples of unmodified brain and four brain tumors: GBM, astrocytoma, meningioma, and neurinoma. To increase the reliability of the analysis, we have combined our mass-spectrometry profiles of lipids with data from transcriptomics and proteomics databases similar to method proposed by Pirhaji and coworkers. We have identified subset of common features differ in unmodified brain and tumor samples. We have also shown that despite the presence of common features each type of tumor has its unique pattern. Detected patterns could be used for development of rapid diagnostics of the tumor progression for biomarker prediction and drug development.

P.10-161-Tue
RAS activation at the Golgi complex prevents tumorigenesis by inducing apoptosis via PTPk-mediated inhibition of ERK activation

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The RAS/RAF/MEK/ERK pathway has been reported to be activated in over 80% of all cutaneous melanomas, making it the focus of many scientific studies in the melanoma field. Discoveries of mutations and aberrant expression of components in this cascade, in particular, BRAF and NRAS render a deeper understanding of the mechanisms responsible for oncogenesis and provide new therapeutic strategies for this deadly disease. Mechanistically, mutated BRAF and NRAS exert most of the oncogenic effects through the activation of the ERK. Ras proteins are distributed in different types of plasma membrane microdomains and endomembranes. We have previously demonstrated that compartmentalization dictates Ras utilization of effectors, the intensity of its signals and the biological response of the cells. Activated Ras enhances proliferation, transformation and tumoral progression in melanoma animals models from most of its platforms, with the exception of the Golgi Apparatus. Interestingly, we have found that Ras signaling from Golgi impairs ERK activation mediated by Epidermal Growth factor (EGF). Moreover, using microarray technology we identified the transcriptional targets of Ras signaling in Golgi and we found that one of these genes was PTPk which is a tyrosine phosphatase receptor type that regulates the phosphorylation state of many important signaling molecules in cancer. PTPk regulates a variety of cellular processes including cell growth, differentiation, mitotic cycle, apoptosis and oncogenic transformation. Using Zebrafish model, we demonstrated that Ras activation at the Golgi Complex induces PTPk expression to impair ERK activation and exert its anti-tumorigenic effect in melanoma in a p53 dependent manner. Moreover, we found that PTPk appears altered in human melanoma and PTPk downregulation correlates with poor survival of patients.

P.10-162-Wed
Theranostics as new methodology using novel polymethinium salts: from diagnostics to therapy

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Theranostics, a new field of medicine combining specific diagnostic tests and subsequent targeted therapy, have been lately demanded for the effective treatment of many human diseases such as cancer. Recently, we reported the synthesis of a novel pentamethinium salt with an incorporated quinoxaline unit and described its structure. Herein, we present the biological and chemical characteristics of this structure and a system based on silica nanoformulations. First, the subcellular distribution of our autofluorescent compound and nanoparticles loaded with the drug was studied in U-2 human sarcoma cells. When the cells were exposed to the methinium system, the drug rapidly accumulated in the mitochondria until cell death. Next, growth inhibition (IC_{50}) was determined by monitoring cell viability. The tested substance showed high cytotoxicity towards the malignant cell lines, but was not cytotoxic to non-malignant BJ fibroblasts. Regarding these results, our findings indicate that theranostics and its specific nano-based diagnostics and therapy could open new pathways of effective cancer treatment.

P.10-163-Mon
Biochemical and biological analysis of novel inhibitors of CDC25 phosphatases acting on melanoma cell lines

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Cell division cycle 25 (CDC25) enzymes are dual phosphatases that regulate cell cycle progression, being also involved in cell response to DNA damage. Their overexpression has been detected in many tumors, including melanoma. In a previous study compound 19 (NSC 28620) showed a potent *in vitro* inhibition on CDC25 activity; furthermore, this inhibitor affected the cell viability of some cancer cell lines. In order to identify more potent inhibitors, which possibly functioned as novel anti-cancer drugs, the structure of NSC 28620 was used as a lead compound to synthesize 29 novel molecules. Among them the most active compounds had *Ki* values in the 0.79–10.0 μM interval. Kinetic studies seem to identify two different mechanisms of inhibition, related to specific functional groups possessed by the inhibitors. The cytotoxic effect of the most active compounds on melanoma cell lines was analysed. MTT assay evidenced that at a low concentration, i.e. 10 μM, essentially one compound (cpd 21) significantly reduced the cell growth of melanoma cells in a time-dependent manner. The effect of cpd 21 on cell viability was further confirmed by the colony-forming assay, that showed a similar trend in the ability to inhibit the clonogenicity of melanoma cells. Moreover, the analysis of the levels of various target proteins indicated that cpd 21 provoked an early increase of pCdk1 and a decrease of CDC25C. An arrest of melanoma cells in G2/M of cell cycle, followed by the activation of a caspase-mediated apoptotic program was also observed during the cell treatment with cpd 21. Conversely, cpd 21 didn't show a cytotoxic effect on BJ-5ta, a nonmalignant fibroblast cell line. Finally, a better investigation on the molecular mechanisms activated by cpd 21 evidenced that the cytotoxic effect of this compound on melanoma cells could be mediated by the reduction of pAkt and an increase of p53 protein levels.

P.10-164-Tue
Novel effect of azithromycin in imatinib-resistant CML cells

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Azithromycin (AZM), is a FDA approved macrolide antibiotic which has been used for respiratory, skin and ear infections. In recent years, possible effectiveness of antibiotics on targeting cancer stem cells has been an attractive research area. CML (chronic myeloid leukemia) is a form of blood cancer which is characterised with the presence of hematopoietic stem cells which are resistant to therapy. This study was designed to examine the potential anticancer effect of azithromycin on K562s (Imatinib sensitive) and K562r (Imatinib resistant) cells. K562r cells were acquired by culturing CML cells with increasing concentrations of Imatinib. We treated K562s and K562r cells with 12.5–500 μM concentrations of AZM before detecting cell viability, apoptosis, MDR function and caspase 3/7 activity with spectrophotometric MTT assay, flow cytometric Annexin V-PE/7AAD and Rho123 staining and caspase 3/7 activity assays respectively. Our results showed that, AZM treatment without Imatinib, inhibited cell viability, induced apoptosis and increased caspase 3/7 activity in K562s cells. AZM treatment without Imatinib did not exhibit a potent effect on cell viability parameters in K562r cells which have high MDR expression. On the other hand, when combined with Imatinib, AZM more potently and significantly ($P < 0.05$) suppressed cell viability, induced apoptosis and caspase3/7 activity in comparison to K562r cells treated with only Imatinib. We also detected decreased MDR function in K562r cells treated with AZM/Imatinib combination compared to Imatinib treated K562r cells. In conclusion, AZM and AZM/Imatinib treatment induced apoptotic markers significantly ($P < 0.05$) in K562s and K562r cells respectively. The apoptotic effects of AZM on K562r cells may have been partly mediated by its effects on the functionality of MDR. However, since AZM is also effective on K562s cells, other mechanisms activated by AZM are needed to be clarified in further research.

P.10-165-Wed
Modulation of ER stress related genes by MSM in HCT-116 colon cancer cells

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Methylsulfonylmethane (MSM) is a small sulfur containing molecule which has been used as a dietary supplement in various inflammatory conditions. Recent studies have shown that MSM may also exhibit apoptotic effects depending on different types of cancers.

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Several mechanisms are involved in inducing apoptosis including ER stress. In our previous study we indicated that MSM induces apoptosis in HCT-116 colon cancer cells regardless of their p53 status. The aim of this study is to determine the role of ER stress in inducing apoptosis in HCT-116 p53 +/+ and HCT p53−/− colon cancer cell lines. HCT-116 p53 +/+ and HCT-116 p53 −/− colon cancer cells were incubated with different doses (200, 400, 600 and 800 mM) of MSM for 24 h. Cells were then collected for RNA isolation, cDNA synthesis and RT- PCR. MSM treatment increased expression levels of CHOP, ATF6 and IREI genes at 200–400 mM concentrations whereas decreased expression of these genes at 600–800 mM concentrations in both cell lines. All concentrations of MSM decreased GRP78 gene expression in both HCT-116 p53 +/+ and HCT-116 p53 −/− cells. PERK was downregulated with all concentrations of MSM except 400 mM in HCT-116 p53 +/+. MSM treatment increased and decreased PERK gene expression in HCT-116 p53 −/− colon cancer cells at 200–400 mM and 600–800 mM concentrations respectively. This study was designed to disclose the role of ER stress in the induction of apoptosis by MSM. Our results showed that different doses of MSM may exhibit opposite effects on the expression of ER stress related genes. According to our data CHOP gene expression increased significantly by 400 mM MSM treatment in both cell lines. In conclusion, our results show that modulation of PERK, ATF6, IRE1 and CHOP may play important roles in the induction of apoptosis by MSM in HCT-116 colon cancer cell lines.

P.10-166-Mon
Interplay between glucose and palmitate uptake in breast carcinoma in vitro

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One of the most studied tumor cells lines *in vitro* is the breast carcinoma MDA-MB-231 cell line. Several studies have proved its glycolytic profile, namely known as the Warburg effect. Glutamine oxidation is also important for its metabolism. Nevertheless, the use of fatty acids for obtaining energy in these cells is still rising. Palmitic acid is the most common saturated fatty acid, containing sixteen carbons in its structure. However, the use of palmitate for metabolic studies in MDA-MB-231 is not very extended due to its pro-apoptotic effect in this cell line after certain time exposure. Nonetheless, in this work we used palmitate as a metabolic fuel for just 30 min in order to see the almost immediate response of the cells to its presence, after a 30 min fast period. Our results show that MDA-MB-231 cells are not able of oxidizing palmitate nor producing lactate from it. Simultaneous presence of palmitate with glucose or with glutamine does not affect glucose nor glutamine uptake in these cells. However, we observed that even low concentrations of glucose increase palmitate uptake in MDA-MB-231 after a 30 min incubation. Treatment with 5 mM 2-deoxyglucose also for 30 min counters this rise, since 2-deoxyglucose diminishes palmitate uptake. Increasing glucose concentration to the same doses of 2-deoxyglucose leads to a prevalence of the glucose effect on palmitate uptake. The exact role of glucose and glucose derivatives should be further studied in order to know more about palmitate metabolism in this cell line. [Our experimental work is supported by grants BIO2014-56092-R (MINECO and FEDER) and P12-CTS-1507 (Andalusian Government and FEDER) and funds from group BIO-267 (Andalusian Government). The "CIBER de Enfermedades Raras" is an initiative from the ISCIII (Spain). This communication has the support of a travel grant "Universidad de Málaga. Campus de Excelencia Internacional Andalucía Tech"].

P.10-167-Tue
MicroRNA-206 reduces cell survival of breast cancer cells by targeting nicotinamide phosphoribosyltransferase and reducing cellular NAD levels

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Background: Nicotinamide adenine dinucleotide (NAD) is a critical coenzyme for redox reactions as well as cellular processes that are important cancer development, including chromatin structure, DNA repair, stress responses, transcription, signaling, apoptosis, differentiation, and improved life span. Nicotinamide phosphoribosyltransferase (NAMPT) is the key enzyme and regulator of the intracellular nicotinamide adenine dinucleotide (NAD) biosynthesis. Cancer cells, have higher rate of NAD consumption and therefore NAMPT is essential for their survival. Thus the aim of this study was to investigate the effect of NAMPT inhibition by miR-206 on breast cancer cell survival. Material & Methods: MCF-7 and MDA-MB-231 breast cancer cells were transfected with miR-206 mimic, inhibitor and their negative controls. NAMPT levels were assessed by real-time PCR as well as western blotting. WST-1 test was used for the assessment of cell survival. Quantification of NAD levels was performed by enzymatic methods. Apoptosis assay was performed by labeling cells with Annexin V-FITC and propidium iodide followed by flow cytometric analysis. Bioinformatics analysis was done to assess whether NAMPT 3′-UTR is a direct target of miR-206 and the results were confirmed by the luciferase reporter assay. Results: NAMPT levels were higher in MCF-7 and MDA-MB-231 cell lines compared to MCF-10 cells. Upregulation of miR-206 reduced NAMPT expression at the protein level, leading to a significant decrease in the cellular NAD levels. NAMPT 3′-UTR was shown to be a direct target of miR-206. miR-206 reduced cell survival and significantly induced apoptosis. Conclusion: Targeting of NAMPT-mediated NAD salvage pathway by miR-206 and the subsequent decline in cell survival might provide a new approach for breast cancer therapy.

Xenobiochemistry and drug metabolism

P.11-001-Mon
Gut hormone GPCRs as potential off-targets for beta-adrenoreceptor blocking agents

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Type 2 diabetes mellitus (T2DM) is one of the most severe side effects induced by pharmacotherapy. Among many drug classes

which were known to disrupt the glucose homeostasis cardiovascular drugs such as beta-blockers are constantly optimized to prolong the harmless treatment time. We hypothesized that one of the mechanisms by which the hyperglycemia risk can be decreased are off-target interactions between beta-blockers and gut hormone G protein-coupled receptors such as glucagon-like peptide 1 receptor (GLP-1R), gastric inhibitory polypeptide receptor (GIPR) and glucagon receptor (GCGR). So-called incretin receptors (GLP-1R and GIPR) are well-known drug targets to treat T2DM. We observed that the strength of enhancing the incretin effect by certain beta-blockers is highly correlated with their impact on the glucose serum levels. Beta-blockers of the highest binding affinity towards gut hormone receptors demonstrated the fewest number of drug-induced T2DM cases in clinical trials. Our observations have been implemented in the freely accessible web service GUT-DOCK (http://gut-dock.biomodellab.eu). Using that docking web service User can test any drug-like molecule for potential off-target interactions with gut hormone receptors. GUT-DOCK was constructed using Autodock VINA and our previously developed web service GPCRM (http://gpcrm.biomodellab.eu) for structure prediction of GPCRs.

P.11-002-Tue
Development of the controlled level of adaptation for toxicological studies

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Toxicological research shell consider multiple parameters, which have a wide range of physiological fluctuations. Heterogeneous distribution of some indicators values can result in results interpreting implications, especially under conditions of low-intensity impact. Indeed, the recognition of the organism's response in the range of physiological adaptation – pseudo-adaptation (compensated hidden pathological process) is a challenging task even for a modern laboratory. For this reason modeling of the additional load that reduces the adaptation potential and accordingly, excludes the possibility of pathological process compensation, is an advanced tool that helps improve the diagnostic reliability of experimental results. Taking into consideration the generally accepted approaches to the toxicological experiments conducting, the process of reducing adaptive potential of experimental animals should be simple, effective and controlled. The modification of diets composition due to changes in the content of macro- and micronutrients corresponds to these requirements. We have conducted the research into developing adaptive potential reduction model in rats through use of diets deficient in essential substances (B vitamins – thiamine, riboflavin, niacin and pyridoxine, iron and magnesium salts). The physiological and biochemical status of rats was evaluated on the basis of hematological, biochemical, morphological, immunological research findings. Upon the data analysis, there were determined the threshold values for the vitamins and minerals content in the diet, allowing for desired reduction in the level of adaptive potential in males and females: 75, 30 and 19% for males and 75, 28 and 18% for females, respectively. The given modification of the diets vitamin-mineral composition can serve as a model for rats' adaptive potential reduction in toxicological studies. This work was supported by Russian Science Foundation grant No. 16-16-00124.

P.11-003-Wed
Identification of covalent adducts of hemoglobin with diclofenac metabolites

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The liver is known to be able to generate toxic metabolites, and it is the main target organ of toxicity caused by xenobiotics. The main cause of the resulting side effects is the formation of reactive metabolites (RM) that form covalent adducts with the protein. Of great interest is the approach based on the electrochemical oxidation of xenobiotics, which makes it possible to generate metabolites, to evaluate their reactivity and the possibility of forming adducts with proteins. This paper investigates the possibility of forming adducts of hemoglobin (HB) with RM of diclofenac (DCl) in vitro. Electrochemical oxidation of DCl was carried out in an electrochemical cell Reactor Cell (Antec Scientific). To assess the possibility of covalent binding, the generated metabolites were incubated with HB, and tryptic hydrolysis was carried out followed by mass spectrometric analysis. An increase in the working electrode potential to 3 V was shown to lead to the formation of the metabolite DCl+2O and the product of its dehydrogenation DCl+2O-2H, in quantities sufficient to detect modified peptides that were not found in earlier studies. Also, new data were obtained on possible sites of RM attachment to HB, and modified peptides (MP) were identified. The main problem in identification is the low proportion of MP in relation to the unmodified peptide. Substantial difficulties are caused by the elution of MP from the sorbent when they are enriched, due to the low amount of the sample. It was decided to carry out metal-affinity chromatography on a Ni containing sorbent to enrich the sample directly on the MALDI target. Accordingly, it was shown that the application of collapsed monolayers of Ni stearate (Langmuir–Blodgett film) onto the target makes it possible to carry out the metal-affinity isolation of the MP from a sample of the minimum volume, thus eliminating the elution step.

P.11-004-Mon
Nanocarrier of apoferritin with encapsulated ellipticine – its construction and properties

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One of the approaches in mitigating the adverse effects of drugs is their encapsulation inside a suitable nanocarrier, allowing for a targeted delivery to disease tissue while avoiding healthy cells. Nanocarriers prepared using ubiquitous proteins or protein cages appear suitable for several anticancer drugs. Apoferritin is the iron-free form of ferritin, a naturally occurring iron-storage protein. Using apoferritin as a nanocarrier has the potential to move

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through the body without inducing any resistance from the immune system of the patient. Furthermore, apoferritin can be modified with recognition ligands to achieve tumour-specific targeting. The simple-to-use encapsulation protocol (creating ApoElli) was developed and the prepared nanocarrier was characterized. The nanocarrier exhibits narrow size distribution, which suggests being suitable for entrapping of the hydrophobic molecule of ellipticine. The release of ellipticine at acidic (6.5) and neutral (7.4) pH was studied. Ellipticine is gradually released from its ApoElli form into the water environment under acidic pH; more than 80% ellipticine was released after 48 hrs incubation at pH 6.5. In contrast, at pH 7.4 only less than 20% was released. ApoElli is also stable after its storage at physiological pH (7.4) up to 2 month at 4°C. Microsomal cytochrome P450 incubated with free ellipticine and/or its ApoElli nanocarrier form in the presence of NADPH were capable of oxidizing ellipticine to its metabolites and generating ellipticine-derived DNA adducts, both under pH 6.5 and 7.4. The ApoElli is toxic to UKF-NB-4 neuroblastoma cancer cells. On the contrary, cytotoxicity of this nanocarrier is significantly lower for non-malignant cells (non-malignant fibroblasts, HDFn cells) than for these cancer cells. Supported by GACR 17-12816S and GAUK 998217.

P.11-005-Tue
Dianthus superbus attenuates angiotensin II-induced renal dysfunction in glomerular mesangial cells

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Glomerular fibrosis is caused by accumulation of extracellular matrix (ECM) proteins in the mesangial interstitial space resulting in fibrosis manifested by either diffuse or nodular changes. *Dianthus superbus* belongs to the Caryophyllaceae family and has been used in traditional medicine as a diuretic, a contraceptive, and an anti-inflammatory agent. The aim of this study was to investigate the effects of *Dianthus superbus* on angiotensin II (Ang II)-stimulated glomerular fibrosis in human renal mesangial cells. *Dianthus superbus* pretreatment attenuated inflammatory factors such as intracellular cell adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1). Additionally, *Dianthus superbus* suppressed transforming growth factor β (TGF-β)/Smad signaling pathway. Collagen type IV, glomerular fibrosis biomarker, was significantly decreased by *Dianthus superbus*. Moreover, *Dianthus superbus* inhibited translocation of nuclear factor kappa B (NF-κB) in Ang II-stimulated mesangial cell. This study further revealed that *Dianthus superbus* significantly improved Ang II-induced reactive oxygen species (ROS) in a dose dependent manner. These findings suggest that *Dianthus superbus* have protective effect against renal inflammation, fibrosis and oxidative stress. Therefore, *Dianthus superbus* may be potential therapies targeting glomerulonephritis and glomerulosclerosis leading to diabetic nephropathy.

P.11-006-Wed
Tyrosine kinase inhibitors vandetanib, lenvatinib and cabozantinib affect oxidation of an anticancer drug ellipticine by cytochromes P450 but not by peroxidases

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Vandetanib, lenvatinib and cabozantinib are tyrosine kinase inhibitors (TKIs) targeting VEGFR subtypes 1 and 2, EGFR and the RET-tyrosine kinase, thus considered as multiple TKIs. These TKIs have already been approved for treating patients suffering from thyroid cancer and renal cell carcinoma, and further clinical trials are ongoing for prostate cancer and glioblastoma multiforme. Ellipticine and its derivatives are other anticancer agents that are effective against certain tumors of the thyroid gland (anaplastic thyroid carcinoma), ovarian carcinoma, breast cancer and osteolytic breast cancer metastasis. Ellipticine anticancer efficiencies are dependent on its metabolism leading both to the activation metabolites causing DNA damage (covalent DNA adducts) and their detoxification to products that are excreted. Ellipticine is oxidized by microsomal cytochrome P450 (CYP) enzymes and peroxidases. Oxidative activation leads to formation of 12-hydroxy- and 13-hydroxyellipticine, reactive metabolites that dissociate to ellipticine-12-ylium and ellipticine-13-ylium, binding to DNA, while formation 9-hydroxyellipticine and the ellipticine dimer are considered to be detoxification products catalyzed by CYPs and peroxidase, respectively. A number of studies testing the effectiveness of individual anticancer drugs alone or in a combination with other cytostatics demonstrated that such combination can have additive and/or even synergistic effects on treatment regimen. The aim of this study was to study the effect of TKIs vandetanib, lenvatinib and cabozantinib on oxidative metabolism of ellipticine. All tested TKIs inhibit oxidation of ellipticine catalyzed by hepatic microsomes, but not by peroxidases (horseradish peroxidase, lactoperoxidase and myeloperoxidase). The mechanism of these effects is studied in details. The study might provide a rationale for the clinical evaluation of the combination of TKIs and DNA-damaging anticancer drugs. Supported by GACR 18-10251S.

P.11-007-Mon
The hepatoprotective capacity of natural products from South Africa

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Drug induced liver injury (DILI) is recognised as a significant clinical problem that accounts for up to 50% of all cases of acute liver failure. DILI has an approximate annual incidence of 20 cases per 100 000 people exposed to medications or dietary supplements. Natural products are well established in traditional medicine, however the therapeutic value of several species has not been thoroughly explored. The aim of this study was to develop and characterize a suitable and cost effective *in vitro* hepatoprotection screening assay. High content analysis measured the hepatoprotective capacity of mushroom (*G. lucidum*, *R. capensis*, *P. ostreatus* and *L. elegans*) and plant extracts (*C. intermedia*, *O. ficus indica* and *K. africana*). All extracts were screened against HepG2 cells using Hoechst- PI dual staining to evaluate the cytotoxicity of tested extracts. Only the ethanolic

G. lucidum extract revealed toxicity against HepG2 cells at physiological concentrations. The accuracy of the hepatoprotection model was confirmed through HepG2 cell-based assays that measured the protective effects against hepatotoxins and assessed the inhibition of liver enzymes (β-glucuronidases and carboxylesterases) by hepatoprotectants. Anti-oxidant capacities of extracts were scored using cell- (CAPe) and chemical-based assays (DPPH, FRAP, ORAC, NO production). *C. intermedia* and *G. lucidum* scored the highest overall anti-oxidant capacity; displaying the most promising DPPH, FRAP, ORAC and CAPe results. *C. intermedia*, *O. ficus indica* and *G. lucidum* presented the highest NO scavenging abilities, while ethanolic extracts displayed better inhibition against the enzymes in comparison to aqueous extracts. Together these assays improve the predictive value of *in vitro* hepatoprotection screening platforms, where *G. lucidum* and *C. intermedia's* promising anti-oxidant capacities or *O. ficus indica's* anti-inflammatory properties may offer novel therapies against DILI.

P.11-008-Tue
Doxorubicin and ellipticine loaded apoferritin and its effect on cancer and healthy cell lines

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Despite advances in cancer diagnosis and therapy, high-risk neuroblastoma (NBL) remains lethal in about 50% of patients because of aggressive cell growth and chemoresistance. Therefore, new agents are needed to improve its prognosis. Apoferritin is a protein composed of 24 polypeptide subunits, structurally arranged to create an internal cavity, which can be artificially employed for carrying of some molecules of interest. The aim of this study was to compare the cytotoxic effects of doxorubicin (DOX) and ellipticine (ELLI) loaded apoferritin (APODOX resp. APOELLI) and free DOX resp. ELLI on NBL cells UKF-NB-4, chemoresistant sublines derived from this cell line and non-malignant fibroblasts (HDFn) *in vitro*. We show here that the effect of APODOX on NBL cells is similar to that of free DOX. The cytotoxicity of APOELLI is lower than cytotoxicity of free cytostatic, but APOELLI induces more double strand breaks than free ELLI. Further, using fluorescence microscopy, we have shown that apoferritin can deliver drugs inside cancer cells and the drug exerts their effect thereof. Entry of APODOX and free DOX into cancer cells was similar. However, entry of APOELLI was significantly lower than entry of free ELLI. The extent of APOELLI enter correlated with formation of covalent ELLI-derived DNA adducts in tested cells; the levels of ELLI-DNA adducts generated by APOELLI were 67% of those formed by free ELLI. The results found in this study seem to be promising, because encapsulation does not affect toxicity of cytostatic and improves drug stability. Moreover, cytotoxicity of drug loaded apoferritin is significantly lower for non-malignant cells than for cancer cells. In addition, apoferritin loaded DOX and ELLI are cytotoxic in hypoxic conditions (1% O_2). We suppose that apoferritin is targeted to the several cancer cells including NBL through TfR 1 and/or SCARA 5 which are expressed in many cancer cells. (*Supported by GACR 17-12816S and GAUK 998217.*)

P.11-009-Wed
Oxidation of a tyrosine kinase inhibitor vandetanib by human cytochromes P450 and flavin monooxygenases in vitro and its effect of DNA adduct formation mediated by an anticancer drug ellipticine

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Vandetanib is a tyrosine kinase inhibitor (TKI) used for treatment of certain tumors of the thyroid gland. It inhibits signalling of epidermal growth, vascular endothelial growth factor or rearranged during transfection. Here, using human hepatic microsomes and recombinant cytochromes P450 (CYPs) and flavin-containing monooxygenases (FMOs) expressed in Supersomes™, oxidation of vandetanib was studied. The vandetanib metabolites were separated by HPLC and identified by mass spectroscopy. Human hepatic microsomes oxidize vandetanib to *N*-desmethylvandetanib, but not to vandetanib-*N*-oxide. Of all tested human CYP enzymes, the CYP1A1, 2C8, 2D6, 3A4 and 3A5 enzymes, mainly in the presence of cytochrome b_5, oxidize vandetanib to *N*-desmethylvandetanib. No vandetanib-*N*-oxide was generated by tested human CYPs. However, FMO enzymes were able to generate this metabolite. Of three human FMOs tested (FMO1, FMO3 and FMO5), FMO1 and FMO3 oxidize vandetanib to vandetanib-*N*-oxide. FMO1 was more effective than FMO3 in this reaction. The results found in this study approved the knowledge showed by the preliminary studies, suggesting that vandetanib is oxidized to *N*-desmethylvandetanib and vandetanib-*N*-oxide, and specified the efficiencies of individual CYPs and FMOs in the reactions. Moreover, they indicated an essential role of cytochrome b_5 in oxidation of vandetanib to *N*-desmethylvandetanib by CYP3A4. Because this CYP is the most important enzyme activating also another anticancer agent that is effective against certain tumours of the thyroid gland, DNA-damaging drug ellipticine, the effect of vandetanib on metabolic activation of this drug was investigated. An inhibition effect of vandetanib on the most efficient anticancer effects of ellipticine, formation of covalent ellipticine-derived DNA adducts, was found. Cytochrome b_5 plays an important role also of this CYP3A4-mediated activity. Supported by GACR 18-10251S.

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P.11-010-Mon
A tyrosine kinase inhibitor lenvatinib is oxidized by human cytochromes P450 and aldehyde oxidase in vitro

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Lenvatinib is an oral, multitargeted tyrosine kinase inhibitor (TKI) of vascular endothelial growth factor receptors (VEGFR1-VEGFR3), fibroblast growth factor receptors (FGFR1-FGFR4), platelet-derived growth factor receptor (PDGFR)α, rearranged during transfection (RET), and v-kit (KIT) signalling networks implicated in tumor angiogenesis. It is used for treatment of certain tumors of the thyroid gland and metastatic renal cell carcinoma. Based on preliminary studies using human hepatic microsomes, lenvatinib was suggested to be oxidized by cytochromes P450 (CYPs), mainly by CYP3A4, to its *O*-demethylated metabolite, a desmethylated form of lenvatinib. However, no direct prove of this suggestion was demonstrated. Therefore, the aim of this study was to investigate the metabolism of lenvatinib by human microsomal enzymes *in vitro* in detail. Utilizing human hepatic microsomes and recombinant CYPs expressed in Supersomes™, the metabolism of lenvatinib was studied. The lenvatinib metabolites were separated by HPLC and identified by mass spectroscopy. Using human hepatic microsomes *O*-desmethyllenvatinib, *N*-depropylated lenvatinib and next one metabolite were produced. Of all tested human CYP enzymes, the CYP1A1, 1A2, 2C19 and 3A4 oxidize lenvatinib to several metabolites. *O*-desmethylated lenvatinib was generated by CYP1A1, 1A2 and 3A4, while CYP2C19 forms another metabolite; its structure has not yet been identified. CYP1A1 and 3A4 are also responsible for oxidation of lenvatinib to *N*-depropylated metabolite. Cytochrome b_5 plays an essential role in the CYP2C19 and 3A4 activities to oxidize lenvatinib. Besides CYPs, aldehyde oxidase (AO) oxidizes lenvatinib forming one metabolite; its structure has not yet been identified. Further characterization of structures of all lenvatinib metabolites formed by the tested enzymatic systems is under way in our laboratory. Supported by GACR 18-10251S.

P.11-011-Tue
Effect of stress on the expression of cytochromes P450 in germ-free mice

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Gut microbiota is involved in a number of different metabolic processes of the host organism, from physiological ones such as digestion of complex polysaccharides, vitamin and amino acid synthesis to pathophysiological processes such as immune and cardiovascular diseases. Gut microbiota has also impact on biotransformation enzymes, directly or indirectly, by modulation of enzymes of the first and second phase of metabolism of xenobiotics. Moreover, metabolism of xenobiotics can be affected by inflammation and stress. There are more and more studies suggesting that stress is associated with changes of immune system, and that inflammation has effect on cytochromes P450. In this study, we focused on the changes of mRNA expression of cytochromes P450 (CYPs), transcriptional factors and enzyme activity of selected liver CYPs of germ-free (GF), stressed GF mice, stressed specific-pathogen-free (SPF) and SPF mice. Generally, GF mice are more sensitive to stress. In stressed GF mice, the mRNA expressions of transcriptional factors (Ahr, CAR, PPARα), CYP 2A5/4 and CYP 2D22 were increased, but the activities of the enzymes were not affected. In the SPF mice, stress caused decrease of expression and activity of CYP 1A1/2 and of the CYP 2C38 mRNA and activity of this enzyme (with diclofenac as a substrate). Interestingly, mRNA expression of CYP 2C29 was increased, however, the activity of this enzyme (with diazepam as a substrate) was not changed. Results indicate an effect of stress on expression of CYP enzymes with clear differences between the mRNA expression of the CYP enzymes in GF and SPF mice. Acknowledgment: Financial support from the GACR project 17-09869S and a UPOL students' project IGA_LF_2017_011 is gratefully acknowledged.

P.11-012-Wed
The effect of a dietary antioxidant on the antioxidant defense system in juvenile rainbow trout, *Oncorhynchus mykiss*

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The objective of the study was to characterize the response of some antioxidant defense system components and fish growth rate on dietary supplement by dihydroquercetin (or Taxifolin), a plant polyphenolic compound with proven antioxidant activity in rainbow trout juveniles. Caged trout (initial mean weight 100 ± 10 g) were fed by commercial feed with or without dihydroquercetin since July to September, 2017. Dihydroquercetin was added to the diet by short two-week courses followed by two-week breaks. No significant differences in growth rate were recorded between dietary groups. However, the mortality in juvenile trout group fed by the antioxidant was two-fold lower (1.97% vs 4.19%). Glutathione-S-transferase (GST), catalase (CAT), peroxidase (Px) activities and GSH level was measured in fish tissues each two weeks. Hepatic antioxidant enzymes such as CAT and Px were not affected by antioxidant-enriched diet; however decreased GSH level and increased GST activity were shown at the end of experiment in rainbow trout juveniles fed by dihydroquercetin. The muscle GST activity was reduced since the first antioxidant treatment course indicating high biomarker sensitivity. The muscle GSH level increased after the third dietary dihydroquercetin course. So, the response to antioxidant exposure was stimulated more readily in the skeletal muscles than in the liver. The results indicate that dihydroquercetin improves fish survival rate through the decrease in oxidative stress response in rainbow trout juveniles. The comprehensive study on the mechanisms involved in the response is required. The KarRC RAS Equipment Sharing Facilities were used in the study. The work was financially supported by the Russian Science Foundation, project no. 17-74-20098.

P.11-013-Mon
Effect of quercetin on mRNA and protein expressions of vitamin D metabolizing CYP27B1 and CYP24A1 in human embryonic kidney cell line (HEK-293)

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Vitamin D is essential compound for life having role not only in the regulation of calcium metabolism but also in the regulation of cell proliferation, immune responses, cardiovascular homeostasis and nervous system. These wide range of actions occur with the enzymatic conversion of vitamin D to 1α,25-dihydroxyvitamin D by cytochrome P450 enzymes (CYPs). While CYP27B1 provides the synthesis of hormonally active form of vitamin D, $1,25(OH)_2D$, CYP24A1 involves in catabolism of vitamin D. Quercetin, as one of the important member of polyphenols, is widely studied due to its abundant consumption with a diet, important role in human health as activators or inhibitors for biochemical reaction, antioxidant and anticancer activities. Recently, interaction between vitamin D receptor and quercetin has been reported at molecular level in a few studies. Quercetin may affect the expressions of vitamin D metabolizing CYP enzymes. The aim of this study was to investigate the effect of quercetin on mRNA and protein expressions of vitamin D metabolizing CYP27B1 and CYP24A1 in embryonic kidney HEK293 cell line. The effects of quercetin on CYP27B1 and CYP24A1 mRNA and protein expressions in HEK-293 cell line were determined by qPCR and western blotting techniques, respectively. Results showed that, quercetin inhibited the proliferation of HEK-293 cells in a concentration dependent manner. IC50 value of quercetin on HEK-293 cell line was found as 60.72 μM. Quercetin treatment did not significantly affect the mRNA expressions of CYP27B1 and CYP24A1. While CYP24A1 protein expression was upregulated significantly (1.38 fold), CYP27B1 protein expression was not affected significantly with quercetin treatment compared to 0.35% DMSO containing untreated cells. These results suggested that quercetin may affect vitamin D metabolism negatively in HEK293 cells due to increase in CYP24A1 protein expression.

P.11-014-Tue
Alteration of protein and gene expressions of Hexokinase II in PC3 cell lines by cisplatin-metformin combination treatment

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Metformin is an antidiabetic drug with anticancer properties. Cisplatin is known as one of the most potent chemotherapeutics for treatment of various types of cancer. In order to overcome cisplatin resistance and toxicity, the drug can be combined with other drugs that sensitize tumour cells to cisplatin. The ability of metformin to potentiate cisplatin-mediated killing of cancer cells in vitro, makes it a plausible candidate for combination with cisplatin-based therapy. The aim of this study is to examine the combined effect of these drugs on protein and mRNA expressions of Hexokinase II, a regulatory enzyme participating in glycolysis as well as cancer promotion. The effects of drugs on prostate cancer cell lines were studied using androgen independent PC3 cell line. Cells were treated with either metformin alone in the range of 1–10 mM, cisplatin alone or a combination of these two drugs. Cytotoxicity of drugs were determined with Alamar Blue Assay and IC50 was calculated. The effects of drugs on Hexokinase II mRNA and protein expressions were determined by qRT-PCR and western immunoblotting techniques, respectively. The intensity of each band was analyzed by Image J software program. IC50 value of cisplatin on PC3 cell line was found as 30 μM. Both alone or combination of drugs were inhibited the proliferation of PC3 cells in a concentration dependent manner. Hexokinase II mRNA and protein expressions were significantly downregulated in metformin/cisplatin treated cells compared to control groups. Epidemiological and experimental researches indicates that men with abdominal obesity is a risk factor for prostate cancer. Parallel with our results, the adjuvant role of this type of antidiabetic drug must be investigated for new cancer treatment options.

P.11-015-Wed
In silico prediction of antidepressant-binding sites on human placental glutathione S-transferase pi

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Antidepressants are commonly used treatments for major depressive disorder worldwide. The lipophilic nature of antidepressants means that they can cross the placental barrier. Glutathione *S*-transferases (GSTs; EC 2.5.1.18) belong to a family of related homodimeric enzymes that are involved in phase II detoxification reactions and in non-substrate ligand binding and sequestration. Here, using protein–ligand docking and interaction profiling, we aim at predicting the mode of interaction between human placental GST pi and two tricyclic antidepressants (namely, amitriptyline and clomipramine) as well as between the enzyme and two selective serotonin reuptake inhibitors (namely, fluoxetine and sertraline) based on *in vitro* results from several enzyme kinetic studies previously conducted and reported by our research group. Amitriptyline and clomipramine appear to interact, through their ring system, with a conserved tyrosine residue (Tyr^{79}) traditionally participating in tocopherol binding, with their hydrophilic portions protruding into an electrostatic region at the dimer interface. On the other hand, fluoxetine and sertraline seem to favorably occupy both the G- and H-subsites of the enzyme's active-site cleft at the same time. Accordingly, these interactions may reduce the therapeutic effect of the prescribed antidepressant on the pregnant mother and also leave the fetus prone to various electrophilic substrates with potentially toxic effects. The present study may additionally serve as a starting point for the structure-based design of anticancer drugs, since GST pi is overexpressed in various human cancers and contributes to multidrug resistance by neutralizing chemotherapeutics.

P.11-016-Mon
Membrane-bound cytochrome P450s of 1A subfamily features similar structural pattern of the trans-membrane segment

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Cytochrome P450 (P450) enzymes are components of a mixed-function oxidase system located in the membrane of endoplasmic reticulum. Using multiscale computational methods, we investigated the structure and dynamics of the full-length membrane-anchored P450 1A2 enzymes. The absence of the structural

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information on the trans-membrane (TM) domain of these two proteins was surpassed by employing a spontaneous self-assembly molecular dynamics (MD). The simulation was performed in randomized dilauroylphosphatidylcholine (DLPC)/water/salt mixture and also in randomized palmitoyloleoylphosphatidylcholine (POPC)/water/salt mixture. The resulting membrane-bound full-length structures of P450 1A2 in different membranes were then optimized using coarse-grained and all-atom MD. The resulting models show that, despite of the different membrane thickness, the upper part of the TM helix in both cases directly interacts with a conserved and highly hydrophobic *N*-terminal proline-rich segment of the catalytic domain. The shallow membrane immersion of the catalytic domain appears to induce a depression in the opposite intact layer of phospholipids, which may help in stabilizing the position of the TM helix directly beneath the catalytic domain. The phospholipid membrane thickness has a direct impact on the TM domain tilt being more inclined in case of the thinner DLPC membrane. Supported by GACR 18-10251S. Access to the MetaCentrum and CERIT-SC computing facilities provided under the programs LM2015042 is highly appreciated.

P.11-017-Tue
Computer prediction of the drug-drug interactions severity

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When several drugs are co-administrated, a drug-drug interaction (DDI) phenomenon may appear. Many DDIs are due to changes in the metabolism of drugs. In this case, DDI is manifested by the effect of one drug on the biotransformation of other drugs, its slowdown (in case of inhibition of drug-metabolizing enzymes) or acceleration (in case of induction of drug-metabolizing enzymes), which leads to a change in the pharmacological action of co-administrated drugs. The severity of DDIs can be classified by applying different classification systems. One of the most advanced is OpeRational ClassificAtion (ORCA) system for the classification of DDI, created for physicians to assess the risk of co-administration of two drugs. ORCA divides DDI into five classes: contraindicated (class 1), provisionally contraindicated (class 2), conditional (class 3), minimal risk (class 4), no interaction (class 5). For computer prediction of the severity of DDI, we collected a training set consisting of about 4000 pairs of 500 drugs that belong to classes 1–3 of DDI in case of co-administration. The prediction of DDI classes is based on a combination of modified MNA descriptors – PoSMNA (Pairs of Substances Multilevel Neighbourhoods of Atoms), and a classification algorithm implemented in the PASS (Prediction of Activity Spectra for Substances) software. The average invariant accuracy of prediction, calculated in the leave-one-out and 20-fold cross-validation procedures, is about 0.9. The Russian Science Foundation (grant No. 17-75-20250) has supported the work.

P.11-018-Wed
Staufen1 reads out structure and sequence features in ARF1 SBS for specific target recognition

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Most posttranscriptional regulation of gene expression is based on RNA elements in mRNAs recognized by RNA-binding proteins (RBPs). Besides primary sequence elements, a second layer of information is embedded in 3′UTRs of mRNAs in the form of RNA structure. Double-stranded RBPs can bind structures in 3′UTRs and then exert their function based on dsRNA target recognition through a combination of structure and sequence. Staufen1 is a dsRBP involved in mRNA transport and localization, translational control and mRNA decay by a staufen-mediated mRNA decay (SMD) pathway. The Staufen1 binding site (SBS) within human ADP-ribosylation factor 1 (ARF1) 3′UTR is one such target and Staufen1 binding to the SBS regulates ARF1 cytoplasmic mRNA levels by the SMD pathway. However, how Staufen1 recognizes specific mRNA targets is still unknown. To reveal how Staufen1 binds specific dsRNA targets, we determined the solution structure of the ARF1 SBS – Staufen1 complex by NMR spectroscopy. Our structure reveals that Staufen1 is indeed a sequence-specific dsRNA binding protein. Staufen1 dsRBD4 recognizes consecutive pyrimidines in the minor groove *via* helix α1, β1β2 loop anchors the dsRBD at the end of the dsRNA in the minor groove and lysines in helix α2 bind to the phosphodiester backbone from the major groove side. Staufen1 dsRBD3 displays the same conserved binding mode and specifically recognizes GC base pairs. While dsRBD4 alone can bind in a single binding register on the ARF1 SBS, dsRBD3 requires interactions with dsRBD4 to display its sequence specificity. Staufen1 mutation of the amino acids involved in minor groove recognition of ARF1 SBS impairs SMD leading to a 2.4-fold increase in ARF1 mRNA levels as compared to wt Staufen1. Further mutagenesis data and the biological implications will be discussed. This project is funded by a grant from the Czech Science Foundation to P.J.L. (P305/18/08153S).

Gaseous molecules

P.12-001-Mon
Challenges in analyzing catabolic products of hydrogen sulfide oxidation

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Hydrogen sulfide (H_2S) is an important endogenous signaling molecule and its determination is complicated by its volatility, propensity to oxidize and dynamic interconversions between free and bound forms. Catabolism of H_2S may be assessed by analyzing the more stable products of its oxidation. We optimized an HPLC method that uses monobromobimane (mBrB) to fluorescently label the H_2S catabolites – sulfite, thiosulfate and thiocyanate. Here we report on challenges in determining these analytes, especially in the preanalytical phase. Firstly, sulfite is known to be unstable at low pH and oxidizes rapidly on air (e.g. urinary sulfite decreased by 20% in 2 h). Secondly, we examined the effect of blood collection tubes on analyte concentrations; EDTA and heparin-lithium (Hep-Li) plasma, and serum were collected simultaneously from 3 subjects. Serum yielded 3-times lower concentrations of thiosulfate than Hep-Li plasma, while EDTA collection tubes were contaminated with thiosulfate and sulfite (3.3, 0.1 μmol/l respectively) preventing their use in practice. Thirdly, matrix effects were observed necessitating the use of plasma- and urine-based calibration standards. Using the optimized mBrB method, we determined typical values in 13 healthy control. In Hep-Li plasma the median concentration of sulfite, thiosulfate and thiocyanate was 0.32 μmol/l (range 0.22–0.59), 0.59 μmol/l (range 0.30–1.07) and 76 μmol/l (range 14.5–165.5), respectively. Median urinary concentrations of sulfite, thiosulfate and thiocyanate were 0.07 μmol/mmol creatinine (range 0.02–0.17), 1.24 μmol/mmol creatinine (range 0.80–2.91) and

5.0 μmol/mmol creatinine (range 1.2–17.2), respectively. This study shows that the novel mBrB method enables simultaneous determination of three markers of H_2S oxidation and that meticulous preanalytical measures have to be observed. This work was supported by the grant AZV 16-30384A and the project RVO-VFN 64165.

Redox biochemistry and signalling

P.13-001-Mon
Expression levels of peroxiredoxins in small intestinal mucosa of children with celiac disease

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Celiac disease (CD) is a chronic inflammatory and immune-mediated disorder triggered by the ingestion of gluten. The pathophysiological mechanism of CD has not yet been fully elucidated; however, recent studies suggest that oxidative stress may play an important role in the pathogenesis of CD. Peroxiredoxins (PRXs) are a family of antioxidant enzymes which detoxify hydrogen peroxide, organic hydroperoxides, and peroxynitrite. Six isoforms of PRXs (PRX-1-6) are expressed in mammalians in different cell compartments. In addition to antioxidant functions, PRXs have also been associated with significant cellular processes such as redox homeostasis, cell survival and proliferation, apoptosis and tissue repair. Although it is shown that PRXs play important role in various inflammatory diseases, the role of PRXs in the generation of CD is yet to be known. Therefore, this study aims to investigate the involvement of PRXs in the pathogenesis of CD. For this purpose, small intestinal biopsy specimens were taken from 7 children with CD and 7 children with histologically normal small intestinal biopsies, by endoscopy. The mRNA and protein expression levels of six PRXs were determined by quantitative real-time polymerase chain reaction and Western blot, respectively. It was found that in small intestinal mucosa with CD, mRNA and protein expression levels of PRDX-4 were significantly decreased while that of PRDX-5 were significantly increased, compared to control group ($P < 0.05$). Our preliminary results reveal that PRXs, in particular PRX-4 and PRX-5, may have a role in the pathogenesis of CD. Acknowledgment: This study was supported by TUBITAK (215S650) and Ege University (03/BIL/2016).

P.13-002-Tue
Redox status of neuronal calcium sensor proteins in photoreceptor system

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In the retina, high metabolic activity and abundance of photosensitized reactions contribute to elevated risk of oxidative stress causing photoreceptor loss and irreversible vision deterioration. Here, we demonstrate that oxidative stress of photoreceptors is associated with alterations in redox status of neuronal calcium sensors (NCSs), signaling proteins regulating various aspects of neuronal function. Western blotting and mass-spectrometry analysis revealed accumulation of oxidized monomers, disulfide dimers and multimers of NCSs involving their conserved cysteine residue under cellular, ex vivo, and in vivo conditions. NCSs oxidation precedes and accompanies light-induced damage of photoreceptors and results in decline in retinal content of some of these proteins. Disulfide dimerization of NCSs is also observed in intact retina suggesting their redox regulation. As exemplified by recoverin and NCS1, NCSs exhibit different susceptibility to oxidation depending on intracellular conditions. The results of in vitro oxidation assay and Ellman's test demonstrate that Ca^{2+} binding potentiates thiol oxidation of recoverin, but inhibits it in the case of NCS1 that instead oxidizes in apo- or Zn^{2+}-bound states. Furthermore, the thiol oxidation differently affects structural features and function of these proteins. Using disulfide dimers and oxidation mimicking mutants it was demonstrated that the oxidation inhibits photoreceptor membrane association of recoverin, but not of NCS1. In addition, it produces constitutively active recoverin and enhances activity of Ca^{2+}-NCS1 in regulation of rhodopsin desensitization by GRK1. Overall, NCSs represent redox-sensitive proteins that may undergo redox regulation in normal photoreceptors and exhibit pronounced structural and functional changes in oxidative stress, what possibly contributes to pathogenesis of neuro-ophthalmological disorders. The study was supported by Russian Foundation for Basic Research (grant #18-04-01250).

P.13-003-Wed
Different responses of hepatocellular carcinoma cell lines to oxidative stress induced by non-thermal plasma

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Reactive oxygen species (ROS) are well-known mediators of oxidative stress, and playing role in activation of several cellular redox processes and signalling pathways, including specific cell death pathways. Recently, non-thermal plasma (NTP), containing chemically active species, has been demonstrated to be a potential tool in anticancer therapy. Previously, many studies have reported that NTP can induce cell death in cancer cells by ROS formation, however, the molecular mechanisms of the interaction between eukaryotic cells and NTP remain elusive. In our study, we assessed the influence of plasma-generated ROS on two human hepatocellular carcinoma cell lines (Huh7 and HepG2). Interestingly, NTP showed greater selective anti-proliferative activity against Huh7 cells relative to HepG2, suggesting that plasma-triggered signalling cascades might be grossly different between cell lines. Our data revealed that air-NTP treatment in Huh7 cells results in increased mitochondrial ROS production and mitochondrial depolarization. Subsequently, ROS accumulation and accompanied mitochondrial dysfunction led to apoptotic cell death in Huh7 cells. Additionally, Caspase-3 activation and phosphatidylserine expression confirmed ROS-mediated apoptosis pathway activation in Huh7 cells. In contrast, in HepG2 NTP treatment leads to activation other specific mechanisms, which resulted in greater degree of resistance to oxidative stress mediated by plasma in comparison to Huh7. This study elucidates the mechanism linking physicochemical signals from the NTP

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cascade to the intracellular cancer death signaling pathway, providing physical, chemical and biological insights into the development of therapeutic techniques to treat cancer related diseases.

P.13-004-Mon
Mapping the morphological and functional alterations of the red blood cells in active maternal smoking during pregnancy

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In today's scenario, maternal smoking during pregnancy is quite prevalent which risks to adverse pregnancy outcomes and abnormal fetal development. The umbilical cord lacks innervations and thereby the main regulator of its vascular tone and blood flow is the nitric oxide (NO) signaling molecules produced by the endothelial nitric oxide synthase (NOS3). Recent evidences show that red blood cells (RBCs) also possess a functional NOS (NOS3 like) that produce and release bioactive NO which maintain the rheological properties of RBCs and may play a compensatory role in case of any dysfunction in the cord endothelium. The aim of this study is to evaluate the morphological and functional alterations in RBCs of heavy smoker mothers and their neonates' cord blood. Our findings on RBCs originated from the smoker's samples that represents (1) distinct morphological variations, followed with microscopic and software analysis (2) significant changes in the activation of NOS, based on immunohistochemistry and flow cytometry data (3) macromolecular damages, developed by 4-hydroxy-2-trans-nonenal staining and lipidomic measurements. (4) Furthermore, atomic force microscopy dissections are done on the RBCs to evaluate their rheological alterations like Young's Modulus to derive on the deformability index, viscosity etc. Our molecular and biophysical data indicates that maternal smoking can exert marked effects on the outcome of *in-utero* development because of the insufficient supply of O_2 which may results in long-lasting (partly related to- epigenetic alteration) health consequences on the developing fetus.

P.13-005-Tue
Caveolin-1 as a regulator of neuronal calcium sensor proteins in phototransduction system

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Caveolin-1 is the major regulatory protein of detergent resistant membranes (DRM), which associating with regulation of signaling activity in different organism systems. Rod cell membranes contain cholesterol-rich DRM, which accumulate visual cascade proteins. In this study, photoreceptor Ca^{2+}-binding proteins recoverin, NCS1, GCAP1, and GCAP2, belonging to neuronal calcium sensor (NCS) family, were recognized as novel caveolin-1 interacting partners. We demonstrated that all studied proteins co-precipitate with caveolin-1 from rod outer segment membranes, and can directly interact with caveolin-1. Pull-down assay, surface plasmon resonance spectroscopy and isothermal titration calorimetry data indicate that there is interaction with full-length caveolin-1 N-terminal domain (1-101) and caveolin-1 scaffolding domain (81-101). Interestingly that this interaction occurs only in absence of calcium ions, what is supported by surface localization of caveolin-1 interaction site in Ca^{2+}-free NCS proteins state. Caveolin-1 increase Ca^{2+}-sensitivity of recoverin, and as a consequence, makes its inhibitory activity to rhodopsin kinase more pronounced, but not interfere with recoverin-rhodopsin kinase interaction. Amount of free Ca^{2+}, required for this process, consider caveolin-1 influence, become in good agreement with physiological conditions of photoreceptor cell. GCAP-2 is upregulated by caveolin-1 in Ca^{2+}-free state, which increase guanylate cyclase activity. It seems that there is a common mechanism of interaction between caveolin-1 and NCS proteins in Ca^{2+}-free state. For recoverin increasing of Ca^{2+}-sensitivity is due to the stabilization of the open conformation of its second Ca^{2+}-binding domain EF2. Obtained data suggest that at low calcium NCS proteins are compartmentalized in photoreceptor rafts via binding to caveolin-1, what enhances their activity or ensures their faster responses on Ca^{2+}-signals. The study was supported by RFBR (grant # 15-04-07963).

P.13-006-Wed
Effect of polyphenols from *Silybum marianum* L. and UVA radiation on Nrf2 signalling pathway in skin cells

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Exposure to solar radiation is one of the most important environmental factors affecting skin physiology. The adverse biological effects of sunlight are mainly associated with ultraviolet (UV) wavelengths. Sunlight is primarily an UVA (315–400 nm) source comprising 90–95% of solar UV rays. UVA penetrates through epidermis deep into the dermis and thus affects both major skin cells, keratinocytes and fibroblasts. UVA initialises a massive production of reactive oxygen or nitrogen species (ROS, RNS) leading to oxidation of lipids, proteins and DNA lesions. ROS/RNS and unstable oxidised products can affect various cellular pathways. One of them is the Nrf2 (*Nuclear factor erythroid-2 related factor 2*) signalling pathway. Nrf2 protein, a redox-sensitive transcription factor is normally bound in the complex with the Keap1 protein (*Kelch-like ECH-associated-protein-1*). Due to the oxidative stress, the affinity of Keap1 to Nrf2 decreases, the Keap1/Nrf2 complex decays and Nrf2 is translocated from the cytosol to the nucleus where it binds to the DNA into promoter sequence of the antioxidant responsive element and stimulates expression of detoxication and antioxidant enzymes. The Nrf2 pathway has been described to be activated by several naturally occurring compounds including polyphenols. Here we have studied effect of silymarin, a standardized extract of polyphenols from *Silybum marianum* L., its flavonolignans silybin, silychristin, silydianin, isosilybin, 2,3-dehydrosilybin and flavonoids taxifolin and quercetin on the Keap1/Nrf2/ARE signalling pathway in human skin cells, primary dermal fibroblasts and epidermal keratinocytes and human keratinocyte cell line HaCaT. Effects were

determined by Western Blot and immunocytochemistry. The results will be discussed in a poster presentation. This work was financially supported by grants GACR 15-10897S, IGA_LF_2018_012 and institutional resources of Palacký University in Olomouc – RVO 61989592.

P.13-007-Mon
Idh2 deficiency accelerates renal dysfunction in aged mice

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The free radical or oxidative stress theory of aging postulates that senescence is due to an accumulation of cellular oxidative damage, caused largely by reactive oxygen species (ROS) that are produced as by-products of normal metabolic processes in mitochondria. The oxidative stress may arise as a result of either increased ROS production or decreased ability to detoxify ROS. The availability of the mitochondrial NADPH pool is critical for the maintenance of the mitochondrial antioxidant system. The major enzyme responsible for generating mitochondrial NADPH is mitochondrial $NADP^+$-dependent isocitrate dehydrogenase (IDH2). Depletion of IDH2 in mice ($idh2^{-/-}$) shortens life span and accelerates the degeneration of multiple age-sensitive traits, such as hair grayness, skin pathology, and eye pathology. Among the various internal organs tested in this study, IDH2 depletion-induced acceleration of senescence was uniquely observed in the kidney. Renal function and structure were greatly deteriorated in 24-month-old $idh2^{-/-}$ mice compared with wild-type. In addition, disruption of redox status, which promotes oxidative damage and apoptosis, was more pronounced in $idh2^{-/-}$ mice. These data support a significant role for increased oxidative stress as a result of compromised mitochondrial antioxidant defenses in modulating life span in mice, and thus support the oxidative stress theory of aging.

P.13-008-Tue
Electric discharge redox technology for biomedical investigation

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The possibility to use a plasma radiation of an electric discharge in air for biomedical research was investigated. A pulse plasma radiation generator with a discharge power of 0.6 J/s was used. Under irradiation and products formed in the discharge itself, long-lived active species are formed in aqueous solution. Active species, generated in the liquid under the action of a discharge, have both oxidative and reducing properties. The formation of nitrous, peroxynitrous acids and peroxynitrite was identified in liquid. The reducing properties were identified by means of free stable radical $DPPH^{\cdot}$ and the reduction of methaemoglobin. In albumin the reduction of –S-S- groups to –SH has been established. Oxidative modification of tryptophan, albumin and hemoglobin molecules in solution after exposure to gas discharge plasma radiation is caused by the formation of complexes with products of plasma irradiation: compounds of nitrogen, nitrogen radicals and hydroperoxides. It was shown, that due to a long lifetime, active species penetrate the skin of the rat. The gas discharge plasma and its radiation have a cytotoxic effect on lymphoid and epithelial neoplastic cells. The cytotoxic effect is seen by disturbances in the structure of the cytoplasmic and nuclear membranes, intracellular contents, in the increase of cytoplasmic membranes fluidity, in the accumulation of oxidative modifications of protein molecules and DNA damage. Tyrosine molecules are less resistant to the action of plasma radiation, which determines the oxidative modification of the erythrocyte proteins of intact animals and animals with experimental sarcoma. Exposure to plasma radiation causes a modification of the tertiary and secondary structure of erythrocyte proteins, disrupt the functional activity of the cell as whole. The obtained data allow developing effective gas-discharge devices for biomedical research and can be used to introduce the innovative plasma technologies in medicine.

P.13-009-Wed
Functional analysis of type VI sulfide:quinone oxidoreductase in photosynthetic purple sulfur bacterium

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Despite its toxicity, sulfide plays essential physiological role in eukaryotes and prokaryotes as neurotransmitter or electron donor. Sulfide detoxification, homeostasis and sulfide-dependent energy conversation processes via electron transfer from sulfide to the membrane quinone pool is catalyzed by sulfide:quinone oxidoreductases (SQR). SQRs are ancient membrane bound flavoproteins, members of disulfide oxidoreductase enzyme family. For catalysis SQRs require FAD cofactor and redox-active disulfide bridge formed between conserved essential cysteines. Based on phylogenetic and structure-based classifications there are six types of SQR proteins. Different type SQRs have diverse catalytic mechanisms might due to the variances in cysteines. *Thiocapsa roseopersicina*, the photosynthetic purple sulfur bacterium possesses a type VI SQR enzyme (SqrF). Biochemical and enzyme kinetic analysis of homologously-expressed and purified recombinant SqrF showed significant differences between SqrF and other type SQRs indicating different catalytic mechanism compared to the known models. To identify the role of cysteine residues of SqrF in the catalytic process cysteine mutant enzymes were created by site-directed mutagenesis, expressed in *T. roseopersicina* and purified by affinity chromatography. Characterization of biochemical and catalytic properties of cysteine mutant SqrF variants, the effect of sulfhydryl-blocking agents on the enzyme activity and FAD cofactor binding of wild type and mutant enzymes identified the reacting disulfide pair of the protein and revealed the function of cysteines in catalytic process of *T. roseopersicina* SqrF. Here, we report the first circumstantial characterization and a model for the sulfide oxidation site and mechanism of type VI SQR enzyme.

P.13-010-Mon
Bach1, a heme-containing mammalian transcription factor: study on structure and function relationship

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The Bach1 protein is the first mammalian transcription factor found to bind heme. Bach1 senses the heme concentration and in the case of heme accumulation it stimulates transcription of heme oxygenase, an enzyme which is responsible for free heme

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degradation. Thus, excess heme is degraded and potentially toxic products are eliminated to CO and biliverdin/bilirubin. In the absence of heme, Bach1 forms a heterodimer with small Maf proteins and binds to enhancers of heme oxygenase gene to repress its expression. Bach1 provides protection effect against oxidative stress damage by induction of heme oxygenase expression. Thus, Bach1 may be a potential therapeutic target of oxidative stress-related diseases. In order to understand and control these processes it is essential to clarify conformational changes caused by heme binding and signal transduction. One method for studying the structural dynamics of proteins under various conditions is hydrogen-deuterium exchange (HDX) coupled to mass spectrometry (MS). This method has been used to distinguish structured (α-helices and β-sheets) protein regions from those that are unstructured, and to monitor the structural changes induced by protein-protein interactions or ligand binding. The data obtained using HDX-MS experiments with the Bach1 wild type protein are in an agreement with the general Bach1 structure: its two functional domains (BTB and ZIP domain) are rather structured, whereas other parts of the protein are sequences without proper secondary structural elements. According to our analyses it is possible that the presence of DNA sequence and/or small Maf proteins is necessary for proper conformational change of Bach1 in the course of heme interaction. Supported by Charles University (GAUK 704217).

P.13-011-Tue
Redox-dependent change in the expression of genes controlling cellular ROS/antioxidants balance under formation of cancer cell resistance to cisplatin

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The most vital functions of cells, including proliferation and apoptosis, are largely depended on the cellular redox-dependent homeostasis which determines by the balance between the production of reactive oxygen species (ROS) and antioxidant defense. The disruption of this ratio can lead to many pathologies including cancer. Here we studied expression of genes, which involve in the control of the cellular redox state, under the development of resistance of human ovarian carcinoma SKOV-3 cell to cisplatin, which has the prooxidant effect. It was found that the formation of cancer cell resistance was accompanied by an increase in the expression of genes encoding key antioxidant enzymes (*SOD2*, *CAT*, *GPX1, HO-1*) in parallel with a significant decrease in expression of the *NOX5* gene, which controls one of major sources of ROS. The coordinated character of changes in the expression of genes can be promoted by enhanced expression of transcription factor Nrf2 in addition with the growth of GSH/GSSG ratio. The obtained results testify to the redox-dependent development of the adaptive antioxidant response as an important process in the mechanism of formation of cancer cell resistance to cisplatin. The publication was prepared with the support of the «RUDN University Program 5-100».

P.13-012-Wed
Interplay between hydrogen sulfide production, L-cysteine transport and *bd*-I cytochrome oxidase function in *E. coli*

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In bacteria, endogenously produced H_2S has been recently recognized as a general protective molecule, which renders multiple bacterial species highly resistant to oxidative stress and various classes of antibiotics. In our previous work we showed that 3-mercaptopyruvate sulfurtransferase (3MST) is the major source of endogenous H_2S in *Escherichia coli*. Furthermore we found that 3MST protects *E. coli* against oxidative stress via L-cysteine utilization and H_2S-mediated sequestration of free iron necessary for the genotoxic Fenton reaction. Here we describe the interplay between H_2S generation, oxidative stress and expression of L-cysteine transporters EamA and CydDC, which export L-cysteine from the cytoplasm into the periplasm. We found that overexpression of CydDC, which is closely related to cytochrome *bd*-I terminal oxidase CydAB, but not EamA transporter, leads to very high sensitivity of *E. coli* cells to hydrogen peroxide. Our results suggest that overexpression of *cydDC* results in reduction of intracellular level of L-cysteine, which is the major source of endogenous H_2S, and thereby prevents H_2S-mediated inhibition of Fenton reaction. Accordingly, the addition of exogenous L-cysteine into the cultural medium leads to suppression of susceptibility of strain overexpressing *cydDC* to peroxide. On the other hand we showed that inactivation of *cydB* gene, encoding *bd*-I cytochrome oxidase, induces significant enhance of sensitivity of *E. coli* strains to hydrogen peroxide, especially in the case of strain with constitutive expression of *mstA*. Exploring of relationship between hydrogen sulfide production, cysteine transport and respiration chain function is the main direction of our further investigations. This work was supported by the Russian Science Foundation grant 17-74-30030.

P.13-013-Mon
Mechanisms of proteasome- and yRpn4-dependent yeast resistance to DNA oxidative damage caused by 4-nitroquinoline-1-oxide

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26S proteasome participates in cell stress responses by degradation of various proteins. Yeast mutated for subunits of 19S proteasome subcomplex shows hyper-resistance to 4-Nitroquinoline-1-oxide (4-NQO) chemical mutagen and carcinogen. These data suggest a negative role for 19S proteasome complex in cell response to 4-NQO, but the underlying mechanism is not clear. To address this question, we impaired Rpn4-dependent transcriptional regulation of *RPT5* encoding subunit of the 19S subcomplex. The mutant strain named YRL accumulates of polyubiquitinated proteins that are the hallmark of impaired proteasome activity but shows strongly increased resistance to 4-NQO. This phenotype is caused by only RPT5 gene deregulation. Since yRpn4 is a proteasome substrate we observed its stabilization in YRL strain. We expect that increased expression of yRpn4 target genes is the cause of YRL hyper-resistance to 4-NQO. We find that genes overexpressed in YRL strain belong to pathways of DNA repair (*RAD52, OGG1, NTG1*), oxidative

stress response (*YAP1, TRX2*), multidrug resistance (*PDR1, YRR1, SNQ2*), cysteine, methionine and hydrogen sulfide biosynthesis (*CYS3, MET3, MET5, NFS1,* and others). Several of these genes are directly regulated by Rpn4 while others are regulated by proteasome subcomplexes both in proteolysis dependent and -independent manner. Consistently, we found that activity of homologous recombination is increased in the mutant strain, and it shows hyper-resistance to oxidative damaging agents like hydrogen peroxide and heavy metals. Our data imply that yeast treated with 4-NQO activates the complex cellular response that is controlled by Rpn4 and proteasome subcomplexes. This work was supported by Russian Science Foundation (project #17-74-30030) and the Program of fundamental research for state academies for 2013–2020 years (# 01201363822).

P.13-014-Tue
Mitochondrial NADP+ dependent isocitrate dehydrogenase deficiency aggravates high fat diet-induced hypertension

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Obesity is a major risk factor for essential hypertension. ROS and oxidative stress is known as a mediator of obesity-induced hypertension. Mitochondrial $NADP^+$-dependent isocitrate dehydrogenase (IDH2) is a producer of mitochondrial NADPH which is an essential factor in glutathione (GSH) and thioredoxin antioxidant systems in the mitochondria. Therefore, we investigate the role of IDH2 on the obesity-induced hypertension. Obesity in *Idh2* gene-deleted ($Idh2^{-/-}$) mice and wild-type ($Idh2^{+/+}$) littermates was induced through feeding of a high fat diet (HFD). HFD accelerated increase in body weight and mean blood pressure (MBP) compared to those in the normal diet (ND) in both mice. HFD-induced increase of MBP was higher in $Idh2^{-/-}$ mice than in $Idh2^{+/+}$ mice. Mitochondrial disruption of kidney tubule cell and oxidative stress in the kidney tissue were observed after HFD feeding in both mice. These HFD-induced changes were greater in the $Idh2^{-/-}$ mice than $Idh2^{+/+}$ mice. mRNA levels of renin, angiotensin converting enzyme, and angiotensin II receptor type I increased in the HFD mouse kidneys and these increases were higher in $Idh2^{-/-}$ mice than in $Idh2^{+/+}$ mice. In conclusion, these results demonstrate that IDH2 gene deletion exacerbates HFD-induced hypertension with greater mitochondrial oxidative stress and activation of RAS system in the kidneys, suggesting that mitochondrial redox balance plays an important role in obesity-induced hypertension.

P.13-015-Wed
Antioxidative effects of selenium containing imidazole compound, selenoneine, on human leukemic K562 cells

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Selenoneine is a selenium containing imidazole compound discovered in the blood of bluefin tuna. People often eating fish also have higher level of selenoneine in their erythrocyte. Since it has strong radical scavenging activity, it is expected to decrease oxidative stress in erythrocyte. This study verified the effect of selenoneine on oxidative stress in human leukemic K562, and analyzed molecular forms of selenium compounds. K562 cells were cultured in the Ham's F12 medium containing 10% FBS with 0–10 μM selenoneine. Oxidative stress was induced by *tert*-butyl hydroperoxide (t-BHP), 2, 2′-azobis (2-amidinopropane) dihydrochloride (AAPH), pyocyanin and S-nitrosoglutathione (GSNO), and cell viability was measured. Molecular forms of selenium compounds in the cells and medium were analyzed by ICP-MS. Selenoneine slightly but significantly improved cell viability in each four types of oxidative stress groups. By addition of 10 μM selenoneine in the culture medium, cell viabilities showed an increase of 14.4% for 0.2 mM t-BHP, 9.2% for 50 mM AAPH, 8.3% for 0.1 mM pyocyanin, 10.4% for 2 mM GSNO, as compared with selenoneine free medium. The oxidative stress conditions induced by these four chemicals can be repressed by antioxidant effects of selenoneine in the cells. ICP-MS analysis showed that almost all selenoneine in the medium was incorporated into K562 cells. Large amounts of selenium were detected in high molecular weight protein fraction by separation with the GPC column Ultrahydrogel 120, and these selenium compounds were detected in the cellular fraction in the presence of 0.2 mM t-BHP. Small amounts of selenium was due to selenoneine. The treatment of high molecular weight fractions in the K562 cell with protease or hydrochloride showed the release of selenoneine from the protein fraction. Therefore, selenoneine may bind to other intracellular protein and form high molecular weight complex to protect from the oxidative stress conditions.

P.13-016-Mon
The relationship of *Escherichia coli* Hyd enzymes with the F_OF_1-ATPase during fermentation of mixture of carbon sources

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Escherichia coli is able to produce molecular hydrogen via reversible membrane-associated four [Ni-Fe]-hydrogenases (Hyd). In this study it was first investigated overall ATPase activity and its inhibition by *N,N′-dicyclohexylcarbodiimide* (DCCD) in *E. coli* wild type and *hypF* mutant lacking all Hyd enzymes during mixed carbon sources (glucose, glycerol, formate) fermentation at different pHs (7.5, 6.5, 5.5). ATPase activity was higher in wild type at pH 7.5 than pH 6.5 and pH 5.5. DCCD-inhibited ATPase activity was 89% and 78% of overall ATPase activity in wild type and *hypF* mutant accordingly at pH 7.5. Overall and F_0F_1-ATPase activity of *hypF* mutant was decreased compared to wild type 1.4 fold and 1.6 fold respectively at alkaline pH. Potassium ions had low effect on overall ATPase activity in wild type and didn't have influence on *hypF* mutant at pH 7.5, but they stimulated DCCD-inhibited ATPase activity in wild type and *hypF* mutant. Effect of DCCD was higher in *hypF* mutant compared with wild type at slightly acidic pH. Potassium ions didn't have role in promotion of overall ATPase activity, but they had stimulating effect on DCCD-inhibited ATPase activity in wild type and *hypF* mutant at pH 6.5. Overall and F_0F_1-ATPase activity of *hypF* mutant was decreased compared to wild type at pH 5.5. DCCD had almost the same effect in wild type and *hypF* mutant at acidic pH. These results establish that alkaline pH is the most optimal condition for operation of F_0F_1-ATPase in *E. coli* during mixed carbon fermentation and deletion of *hypF* gene is negatively affected not only on overall ATPase activity, but also on F_0F_1-ATPase activity at pH 7.5. Taken together it might be concluded that proton ATPase activity is highly dependent on Hyd enzymes and this relationship could be due to maintaining intracellular pH and thus proton motive force generation.

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P.13-017-Tue
Deciliation of kidney primary cilium into urine by acute kidney injury

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The primary cilium protrudes into the kidney epithelial cell surface. In kidney epithelial cells, the alteration of primary cilia length including shortening has been suggested as a result or cause of various kidney diseases including acute kidney injury (AKI). Therefore, we hypothesized that alterations of primary cilia length by lengthening, shortening and deciliation reflect kidney condition. Here, we investigated the correlation between alterations of primary cilia length in the cells and deciliation into urine and renal injures in the two deferent acute kidney injury (AKI) animal models which are ischemia/reperfusion (I/R)- and cis-diamminedichloroplatinum II (cisplatin, an effective anticancer drug) nephrotoxicity-AKI models. Here, we found that I/R and cisplatin injection induced shortening of primary cilia of kidney epithelial cells and deciliation into urine and that treatment of (2-(2,2,6,6-Tetramethylpiperidin-1-oxyl-4-ylamino)-2-oxoethyl) triphenylphosphonium chloride monohydrate (Mito-Tempo, a mitochondria-targeted antioxidant), prevented the shortening of primary cilium length and deciliation. These findings indicate that I/R and cisplatin induce shedding of kidney primary cilia via increasing oxidative stress, consequently inducing shortening of tissue primary cilia length and release of primary cilia proteins into urine. This result suggests that the presence or increase of primary cilia proteins in urine and the alteration of tissue primary cilia length could indicate kidney cell damage.

P.13-018-Wed
Relationship of dcu transport system and proton ATPase during glycerol fermentation

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The C4-dicarboxylate or *dcu* family of transporters are exchanging succinate, malate, fumarate etc. Depending on external conditions *dcu* system can transport succinate with H^+ symport. In this study proton ATPase activity was investigated in *E. coli* wild type, *dcuD*, *dcuABC* and *dcuABCD* mutants during glycerol fermentation at pH 6.5 and pH 5.5. Overall ATPase activity in *E. coli* wild type was ~1.9 fold higher at pH 6.5 than pH 5.5. Moreover, proton ATPase activity was detected only at pH 6.5 where it represent 72% of overall ATPase activity. Only in *dcuD* mutant overall ATPase activity was decreased by 18% but contribution of other *dcu* subunits on proton ATPase activity was absent. Interestingly, in wild type cell assays K^+ ions had negative effect on overall ATPase activity but in *dcuD* mutant K^+ ions decreased the contribution of proton ATPase by ~2.3 fold. It is possible if K^+ ions had effects directly on the activity of proton ATPase or indirectly via *dcu* transport system. At pH 5.5 overall ATPase activity in *dcuD* and *dcuABCD* mutants decreased ~1.5 fold compared to wild type. But in *dcuABC* mutant ATPase activity was increased ~70%. K^+ ions had enhancing effects on ATPase activity in all mutants except wild type. The contribution of proton ATPase was highest ~90% in *dcuABC* mutant but in *dcuD* and *dcuABCD* mutants it was 50% and 32% respectively, compared to wild type. In wild type assays K^+ ions resulted in increase of proton ATPase activity ~50% but in *dcuABCD* it was decreased by 40%. Taken together it might be concluded that at pH 5.5 proton ATPase and *dcu* transport system has direct relationship and K^+ ions might be important for this interaction. The absence of *dcu* subunits drives proton ATPase mainly for proton transport across the membrane. Moreover, H^+ ATPase activity was higher at pH 6.5 than at pH 5.5 but no any direct interaction might be suggested.

P.13-019-Mon
Transcriptional activation of the nudC gene encoding intracellular redox balance regulator from *Pseudomonas syringae*

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In pathogenic bacteria redox homeostasis, reflected by the $NADH/NAD^+$ ratio, not only indicates the metabolic state of cells, but it is an important determinant of biofilm morphology, a virulence-related phenotype. Our research is focused on the functional analyses of NudC, a cellular redox balance regulator from plant pathogen *Pseudomonas syringae* DC3000. This protein belongs to the widely distributed Nudix pyrophosphatase family, which catalyze the hydrolysis of a variety of nucleoside diphosphate derivatives. It has been shown that Nudix proteins play important regulatory functions in pathogenesis and in response to different stresses. Previously, we have established that the *P. syringae* mutant deficient in NudC protein, displayed significant defects in motility, biofilm formation and Arabidopsis host colonization. The *nudC* mutant cells accumulated NADH in the stationary phase of culture growth, causing severe phenotypic disorders. Strict control of redox homeostasis by the NudC protein, indicated that the expression of *nudC* is tightly regulated. With the help of bioinformatics tools and databases, hypothetical position of the *nudC* promoter was established. The transcriptional fusions of the studied promoter with *lacZ* gene have confirmed *in silico* assessments. Northern blot assay was used to detect *nudC* transcript in exponential and stationary phase of bacterial growth. It was established that the activity of the *nudC* promoter elevates in response to change of growth conditions. Moreover, β-galactosidase assays conducted in the *nudC* mutant suggested that transcription of the *nudC* gene is negatively regulated by the NudC protein. This work was supported by grant no: umo-2014/15/B/NZ6/02562 from The National Science Center.

P.13-020-Tue
Oxidative stress and voltage-gated sodium channel activity in human breast cancer cells

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In several carcinomas, including breast cancer, functional up-regulation of voltage-gated sodium channels potentiates various components of the metastatic cascade. Metastasising cells experience oxidative stress involving intracellular reactive oxygen species (ROS) production. We aimed to investigate how voltage-

gated sodium channel activity play a role in the response of cancer cells to oxidative stress. We used the strongly metastatic human breast cancer cell line MDA-MB-231, which expresses functional voltage-gated sodium channel, as a model. Both basal and H_2O_2-induced oxidative stress were measured using 2,7-dichlorofluorescein diacetate (DCF-DA). voltage-gated sodium channel activity was blocked by tetrodotoxin or ranolazine. Application of tetrodotoxin or ranolazine (both 10 μM) to resting cells significantly increased ROS levels by 61% and 86%, respectively, consistent with there being (i) a basal level of oxidative stress and (ii) ongoing voltage-gated sodium channel activity. Application of H_2O_2 (200 mM) for 30 mins significantly elevated the ROS level by 100%. Co-application of H_2O_2 with tetrodotoxin or ranolazine significantly increased ROS production further, by 154% and 131%, respectively. Functional voltage-gated sodium channel expression (in particular the persistent current – I_{NaP}) endowed metastatic breast cancer cells with significant advantage in resisting oxidative stress. Thus, the effectiveness of therapeutic regimens (e.g. chemotherapy or radiotherapy), aiming to kill cancer cells, can be boosted by combination with voltage-gated sodium channel/I_{NaP} blockers.

P.13-021-Wed
Glutathione S-transferase; moonlighting protein in haem homeostasis of tick digest cells

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Haem and iron homeostasis in most eukaryotic cells is based on a balancing flux between the opposing pathways of haem synthesis and haem degradation. Ticks, blood-feeding arthropods, encode neither haem biosynthetic nor haem-degrading enzymes. Excess of acquired host haem thus needs to be disposed of by selective accumulation, export, and scavenging. RNA-seq analyses of the *Ixodes ricinus* midguts from blood- and serum-fed females identified an abundant transcript of *glutathione S-transferase* (*gst*) to be substantially up-regulated in the presence of red blood cells in the diet (blood-meal). We determined the full sequence of this encoding gene, *ir-gst1*, and found that it is homologous to the delta/epsilon-class of GSTs. We have confirmed that *ir-gst1* expression is induced by dietary haem(oglobin), and not by other components of red blood cells. Kinetics evaluation of recombinant *Ir*GST1 was performed by model and natural GST substrates, conforming its ancestral catalytic properties. The enzyme was also shown to bind haemin *in vitro* as evidenced by inhibition assay, VIS spectrophotometry, gel filtration, and affinity chromatography. In the native state, *Ir*GST1 forms a dimer which further polymerises upon binding of excessive amount of haemin molecules. Phylogenetic analysis revealed that homologous group of *Ir*GST1 is formed only in tick species, and not in other mites or blood-feeding insect. It suggests that these enzymes exaptated from its ancestral enzyme only after formation of ticks during Arthropods evolution and gained their haem binding properties. Due to susceptibility of ticks to haem as a signalling molecule, we speculate that the expression of *Ir*GST1 in tick midgut functions as intracellular buffer of labile haem pool to ameliorate its cytotoxic effects upon haem liberation of haemoglobin hydrolysis. Acknowledgements: The project is supported by Czech Science Foundation No. 18-018132S (PK).

P.13-022-Mon
Autophagy protects neurons and astrocytes from bilirubin-induced cytotoxicity

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Unconjugated bilirubin (UCB) neurotoxicity involves oxidative stress, calcium signaling and ER-stress. The same insults also induce autophagy, a process of "self-eating", with both a pro-survival or a pro-apoptotic role. Our aim was to study the outcome of autophagy activation by UCB in the highly sensitive neuronal SH-SY5Y cells and in the resistant astrocytoma U87 cells. Upon treatment with a toxic dose of UCB, the conversion of LC3-I to LC3-II was detected in both cell lines. Inhibition of autophagy by E64d before UCB treatment increased SH-SY5Y reduction of cell viability from 40% to 60% and made U87 cells sensitive to UCB. In SH-SY5Y cells autophagy related genes ATG8(5 folds), ATG18 (5 folds), p62 (3 folds) and FAM 129A (4.5 folds) were induced 8 h after UCB treatment while DDIT4 up-regulation (13 folds) started at 4 h. mTORC1 inactivation by UCB was confirmed by phosphorylation of 4-EBP1. UCB induced LC3-II conversion was completely prevented by pre-treating the cells with the calcium chelator BAPTA and reduced by 65% using the ER-stress inhibitor 4-PBA. Pre-treatment with the PKC inhibitor reduced LC3 mRNA by 70% as compared to cells exposed to UCB alone. Finally, autophagy induction by Trifluoroperazine (TFP) increased the cell viability of rat hippocampal primary neurons upon UCB treatment from 60% to 80%. In SH-SY5Y cells, TFP pre-treatment blocked the UCB-induced cleaved caspase-3 protein expression, decreased LDH release from 50% to 23%, reduced the UCB-induction of HO1, CHOP and IL-8 mRNAs by 85%, 70% and 97%. Collectively these data indicate that the activation of autophagy protects neuronal cells from UCB cytotoxicity. The mechanisms of autophagy activation by UCB involves mTOR/ER-stress/PKC/calcium signaling.

P.13-023-Tue
Recovery of NAD+ level and DNA repair efficiency by NAD+ precursors in cultivated human cells after genotoxic stresses

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NAD is critical for the regulation of cell response to genotoxic stresses where it is used as a substrate for poly (ADP-ribose) polymerases (PARPs). PARP1 is hyperactivated and auto-poly (ADP-ribosyl)ated in response to DNA strand breaks. It accumulates poly (ADP-ribose) (PAR) on chromatin at the sites of DNA damage and facilitates recruitment of DNA repair factors. In this study we examined the effect of administration of NAD+ precursors on the level of NAD+ and DNA repair after the treatment of human dermal fibroblasts (HDF) and HEK293 cells with H2O2 or X-ray irradiation. Using immunofluorescence microscopy and immunoblotting, it has been shown that hyperactivation of PARP1 in response to oxidative stress leads to a very fast and intensive accumulation of PAR in HEK293 cells and to a less PAR accumulation in HDF. Significant drop of NAD+ was observed 30 min after H2O2 treatment in both cell lines, and, at 24 h, the content of NAD+ was recovered to the control level. Administration of NAD+ precursors (nicotinamide riboside, NR or nicotinic acid riboside, NAR) before and during treatment with H2O2 led to a partial recovery of NAD+ level. Effectiveness

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of DNA single strand break (SSB) repair after H2O2 treatment was estimated by immunofluorescence microscopy using an antibody to phosphorylated H2AX (gH2AX), the marker of DNA double-strand breaks (DSBs) formed from unrepaired SSBs located in opposite DNA strands. Administration of NAR diminished gH2AX staining indicating that SSB repair is more efficient in the presence of NAD+ precursor. Induction of DSBs after X-ray irradiation of HDF at the dose of 5 Gy only slightly decreased the level of NAD+. The rate of DSB elimination was not changed after pretreatment with FK866, an inhibitor of the main pathway of cellular NAD+ synthesis. These data suggest that intensive poly ADP-ribosylation is not necessary for the efficient DSB repair. This work was supported by the Russian Science Foundation grant 16-14-10240.

P.13-024-Wed
Phragmitis Rhizoma attenuates hydrogen peroxide induced oxidative stress and apoptosis by regulation of the Nrf2 signaling pathway in Chang liver cells

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Phragmitis Rhizoma (PR), the roots of *Phragmites communis* Trinius, has long been used as a traditional medicinal herb to remove heat, promote the production of body fluids, induce excretion of urine and suppress melanogenesis. However, the molecular mechanisms of its anti- oxidative activity have not been clearly elucidated yet. In the present study, we investigated the potential therapeutic efficacy of the PR against hydrogen peroxide (H_2O_2)-induced oxidative stress and apoptosis in Chang liver cells. It was found that exposure of Chang liver cells to H_2O_2 caused a reduction in cell viability by generation of intracellular reactive oxygen species (ROS), induction of DNA damage, disruption of mitochondrial membrane permeability and apoptosis. However, pretreatment of PR before H_2O_2 exposure effectively attenuated these changes, suggesting that PR prevented H_2O_2-induced oxidative stress and mitochondria-dependent apoptosis. Furthermore, the increased expression and phosphorylation of nuclear factor erythroid 2-related factor 2 (Nrf2) and up-regulation of heme oxygenase-1 (HO-1), a phase II antioxidant enzyme, were detected in PR-treated Chang liver cells. We also found that zinc protoporphyrin IX (ZnPP), a HO-1 inhibitor, attenuated the protective effects of PR against H_2O_2-induced oxidative stress and apoptosis. Collectively, these findings indicate that PR enhances cellular antioxidant defense capacity through the inhibition of ROS generation and the activation of the Nrf2 signaling pathway, thus protecting Chang liver cells from H_2O_2-induced oxidative stress and apoptosis. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) grant funded by the Korea government (2015R1A2A2A01004633 & 2017R1D1A1B03032689)].

P.13-025-Mon
Ultrasound-based imaging of reactive oxygen species overproduction associated with atherosclerosis in hypercholesterolemic apolipoprotein E-deficient mice

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Imaging of critical pathological processes such as oxidative stress, which is typically correlated with robust inflammatory reactions and the severity of atherosclerosis is challenging. The aim of this study was to develop at preclinical level an ultrasound-based imaging method to assess reactive oxygen species (ROS) overproduction in atherosclerosis employing sterically stabilized targeted liposomes (Lp) encapsulating allylhydrazine (ALH). ALH reacts with ROS and results in the generation of echogenic gas (nitrogen and propene)-filled microbubbles. Fluorescent labeled, sterically stabilized Lp entrapping the ALH were prepared using the extrusion method. Peptides that recognize the vascular cell adhesion molecule-1 were conjugated to the surface of Lp. Male ApoE−/− mice were randomized to receive normal (ND) or high-fat cholesterol-rich diet (HD) for 10 weeks. The mice were treated via retro-orbital injection with Lp incorporating ALH. After 10 min, the changes in echo intensity triggered by ROS-induced microbubbles were detected by scanning vascular territories prone to atherosclerosis (aortic arch, thoracic aorta) by high frequency, high resolution imaging (Vevo® 2100). Pre-formed, nitrogen- and perfluorobutane-filled microbubbles were used as positive control. High resolution fluorescence imaging (IVIS™ Caliper) indicated the high uptake of Lp in the aortic atherosclerotic lesions and in organs (liver, lungs, kidney). Significant increases in acoustic impedance were detected in the aortic arch territories of ApoE−/− (HD) mice compared to ApoE−/− (ND) animals. ROS-induced ALH echo signals correlated with the severity of atherosclerotic lesions (Oil red O) and ROS overproduction (dihydroethidium) in ApoE−/− mice. Acoustic detection of ROS via ALH-induced chemically-generated gas microbubbles may be a reliable strategy to routinely diagnose early and advanced atherosclerosis. Work supported by UEFISCDI (PN-III-P2-2.1-PED-2016-1308, PN-III-P4-ID-PCE-2016-0665).

P.13-026-Tue
Regulation of autophagy by mitochondrial electron transport chain complex III

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We have shown that mitochondrial complex III inhibitors decrease autophagy activity, however mechanism of this phenomena remains unclear. We assumed that in this process an important role is played by dihydroorotate dehydrogenase (DHODH) that could be inhibited by myxothiazol. DHODH is localized in the inner mitochondrial membrane and catalyzes the oxidation of dihydroorotate to orotate that is used for biosynthesis of pyrimidines that important for further phospholipids biosynthesis. The activity of this enzyme is associated with the activity of complex III as DHODH uses ubiquinone as the electron acceptor. We demonstrated that incubation of cells with complex III inhibitor myxothiazol as well as with DHODH inhibitor leflunomide leads to significant pyrimidines depletion, p53 activation and reduces both basal and rapamycin induced autophagy in Rko and Hela

cells, while adding to the cells incubation medium of uridine, cytidine or cytidine diphosphate choline neutralizes these effects. Adding of phosphatidylethanolamine or phosphatidylcholine does not affect the level of pyrimidines in the cell, but prevents the inhibitory effect of myxothiazol on autophagy. We assume that low levels of pyrimidines could influence the autophagy at least in two ways: by p53 protein activation and by inhibition of biosynthesis of a number of key phospholipids in the cell that may play a signaling role in the mechanism of autophagy. Our results could be important for the clinical application, as DHODH inhibitors are actively investigated for the use in treatment of autoimmune and immune abnormalities, multiple sclerosis and some cancers, however the influence of these inhibitors on autophagy is disregarded.

P.13-027-Wed
AQP5-mediated hydrogen peroxide permeation is altered by nutritional compounds and induces cell resistance

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Aquaporins (AQPs) are a family of transmembrane proteins present in all types of organisms and involved in the selective transport of water and small solutes such as glycerol. A few isoforms can also permeate H2O2 (peroxiporins). So far, 13 aquaporins (AQP0-12) were identified in humans and reported to be involved in a wide range of physiological functions, as water/salt homeostasis, exocrine fluid secretion and epidermal hydration, and human diseases including glaucoma, cancer and obesity. High level of H2O2 misbalance cell redox reactions and may induce tumorigenesis. Recently, we assessed AQP-mediated hydrogen peroxide permeation of mammalian AQPs individually expressed in yeast and reported the rat AQP5 can mediate H2O2 transport. Sequence alignment of human and rat AQP5 isoforms show a sequence identity of 91%. Thus, we next investigated H2O2 permeation by human AQP5 and related cell resistance to oxidative stress. The results indicate that, similarly to AQP3 and AQP8, human AQP5 also permeates H2O2 and importantly, induces cell resistance under oxidative stress. In addition, the effect of antioxidant compounds on hAQP5-mediated H2O2 accumulation and cell growth was investigated. Curcumin and naringenin enhanced cell resistance in AQP5-transformed yeast cells and anti-proliferative properties known for these compounds were attenuated in yeast cells expressing human AQP5. These data propose the AQP5 isoform as a key player in oxidative stress resistance and point to a novel mechanism of AQP5 involvement in tumorigenesis.

P.13-028-Mon
The role of glutathionylation in redox regulation of Na,K-ATPase receptor function

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Na,K-ATPase maintains the homeostasis of Na^+ and K^+ in animal cells. Na,K-ATPase is redox–sensitive, and glutathionylation is the key regulator of its activity under hypoxia. Na,K-ATPase is also the receptor for cardiotonic steroids, in particular, ouabain. One of the main ways of signal transmission upon binding of ouabain is the activation of Src kinase, which forms a complex with Na,K-ATPase. Earlier we observed that under hypoxia receptor function of Na,K-ATPase is inverted: binding of ouabain leads to decrease of ROS and GSH and decrease of Src kinase activating phosphorylation. In this study, to reveal the mechanism of redox regulation of Na,K-ATPase receptor function, we exposed murine fibroblast cells to hypoxia or ouabain, monitored the level of ROS and GSH and studied the influence of Na,K-ATPase glutathionylation on its interaction with Src. We have found that ROS growth occurs within 10 min after hypoxia or ouabain treatment, while GSH level – only after 30 min of affection, which may be an adoptive response to oxidative stress, caused by hypoxia or ouabain. We have observed that activation of Src coincides in time with the growth of ROS under hypoxia or ouabain treatment. We have demonstrated that glutathionylation of Na,K-ATPase can be caused not only by hypoxia, but also by ouabain treatment, and it is accompanied by Src activation. The proposal mechanism of redox regulation of Na,K-ATPase receptor function is as follows: exposure to hypoxia or binding of ouabain to Na,K-ATPase causes growth of ROS, which leads to glutathionylation of Na,K-ATPase. Glutathionylation, in turn, prevents the interaction of Na,K-ATPase with the kinase domain of Src, resulting in the activation of Src. Under hypoxia ouabain binding decreases the level of ROS and induces deglutathionylation of Na,K-ATPase with the consequent deactivation of Src. The study was supported by Russian Science Foundation (grant #14-14-01152).

P.13-029-Tue
Utilization of Se-containing imidazole compound, selenoneine, extracted from tuna and fisheries wastes

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Selenoneine, 2-selenyl-Nα,Nα,Nα-trimethyl-L-histidine, is a strong antioxidant found in the tissues of tuna and other fish that can mediate the prevention of autoxidation of heme proteins, such as hemoglobin and myoglobin. We measured concentrations of selenoneine, total selenium, and total mercury in the red muscles of tuna species. Red muscles of yellowfin tuna contained the high levels of selenium at 5.6–21.2 mg/kg, and almost all selenium in tuna muscles was identified to be selenoneine by HPLC-ICP-MS analysis. We developed selenium-rich surimi products from the red muscles of yellowfin tuna. The surimi product contained selenoneine at 0.28 mg Se/kg. We also concentrated and purified selenoneine from the waste water and internal organs of the surimi processing of fish. After filtration of the boiling water by reverse osmosis membrane and column chromatography with cation exchange and Sepabeads resins, selenoneine was purified by reverse-phase HPLC. The tuna surimi products and the purified selenoneine concentrate can be applied to be the selenium-rich functional food and feed to enhance selenium-mediated antioxidant functions.

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Biochemical processes at cellular membranes

P.14-001-Mon
STIM1 deficiency triggers mitochondrial impairment and senescence by upregulation of L-type voltage operated Ca(2+) entry in SH-SY5Y cells

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STIM1 is a transmembrane protein located at the endoplasmic reticulum (ER), with a role in Ca^{2+} mobilization and signaling. As a sensor of the Ca^{2+} level within the ER, STIM1 modulates plasma membrane (PM) Ca^{2+} channels to regulate the store-operated Ca^{2+} entry (SOCE). However, STIM1 also inhibits the voltage-operated calcium channel $Ca_v1.2$ by direct binding of STIM1 to the channel and by enhancing the channel internalization from the PM. Besides, it has been shown that the presenilin-1 (PS1)-associated γ-secretase interacts with STIM1 in neuroblastoma SH-SY5Y cells, familial Alzheimer's disease patient fibroblasts, and mouse primary cortical neurons, and that STIM1 is cleaved at the transmembrane domain, where the protein shows a target sequence for γ-secretase. To understand better the role of STIM1 in neurodegenerative diseases, we designed a strategy to knock-out the expression of STIM1 gene in SH-SY5Y neuroblastoma cell line, as an ex vivo model to study the phenotype of STIM1-deficient cells. As a result, we proved that STIM1, which was silenced by CRISPR genome editing, is not required for differentiation but it is essential for cell survival of differentiating cells. Differentiated cells showed inner mitochondrial membrane depolarization, measured with tetramethyl-rhodamine, as well as senescence, measured with the substrate of the senescence-associated β-galactosidase C12FDG. In parallel, STIM1-KO cells showed an upregulation of CACNA1C gene transcripts, which correlates with a potentiated Ca^{2+} entry in response to depolarization with KCl. The stable knocking-down of CACNA1C transcripts with specific shRNA restored mitochondrial function, and dropped senescence to basal levels, confirming the essential role of $Ca_v1.2$ channels in the cell death triggered by STIM1 deficiency. Funded by grants BFU2014-52401-P and IB16088 of the Spanish Ministerio de Economia and Junta de Extremadura.

P.14-002-Tue
Effect of melatonin and resveratrol on renal expression of aquaporins 1 and 2 in 2-year-old male and female rats

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Water channels (aquaporins; AQPs) facilitate the passive movement of water across cell membranes. In mammalian kidneys, AQP1 and AQP2 mediate water reabsorption from primary urine across the apical membrane of proximal and distal nephron segments. Studies indicate that renal functional/structural changes in humans and experimental animals develop with age. Aging promotes fluid loss in tissues which could reflect changes in the expression of AQPs along the nephron. We investigated the possible age-ameliorating effects of two potential geroprotector substances, melatonin (endogenous hormone) and resveratrol (plant flavonoid), on AQP1 and AQP2 expressions in the rat model of experimental aging. Three month old rats of both sexes were given melatonin or resveratrol for 21 months via drinking water (~1 mg/kg b.w./day), whereas control animals drank vehicle (0.01% ethanol in drinking water). 24-h urine was collected from animals using metabolic cages. The renal expression of AQP1 and AQP2 was determined using immunofluorescence cytochemistry tissue cryosections and Western blot analysis in isolated total cell membranes. Results showed that melatonin and resveratrol had no effect on renal AQP1 expression in both sexes. AQP2 expression in male and female kidneys of resveratrol-treated rats also did not change compared to controls. Melatonin exerted a sex-dependent AQP2 upregulation in the female kidney inner stripe and papilla. However, no differences were found in the 24-h urine outputs between treated and control animals, indicating that melatonin and resveratrol did not impact the renal AQP expression to significantly alter urine production in aging rats. (Croatian Science Foundation project IP-2013-11-1481).

P.14-003-Wed
Stress related changes in Na^+/K^+-ATPase activity and way of action of creatine

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Negative influence of psycho-social stress is widely common. In a recent years, Creatine (Cr) that has been known for its involvement in the high energy phosphate shuttle has been actively discussed for its wide range of impact on different cellular systems and amongst them membrane pumps. The Na^+/K^+-ATPase is one of the enzymes that is influenced by various factors and works for maintaining balance in the cell and thought to be tightly functionally bound to the NMDA Receptor in neural system. Although the data show no significant changes in Na^+/K^+-ATPase activity in hippocampus of control group rats under the 30-days intraperitoneal injection (i.p.) of Cr (140 mg/kg), the valuable results were taken for the stressed ones, were the enzyme activity was taken back to the normal value. In addition to the normalization Na^+/K^+-ATPase activity, the 30-days i.p. injection of Cr had positive influence on stress induced increase in Ca^{2+} levels. Such changes were predicted to be due to the modulation of NMDA Receptor as no changes were seen in control group. The idea was strengthened by *in vitro* experiments with Glutamate/Glycine and Ketamine and co-addition them with the Cr in the reaction media of hippocampal slices. Cr was seen as a blocker of Glutamate binding site and prevent from the stimulation of NMDA receptor. Finally it can be concluded that there is no direct relations between Cr and Na^+/K^+-ATPase, but under the certain pathological conditions it can maintain the homeostasis of the system by indirect influence on the Na^+/K^+-ATPase via NMDA receptor, thus retrieving the membrane potential and diminishing the negative influence of psycho-emotional stress. However, the exact mechanisms of this interaction need more detailed research and clarification.

P.14-004-Mon
Pore–forming ability of cyclic lipopeptides of *Bacillus subtilis*

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Cyclic lipopeptides (CLPs) are biosurfactants produced by various bacterial and fungal strains. They are characterized by a structural diversity and broad spectrum of action. In particular, CLPs are able to interact with biological membranes that are resulted to membrane degradation or formation of transmembrane pores. In literature CLPs are enthusiastically discussed as a new class of antibiotics. Surfactin (SF), iturin (IT), mycosubtilin (MS) and fengycin (FE) are surface active compounds produced by *Bacillus subtilis*. Here the ability of SF, IT, MS and FE to form ion–permeable nanopores in bilayers of different composition has been studied. It has been shown that the one-side addition of CLPs to 1 M KCl induces the step-like current fluctuations corresponding to the openings and closures of transmembrane pores in DOPC-membranes. Pores formed by SF, IT and MS are characterized by the multilevel conductance in the range of units to hundreds pS. Amplitude of FE–pores does not exceed 10 pS. The dwell time of SF–, IT–, MS– and FE–pores is about several hundreds ms. The alteration in the lateral pressure profile, the surface and dipole potentials of the membrane does not lead to significant changes in the pore-forming activity of SF, IT and MS. On the contrary, the introduction of positively charged lipid, DOTAP, into bilayer–forming solution and the addition of the well-known dipole modifiers, phloretin and RH 421, which reduces and increases the dipole potential of the bilayer, respectively, greatly contributes to the integration of the negatively charged FE–molecules into model membranes. The mechanisms of the independence of the pore-forming ability of negatively charged SF on the electrical properties of bilayers are discussed. This work was supported by The Russian Foundation of Science (# 14-14-00565-P).

P.14-005-Tue
The effect of neurotransmitter and synaptic factor on the synaptic membrane anion-activated ATPases

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The anion activated ATPases represent the enzyme systems which are activated by anions. The HCO^-_3- and Cl-anion dependent Mg-stimulated ATP hydrolysis is reported in the literature. They play a principal role in the regulation of intracellular pH, cell volume, nerve excitability and determination of rest potential. Cl-anion and HCO^-_3-anion activated Mg^{2+}-dependent ATPases satisfy the necessary kinetic condition of transport ATPases and they belongs to the P-type ATPases group. It has been assumed that Cl-ATPase, like Na,K-ATPase, presumably takes part in the regulation of synaptic transmission mechanisms. The neurotransmitters (5-hydroxytryptamine (5-HT), dopamine (DA)) and synaptosomal factor (SF) represent modifiers of Na,K-ATPase system. In the cytosol of nerve ending endogenous factor SF with MW 60kD was found, which regulates the synaptic Na,K-ATPase system. It is remarkable that this factor with neurotransmitters activates the synaptic Na,K-ATPase. The effects of dopamine (DA) and endogenous synaptosomal factors on anion ATPases (Cl-activated ATPase E.C. 3.6.3.11, HCO^-_3-activated ATPase E.C. 3.6.1.3.) were studied in the rat brain fraction greatly enriched in synaptic junctional complexes (SJC). NT and SF each separately inhibit Cl-ATPase, whereas their combined action (SF/NT) resulted in the withdrawal of the inhibitory effect and passed into activation. The effect of NT and SF on Mg^{2+}-dependent HCO^-_3-ATPases is not significant. The neurotransmitter's regulation by a synaptic factor is universal for those transport enzyme systems, which are involved in synaptic transmission and does not extend to other membrane enzymes.

P.14-006-Wed
Effectiveness of three families of antifungal peptides is influenced by lipid composition of the yeast plasma membrane

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Widespread and prolonged use of antifungals in recent years has led to the rapid emergence of multidrug resistant strains of *Candida*. Therefore, novel treatments for fungal infections are urgently needed. Naturally occurring antimicrobial peptides are promising candidates for development of new antifungal drugs. We focused on three groups of short cationic peptides isolated from the venom of bees and their synthetic analogs (lasioglossins, halictines and hylanins), which rapidly permeabilize plasma membrane and thus circumvent the pathogen's intracellular resistance mechanisms. The diS-C_3(3) fluorescence assay enabled to detect the level of yeast-membrane damage. We compared peptides' potency against six pathogenic *Candida* species (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. dubliniensis*) and laboratory model yeast *Saccharomyces cerevisiae*. We analyzed lipid composition of the yeast species to explain mechanism of action of tested antifungal peptides. Although direct interaction of peptides with ergosterol (or mammalian cholesterol and plant stigmasterol) is negligible in comparison with another plasma-membrane damaging antifungal amphotericin B, diminished ergosterol content resulting from terbinafine pretreatment caused resistance of *C. glabrata* to the peptides. Lasioglossin activity exhibited the poorest dependence on ergosterol, and contrary, hylanin was the most sensitive to ergosterol content. All three groups of peptides strongly interacted with phosphatidylglycerol, phosphatidic acid, cardiolipin and dihydrosphingosine, partly with phosphatidylinositol and only lasioglossin interacted with phytosphingosine. We thank Dr. Čeřovský for providing the antimicrobial peptides. This work was supported by LQ1604 NPU II provided by MEYS and CZ.1.05/1.1.00/02.0109 BIOCEV provided by ERDF and MEYS. Also the GA CR project 16-03398S and the support within the bilateral Mobility project SAV-16-12 are acknowledged.

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P.14-007-Mon
Two structural features of the 3′ region of the carnitine palmitoyltransferase 1a (CPT1a) gene specifically observed with human but not with rodents

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Fatty acids are catabolized by the processes of β-oxidation at mitochondrial matrix space. However, as fatty acids cannot penetrate the inner mitochondrial membrane, they are translocated by the processes of carnitine system. Carnitine palmitoyltransferase 1 (CPT1) is known as a rate limiting enzyme of the carnitine system, and three isozymes of 1a, 1b and 1c are known to exist in mammals. The human gene encoding mitochondrial CPT1a was thought to be consisted from 19 exons. However, a recent study revealed the possible existence of 20th exon, which is alternatively used with 19th exon. The use of 20th exon encodes a protein having different C-terminal amino acid sequence from that of ordinary protein, and its physiological roles are still uncertain. In the present study, we first examined whether 20th exon of CPT1a gene is also present in rodents. The genomic regions corresponding to the 20th exon of human CPT1a gene were not conserved in rats and mouse genomes. Moreover, possible genomic regions encoding amino acid sequence similar to that coded by 20th exon of human CPT1a gene were not observed with rats and mouse genomes. Based on these results, we concluded that 20th exon of CPT1a gene is not present in rodents genomes. We next focused on the fact that length of 19th exon of human CPT1a gene was longer than those of rodents. This difference was found to be caused by insertion of the Alu sequence into the 19th exon of human CPT1a gene, and possibly due to this insertion, the cDNAs of the human CPT1a having diverse 3′ length were registered. Based on these results, the 3′ region of human CPT1a gene was concluded to have unique structural properties.

P.14-008-Tue
The phage Vp16 peptide deformylase ribosome binding investigation depicts a new spatiotemporal orchestration of the ribosome binding proteins

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Prokaryotic proteins must be deformylated before the removal of their first methionine. Peptide deformylase (PDF) is indispensable and guarantees this mechanism. Unpredicted modified bacterial PDF genes have been retrieved from many viruses. Sequence comparisons with other known PDFs reveal that viral PDFs are devoid of the key ribosome-interacting C-terminal region. Little is known regarding these viral PDFs, including the capacity of the corresponding encoded proteins to ensure deformylase activity. We have already shown for the first time that viral PDFs, including the shortest PDF identified to date, *Vp*16 PDF, display deformylase activity in vivo. Large-scale N-terminomics characterization reveals that *Vp*16 PDF has substrate specificity similar to that of other bacterial PDFs. However, our integrated biophysical and biochemical approaches reveal hidden and unique functions of the unusual C-terminus. The high-resolution crystal structures of *Vp*16 PDF, free or bound to the potent inhibitor actinonin, reveal a classical PDF fold and an unexpected crucial role for the ultimate residue tethering the active site. Our study underscores the structural and molecular characteristics of the unusual C-terminal Ile residue that sustains deformylase activity in the absence of the otherwise indispensable C-terminal domain. We investigate the ability of *Vp*16 PDF to bind the bacterial ribosome highlighting the differences with the *E. coli* PDF and the chimerae between the two proteins. We provide the first evidence that this phage PDF is able to bind bacterial ribosome more efficiently than *E. coli* PDF. Differently from *E. coli* PDF, *Vp*16 PDF-ribosome binding was increased when a specific nascent chain was present on the ribosome. The ribosome-binding comparison among different *chimerae* between *Vp*16 PDF and *E. coli* PDF reveals different modes of action of the two PDFs on the ribosome.

P.14-009-Wed
Plasma membrane transporter SLC6A14 is controlled by cytosolic heat shock proteins

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SLC6A14 is a member of solute carrier (SLC) family 6 of plasma membrane transporters specific towards amino acids, neurotransmitters, and osmolytes. SLC6A14 transports all neutral and basic amino acids in a Na/Cl – dependent way and it is overexpressed in many types of cancer. Both N- and C-termini of SLC6A14 are localized on the cytosolic side. Our analysis of SL6A14 interactome by mass spectrometry revealed, among others, the presence of cytosolic heat shock proteins (HSPs) and co-chaperones. We studied interaction of SLC6A14 with HSP90beta and HSP70 (HSPA14), identified as possible transporter partners. Immunofluorescence experiments demonstrated the strongest co-localization of both HSPs with overexpressed rat SLC6A14 in transiently transfected HEK293 cells after 24 h. The direct interaction between HSPs and SLC6A14 was confirmed using the proximity ligation assay. Interaction of the transporter with HSP90beta was inhibited by radicicol, known to bind to HSP90 ATP-binding site, while interaction with HSPA14 was attenuated by its inhibitor – VER155008. Cell surface proteins biotinylation demonstrated a dramatic decrease of SLC6A14 presence in the plasma membrane upon treatment with either radicicol or VER155008, what resulted from the diminished level of the total transporter protein. Distortion of SLC6A14 proper folding by both HSPs inhibitors directed the transporter towards endoplasmic reticulum-associated degradation, a process reversed by the proteasome inhibitor – bortezomib. These results indicate that a plasma membrane protein folding can be controlled not only by chaperones in the endoplasmic reticulum, but also those localized in the cytosol. Moreover, these observations may have a potential therapeutic significance, since the use of HSPs inhibitors could decrease amino acid supply to quickly proliferating cancer cells with a high expression of SLC6A14. This study was financed by a grant 2015/19/B/NZ3/00049 from the National Science Centre in Poland.

P.14-010-Mon
The effect of dopamine and synaptosomal factor on the Mg-dependent Ca-ATPase

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Solution of many problems faced by contemporary neurobiology, including neurochemistry, depends to a large extent on the

comprehensive knowledge of the processes going on within a synapse, since the processes in question determine the coordinated integral activity of the central nervous system. In spite of the fact that much in this respect is already known, many problems still remain undefined, or insufficiently studied. In order to settle them, it is necessary to study the individual processes taking place within a synapse and to define their role in the complex sequence of process chain during synaptic transmission. Ca^{2+} and respectively its transport systems take one of the important places in these processes. One of the significant transportable systems of Ca^{2+} is Mg^{2+}-depending Ca-ATPase. Activity of this latter is controllable. Study of regulation Ca-ATPase activity is especially important with neurotransmitters, in our case with dopamine, for the neurotransmitters are directly involved in synaptic transmission Study of the effect of dopamine and synaptosomal factor (factor localized in the synaptic cytosol) on the Ca-ATPase activity, localized in the albino rat brain synapses and microsomal fraction revealed: 1) Dopamine activates Ca-ATPase activity, localized in synapses; 2) Dependence of Ca,MgATPase activity on dopamine concentration has bell like shape, maximum at 0.05 mM concentration; 3) Dopamine does not effects Ca,MgATPase (localized in albino rat brain synaptosomal membrane) transport stoichiometry; 4) Synaptosomal factor does not affect CaATPase activity; 5) Together with dopamine and synaptosomal factor inhibits Ca-ATPase activity, localized in synapses; 6) Dopamine and synaptosomal factor do not have effect on Ca-ATPase activity localized in microsomal fraction.

P.14-011-Tue
Exit of SLC6A14, an amino acid transporter B (0,+), from the endoplasmic reticulum depends on the interaction with a cargo recognizing COPII element SEC24C

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The solute carrier 6 (SLC6) gene family encodes for transporters of neurotransmitters, amino acids, osmolytes, and energy metabolites. SLC6A14 [ATB(0,+)] is a Na^+/Cl^--dependent transporter for neutral and cationic amino acids. Moreover, its expression is increased in certain cancers. In the initial step of trafficking to the plasma membrane, it has to interact with the cargo recognizing protein SEC24 within the coatomer II (COPII) complex to exit the endoplasmic reticulum (ER). Trafficking of rat SLC6A14 to the plasma membrane was studied in a heterologous expression system in HEK293 cells to understand, which of the four paralogues of SEC24 was required for its ER export. Immunofluorescence and biotinylation analyses showed that more than half of SLC6A14 reached the plasma membrane in its fully glycosylated form after 48 h. Experiments with dominant negative mutants of COPII component Sar1A-T39N verified that the export from the ER was COPII-dependent. In fact it was exclusively dependent on a direct interaction with SEC24C, as shown by immunoprecipitation, immunofluorescence and the proximity ligation assay. Interestingly, co-transfection with a dominant mutant of SEC24C did not only decrease the surface levels of SLC6A14 but also its total cellular amount, a finding also seen after co-transfection with Sar1A-T39N. This suggested that a portion of ER-resident SLC6A14 was subject to ER-associated degradation, a conjecture, confirmed by experiments with the proteasome inhibitor bortezomib. This work was supported by the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement no. 665735 (Bio4Med) and by the funding from Polish Ministry of Science and Higher Education within 2016–2020 funds for the implementation of international projects (agreement no 3548/H2020/COFUND2016/2).

P.14-012-Wed
Length of the cytoplasmic C-terminal part of yeast Nha1 Na^+/H^+ antiporter influences its plasma-membrane targeting in cells lacking Erv14 COPII cargo receptor

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All living cells spend a lot of energy to maintain their intracellular alkali-metal-cation homeostasis. In yeasts, various transporters work in plasma and intracellular membranes to ensure proper alkali-metal-cation concentrations both in the cytoplasm and in the organelles. In the model yeast *Saccharomyces cerevisiae*, the plasma-membrane Na^+/H^+ antiporter Nha1 interacts with the COPII cargo receptor Erv14. The lack of Erv14 results in intracellular stacking of Nha1 and affects its function. Erv14 binds the transmembrane domains of Nha1, but, interestingly, no intracellular stacking of Nha1 in *erv14Δ* cells can be observed when Nha1 lacks its long hydrophilic C-terminal part. To reveal the role of Nha1's C-terminus in its intracellular stacking in *erv14Δ* cells, we first studied the function and localization of Nha1 homologues from various yeast species produced in *S. cerevisiae* cells either lacking or possessing Erv14. Studied Nha antiporters significantly differ in the lengths of their C-terminal parts and our results suggest that Erv14 is especially important for proper plasma-membrane delivery of antiporters with long C-termini. Second, we prepared several mutated versions of *Sc*Nha1 with truncated C-terminal part and studied their function and localization in cells with or without Erv14 protein. This approach allowed us to reveal which region of Nha1's C-terminus is responsible for the antiporter's requirement of Erv14 in its proper plasma-membrane targeting. This work was supported by a GACR grant 17-01953S.

P.14-013-Mon
Trk transporters mediate potassium uptake and contribute to pH homeostasis in *Candida* species

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The regulation of ion and pH homeostases is an essential process critical for cell viability. The maintenance of high intracellular concentrations of potassium and neutral pH is important for a variety of cellular functions including cell volume, DNA integrity, protein modification and trafficking. It is becoming increasingly evident that the coordination between primary H^+-ATPases and transport systems involved in the influx and efflux of potassium allows this pH maintenance to occur. Genes encoding three types of potassium uptake systems have been found in genomes of pathogenic *Candida* species. *In silico* studies revealed that *Candida spp.* differ in the number and type of potassium transporters. Whereas *C. albicans* possesses genes for all three known types of transporters (Trk1, Hak1, Acu1), *C. glabrata* and *C. krusei* genomes contain only genes encoding a putative

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Trk1 uniporter. In the present study, we show 1) the comparison of *Candida* Trk proteins *in silico* and *in vivo* upon expression in an *S. cerevisiae* mutant lacking its own potassium uptake systems, 2) the importance of *C. glabrata* Trk1 not only to potassium uptake but also to pH_{in} and plasma-membrane potential maintenance, and 3) a detailed characterization of putative *C. krusei* Trk transporters. Taken together, our results find the Trk-type potassium uptake systems in *Candida* cells to be a promising target in the search for their specific inhibitors and in developing new antifungal drugs. Acknowledgments: This work was supported by grants from the Czech Science Foundation (GA CR 16-03398S) and from the FP7-PEOPLE-2013-ITN ImresFun (606786).

P.14-014-Tue
Yeast VDAC2 forms voltage-dependent channels displaying high-conductance states of opposite selectivity

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The Voltage-Dependent Anion Channel (VDAC) represents the most abundant family of pore-forming proteins located into Mitochondrial Outer Membrane. The main isoform VDAC1 is conserved from yeast to human and allows metabolites exchange between cytosol and mitochondria. In fact, the genetic inactivation of the corresponding gene *por1*, in the budding yeast *Saccharomyces cerevisiae*, results in a complete impairment of yeast growth in non-fermentable condition. Yeast genome is endowed with a second gene, *por2*, encoding for the less-known isoform VDAC2. Since the discovery, the function of yVDAC2 was unclear. In one hand, *por2* inactivation has no consequence for the yeast growth, suggesting that the protein might be devoid of a channel function. On the other hand, yVDAC2 restores yeast growth in VDAC1-null cells when overexpressed, supporting the channel activity. In this work, we have elucidated for the first time the functional activity of yVDAC2. The protein was purified from VDAC1-null yeast cells or produced as recombinant protein, and the activity of both native and recombinant yVDAC2 were analyzed after protein reconstitution in artificial membranes. Results, obtained at the Planar Lipid Bilayer system, have shown that yVDAC2 is able to form voltage-dependence channels, characterized by a conductance of about 3.5 nS in 1M KCl. Moreover, the application of a KCl gradient between two sides of the membrane resulted in shifts in the current/voltage curve slope, allowed for identification of up to three different states with different calculated parameters of ionic selectivity for yVDAC2. Interestingly, two of them appear to be high-conductance states but with opposite selectivity. In conclusion, our results obtained with two different approaches give the definitive message that yVDAC2 is another member of the VDAC family.

P.14-015-Wed
Effect of hypoxia on the proteins composition of rat erythrocyte membrane

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Side-by-side with passive uptake and release of oxygen and metabolically-derived gases erythrocytes exhibit diverse oxygen-sensitive responses involved in regulation of their own and neighboring cell functions. To goal of this study was to determine if the attenuated integrity of deoxygenated RBC could be linked to any changes in the composition erythrocyte membrane. Twenty-min exposure to oxygen-free environment decreases RBC integrity documented by 3-fold elevation of hemoglobin release. Electron magnetic resonance spectroscopy of spin-labeled stearic acid analogues did not detect any action of hypoxia on the membrane fluidity. The proteomics technology in combination with relative label free quantification analysis allowed us to compare the content of 1176 membrane-bound proteins in the ghosts obtained normoxic and hypoxic RBC. Using this approach we found that sustained deoxygenation of rat erythrocytes alters the composition of membrane-bound proteins including 2–3 fold elevation of the content of hemoglobin and glycophorin-C. Mapping the identified proteins in the KEGG pathway database we found that the proteins of multi subunit Cullin-Rbx E3 ubiquitin ligase complex presented in normoxic RBC ghosts but not in the hypoxic samples. We hypothesized that Cullin-Rbx E3 complex, associated with RBC membrane in normoxia, provides detection and deletion of glycophorin-C and other membrane proteins damaged by reactive oxygen species. In hypoxic conditions, deoxy-Hb binds to band 3 protein, resulting in dissociation of Cullin-Rbx E3 complex from RBC membrane and impaired clearance of damaged cytoskeleton proteins including glycophorin-C. We believe that these changes underlie attenuated integrity of RBC membrane and contribute to augment release of hemoglobin and ATP seen in hypoxic conditions. This work was supported by grants from the Russian Foundation for Basic Research (#18-04-00063) and the Russian Scientific Foundation (#16-15-10026).

P.14-016-Mon
Novel fluorescent NBD-labeled 20-hydroxycholesterol-like pregn-5-en-3β-ols: interactions with yeast cells and mammalian STARD1 protein

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Two novel 20-hydroxy-pregn-5-en-3β-ols with NBD-label in their side-chains, attached to C20 via -NH_2-CH_2CH_2- (1) and -NH_2-CH_2CH_2CC- (2) linkers, were synthesized aiming to create fluorescent probes with 20-hydroxycholesterol-like scaffold. We estimated their interactions with whole cells of yeasts Yarrowia lipolytica and Saccharomyces cerevisiae at aerobic conditions. Using confocal microscopy the compounds were found to stain

different compartments of the yeasts, including intracellular. Using chromatography and mass-spectrometry the steroids were also found to undergo partial conversion into 3-O-acetates by S. cerevisiae, but not Y. lipolytica, which was in accordance with computational BLAST search of S. cerevisiae Atf2p homologues. Also the compounds' binding with mammalian STARD1 protein were observed. Compound 2 is also promising as a Raman probe due to alkyne insertion into its side chain. Basing on both the results and Autodock Vina-aided docking simulations with various sterol-binding proteins, e.g. STARD1 (3pl0), Osh4 (1zhw), Osh1 (5wvr) and Npc1 (3gkj), we speculate about possibilities of 20-hydroxycholesterol-like compounds to affect yeast cells or mammalian mitochondria as well as future perspectives of such compounds synthesis and usage for studies of 20-oxysterol trafficking and signaling. This work was supported by joint BRFFI (X16P-065) - RFFI (16-54-00139) grant, Belarusian SPSI grant 20161380 and RSF grant 17-74-10053.

P.14-017-Tue
Characterization of the GM1 oligosaccharide transport across the blood-brain-barrier

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Ganglioside GM1 has demonstrated to attenuate Parkinson Disease (PD) symptoms in clinical and preclinical trials. Nevertheless, the GM1 efficacy revealed *in vitro* is critically reduced *in vivo*, because of the amphiphilic behavior that limits the passage across the blood brain barrier (BBB). *In vitro* and *in vivo* experiments showed that GM1 exerts neurotrophic functions by interacting with plasma membrane (PM) proteins throughout its oligosaccharide portion (OligoGM1). Furthermore, OligoGM1 intravenously or subcutaneously injected into mice is absorbed and taken up by different organs and tissues, including brain. In order to take advantage of GM1 oligosaccharide properties and to overcome GM1 pharmacological limitation, this study has been aimed by the investigation of the OligoGM1 transport through the BBB, by using a human *in vitro* model for human brain-like endothelial cells (hBLEC). Ruled out the toxicity of OligoGM1 on hBLEC, the OligoGM1 transport across the hBBB has been analyzed, finding out a 20 fold higher rate than GM1 and a time and concentration dependence. In order to characterize the OligoGM1 passage, a direct evaluation of the OligoGM1 interaction with the ABC-transporters was carried on, leaving out this way for OligoGM1 transport. Moreover, inverse- and 4°C-transport experiments were performed excluding the implication of the active transport for OligoGM1 passage across the hBLEC, leading to consider the passive-paracellular route. Furthermore, after the hBLEC transport, OligoGM1 maintained its stability and capacity to induce neuritogenesis in the mouse neuroblastoma cells line Neuro2a. This preliminary study has improved the knowledge about the GM1 pharmacological potential by proving that OligoGM1 can cross advantageously the BBB, offering a new promising therapeutic strategy.

P.14-018-Wed
GM1 neuroprotective properties are related to GM1 oligosaccharide

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In light of its neurotrophic and neuroprotective properties, GM1 ganglioside has been considered as a master regulator of the nervous system. Recently, we demonstrated in a mouse neuroblastoma cell line N2a that the oligosaccharide portion of GM1 (OligoGM1) is responsible for the ability of GM1 to induce neuritogenesis by the activation of the TrkA-MAPK pathway. This means that the specific role of GM1 in neuronal differentiation is determined by a direct interaction between its oligosaccharide portion and specific proteins expressed on the plasma membrane. In order to understand if the exogenous administration of OligoGM1 and the resulting activation of TrkA pathway is able to trigger crucial biochemical signaling, we performed a proteomic analysis on N2a cells treated with 50 μM OligoGM1 for 24 h. The analysis led to the identification and quantification of 744 proteins; 324 proteins were identified only in OligoGM1-treated cells. Interestingly, the OligoGM1-only proteins are mainly involved in biochemical mechanisms, many of which offer neuroprotective potential reflecting the GM1 neuroprotective effect. To confirm that the neuroprotective effect of GM1 is due to its oligosaccharide portion, OligoGM1-treated N2a cells were administered with MPTP for 48 h. We found that MPTP-induced mortality was reduced by 50% in OligoGM1-treated cells respect to untreated ones. Furthermore, we saw that the administration to N2a cells of dichlorobenzamil (DCB), a potent inhibitor of the Na^+-Ca^{2+} antiporter, which results in elevating intracellular Ca^{2+}, is responsible for cell death after 24-h exposure. In presence of 50 μM OligoGM1 the DCB-cell mortality is definitely reduced, suggesting that the modulation of Ca^{2+} flux might be one mechanism in the neuroprotective effect due to exogenous administration of OligoGM1. Our results suggest that the molecular mechanisms underlying the neuroprotection effects induced by GM1 depend on its oligosaccharide chain.

P.14-019-Mon
The subcellular localization mechanisms of type II membrane proteins

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Type II membrane proteins are inserted into endoplasmic reticulum (ER) at the early stage of protein subcellular localization due to the recognition of the signal-anchors by ER translocons. However, the evidential transport mechanisms of transmembrane protein localization from ER to the Golgi or plasma membrane have not been elucidated. Understanding the mechanism of protein subcellular localization is believed to be crucial for control of protein subcellular localization. In this study, to elucidate transport mechanisms of transmembrane proteins, the amino acid propensities around the signal-anchor and C-terminus regions were calculated. The transmembrane protein dataset was classified into three groups: plasma membrane proteins, ER membrane proteins and Golgi membrane proteins. Especially, the trans-Golgi membrane protein dataset was extracted from the Golgi group. The discrimination accuracy of each group was estimated by the discrimination scores which were calculated by the position-specific scoring matrix (PSSM) to evaluate whether the transmembrane protein localization is determined by the

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sequences around the signal-anchor and C-terminus. The Golgi and plasma membrane proteins could be discriminated with high accuracy (> 90%) using the PSSM created by the signal-anchor sequences. The trans-Golgi membrane proteins are well distinguished from plasma membrane proteins using the PSSM created by the C-terminal sequences. These results suggested that the amino acid propensities around signal-anchor were related to the Golgi to plasma membrane localization mechanism. The C-terminal sequence was considered to related to the trans-Golgi-plasma membrane transport. To verify this presumption by experimental methods, the GFP fusion proteins with signal-anchors of the representative proteins selected from each group were designed. The subcellular localization of these GFP fusion proteins expressed in HeLa cells were observed by confocal laser fluorescence microscope.

P.14-020-Tue
Comparative analysis of GPI modification mechanisms between human and rice plant proteins

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Glycosylphosphatidylinositol (GPI) is one of the protein post-translational modification molecules. GPI-anchored proteins (GPI-APs) in mammalian cells are known to localize themselves to raft domain via the endoplasmic reticulum (ER). GPI-APs have two signal sequences, signal-peptide (SP) and GPI-attachment signal (GPI-AS), which are N-terminal ER localization sequences and C-terminal signal sequences for GPI modification, respectively. Plant proteins also have GPI modification systems as well as mammalian proteins, can translocate to the cell wall or the raft region on the cell membrane. However, because few plant proteins have been isolated as GPI-APs, the plant GPI modification mechanisms have not been clarified. In this study, GPI modification mechanisms were compared between plant and human proteins using bioinformatics and experimental approaches. By the bioinformatics analysis, some differences between plant and human GPI-APs were found in the physicochemical characteristics, especially in SPs. Furthermore, the wild-type and chimera CEBiP (Chitin Elicitor Binging Protein) were expressed in the HeLa cells, and the subcellular localization of the wild-type and chimera CEBiP was observed by the immunostaining method. The results indicated that one of the differences of the GPI modification mechanisms between human and plant proteins is the protein transport system which depends on SPs.

P.14-021-Wed
Dualism of action of local anesthetics on ionic channels formed by antimicrobial agents

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Despite a long history of an application, the precise mechanisms of action of local anesthetics (LAs) are still under debate. The question is whether the effects are induced by acting on functional proteins, on membrane lipids, or on both. It is generally recognized that LAs block sodium channels, but membrane protein-interacting theory is not able to explain all of the pharmacological and toxicological features of these agents. To study the molecular mechanisms of the influence of LAs on ionic channels we used pore-forming antimicrobial agents reconstituted into planar lipid bilayers. We found that the effects of LAs on the conductance of single ionic channels induced by well-known peptide antibiotic, gramicidin A, and by antifungal cyclic lipopeptide, syringomycin E, were caused by their influence on membrane electrostatic potentials; the regulation by amide-linked compounds, in particular, lidocaine, prilocaine, and mepivacaine, was related to the membrane surface potential, whereas modulation by ester-bound LAs, in particular, tetracaine, was predominantly due to changes in a dipole potential. Moreover, at high electrolyte concentration in the membrane bathing solution amide-linked LAs block ion passage through syringomycin channels; they permeate a pore from its wider lipid mouth; this leads to a sharp drop in channel lifetime and conductance at negative transmembrane voltages. The cooperativity of interaction observed with lidocaine indicated the existence of specific binding sites. LAs affecting lipid packing, especially, tetracaine, led to a significant increase in the steady-state membrane conductance induced by antifungal polyene macrolides, nystatin and amphotericin B, syringomycin E, and antimicrobial peptides, magainin and melittin. The results were consistent with a lipid-mediated mode of action of the LAs via decreasing a curvature stress at the formation of toroidal pores. The work was supported by the Russian Science Foundation (# 14-14-00565-P).

P.14-022-Mon
RNA-binding to negatively charged membrane surfaces affects the structure of the membrane and of the RNA

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Based on the RNA world hypothesis, early RNA molecules are the origin of life. However, this idea begs the question of what actually occurred in primordial cells when RNA was encapsulated within a membranous compartment. Did the RNA anyhow "interact" with the lipid bilayer? And did such an interaction anyhow impact the structure and integrity of the membrane or the RNA molecule? Recently, RNA molecules were identified that were suggested to interact with phosphatidylcholine (PC) bilayers, which are net uncharged. We now have synthesized these RNA molecules *in vitro* and have analyzed their interaction with model membranes in great detail. While we did not observe any impact of the RNA molecules on the organization of PC membranes, we detected formation of liposome aggregates. However, in presence of the RNA molecules the phase transition temperature of phosphatidylglycerol (PG) liposomes was shifted to higher temperatures. Since NaCl exterminated this effect, binding of RNA to the PG surfaces appears to be electrostatically driven. Yet, this was a rather unexpected finding, as PG membranes carry a negative surface charge, and the negatively charged RNA appears to interact specifically with negatively charged membrane surfaces. Furthermore, interaction with the negatively charged PG, but not with the zwitterionic PC, does affect the RNA stability and decreases the RNA melting temperature. In summary, we could show that RNA molecules can interact with membrane surfaces, and such an interaction does affect the physico-chemical properties of one other.

P.14-023-Tue
Presence of cholesterol and acyl chain composition affects interactions between phosphatidic acid and peripheral proteins

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Cellular membranes consist of enormous variety of lipid species. One of them is phosphatidic acid (PA), the simplest diacyl-glycerophospholipid, which is minor lipid in membranes, but is a key molecule in membrane lipids synthesis pathways and acts as a signaling molecule. Until now more than 20 proteins involved in various cellular processes have been characterized as PA binders. The possibility of one lipid species playing multiple functions may stem from its structure. We assumed that the length and saturation of PA acyl chains may influence its interaction with proteins. Cellular PA species differ in its acyl chain composition. Moreover, recent findings show, that cholesterol has a strong impact on head group conformations of neighboring phospholipids, which may affect the affinity of proteins to the lipid bilayer. We study the specific recognition of PA by FRB domain of mTOR, a well-known PA partner. We tested three different PA species: 16:0–18:1 (POPA), 18:0–20:4 (SAPA), 16:0 (DPPA) in presence and absence of cholesterol. Using LUVs as membrane model systems and flotation assay we demonstrated that different PA species show the diverse binding ability to FRB domain and the presence of cholesterol modulate it, although the domain does not bind to cholesterol itself. To confirm this results we tested the interaction between GUVs with the same lipid composition and FRB-GFP using confocal microscopy. Quantification of the fluorescence signal gathered from the protein was used to determine binding characteristics. Moreover, to estimate binding affinity, FRET technique on LUVs and FRB was implemented. We propose that, due to the acyl chain diversity, presentation of PA within the membrane varies affecting interaction with FRB domain. Furthermore, cholesterol has also an impact on PA presentation in the membranes. Our results are of great importance for understanding interactions of signaling lipids with their receptors.

P.14-024-Wed
High affinity interaction of MPP1 with flotillins in vitro

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Research of the molecular mechanism of raft domain formation is essential in understanding lateral membrane organization, and its physiological role. MPP1 (Membrane Palmitoylated Protein 1) is peripheral membrane MAGUK-family protein that consists of four domains commonly known in the literature: PDZ domain, which interacts with glycophorin C and whirlin, SH3 domain of unknown function in this protein, D5 domain, which interacts with 4.1 protein and GUK domain, which is catalytically inactive and interacts with other proteins. MPP1 was found recently to be engaged in proper lateral organisation of plasma membrane of both erythrocytes and erythroid precursors. Our recent research identified flotillins, which are the raft marker proteins, as molecular partners for MPP1 in plasma membrane. Here we present data on in vitro kinetics of this interaction and also attempt to identify specific region of MPP1 responsible for interaction with flotillins. Surface plasmon resonance was our method of choice to measure dissociation constants for protein-protein interactions. Biacore T200 (GE Healthcare) was used for this purpose with Ni-NTA chip. Recombinant proteins, bacterially expressed, histagged flotillin-1 and flotillin-2 were immobilized on the chip. Recombinant constructs of full length MPP1 and its four domains were bacterially expressed and purified in GST-free form by using PreScission Protease. These proteins were used as analyte in the SPR experiments. In result of these experiments we were determined K_D (equilibrium dissociation constants) for interaction between MPP1 and both flotillins 1 and 2. We found that two domains: D5 and SH3 bound both flotillins with high affinity, similar to this characteristic for full length protein. Our data gives more details into newly, recently described MPP1-flotillins interaction. We believe that this interaction plays an important role in the lateral membrane organisation. Supported by NCN Grant DEC-2016/21/B/NZ1/02821.

P.14-025-Mon
Quantified membrane permeabilization and antimicrobial gram-selectivity

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Membranes act as first barrier for entering cells, they ensure integrity of cells and compartments, and are putative targets in antimicrobial activity. Membrane permeabilization is often studied by fluorescence spectroscopy or microscopy. However, typical data analysis did not reveal the full potential of experimental leakage data. Our new concept for the quantification of membrane permeabilization is valid for any kind of intended or unintended permeabilization process, such as experimental artefacts in fluorescence microscopy of GUVs, *in vitro* studies of antimicrobial, anticancer and cell penetrating peptides and their synthetic mimics, pore-forming peripheral and transmembrane proteins, transfection agents, and drug delivery vehicles. Our statistical analysis and kinetic studies of lipid membrane permeabilization yield the strength of an individual leakage event. The thorough quantification of leakage with our concept gives hints for the molecular mechanism of permeabilization, such as asymmetry stress, curvature stabilization (toroidal pores, defects), assembled (oligomeric) pores and combinations of these. We will show the usefulness of the concept by a systematic and mechanistic assessment of a series of Gam-selective synthetic antimicrobials and their selectivity for certain lipid membranes. Natural antimicrobial peptides and even more so their synthetic mimics are promising and dearly-needed alternatives to antibiotics. We find selectivity for Gram-positive bacteria to be related to the facilitation of assembled pores. We provide indicators for these selective assembled pores. On the other hand, unwanted haemolysis is associated to the asymmetry-stress permeabilization mechanism and we give a design guideline for non-haemolytical antimicrobials. Our quantification of membrane permeabilization increases the value of model studies for biological and biochemical processes at membranes.

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P.14-026-Tue
Functional analysis of lipase transcription factor induced by stearyl alcohol in *Ralstonia* sp. NT80

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Stable and high stereo-selective lipase and esterase have attracted much attention from viewpoints of industrial usage. In the course of our studies searching for efficient inducers for lipase, among various additives, we have found that fatty alcohols and polyoxyethylene (n) alkylethers act as the most effective super-inducers for induction of thermo-stable and stereo-selective lipase production by several *Pseudomonas*-like bacteria. The addition of fatty alcohols brought about more than several hundred-fold enhancement of the lipase activity compared to the case with no additive. This means several dozen-fold enhancement of lipase activity compared with olive oil grown case. We also found that when *Ralstonia* sp. NT80, one of the *Pseudomonas*-like bacteria, were grown on fatty alcohols, not only lipases but a large amount of an extracellular secreted 14kD protein, EliA (effector protein of lipase induction), was strongly induced. EliA was identified to be a surface-active agent from the following facts: Purified EliA reduced surface tension of water and possessed n-alkane emulsifying activity. Both lipase activity and the amount of lipase gene transcript in EliA gene deletion mutant-strain were remarkably reduced compared with those in wild-type strain, so much so that EliA seems to be concerned in the lipase super induction by fatty alcohols. EliA gene seems to be under a different transcriptional regulation from that of lipase gene as a result of 5′-upstream region analysis. Our results indicate that lipase expression, induced by stearyl alcohol, in *Ralstonia* sp. NT80 is under the control of the two-component system (sensor kinase and response regulator).We propose a model of lipase super-production system as follows; uptake of a small amount of lipase super-inducer, induction of EliA protein and secretion, emulsification of stearyl alcohol by EliA protein etc., uptake of a large amount of lipase super-inducer, and strong induction of lipase and secretion.

P.14-027-Wed
Structure and substrate specificity of essential bacterial Apolipoprotein N-acyltransferase (Lnt) from different bacterial species

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The bacterial lipoprotein modification pathway represents an attractive target for antibiotic development. It is essential for the viability of proteobacteria, which include many important human pathogens. The three enzymes involved in the maturation of lipoproteins are integral membrane proteins with catalytic domains facing the bacterial periplasm. This topology makes the lipoprotein biogenesis pathway accessible to small molecules that will be capable of inhibiting enzymes without a need to traverse the relatively impermeable cytoplasmic membrane. Apolipoprotein *N*-acyltransferase (Lnt) catalyzes the third and last step in the post-translational modification of bacterial lipoproteins. We have developed an *in vitro* assay for Lnt, based on purified Lnt enzyme, a synthetic diacylated peptide and pure phospholipids. By monitoring the formation of mature triacylated peptide we have shown that Lnt of *E. coli* has substrate specificity for phospholipids with a small polar headgroup, and saturated *sn*-1 and non-saturated *sn*-2 acyl chains. Phospholipids with branched fatty-acids or bulky reactive groups are not substrates for Lnt. Our kinetics studies showed that Lnt follows a two-step ping-pong mechanism. In the first step, the *sn*-1 of phosphatidylethanolamine reacts with the active site cysteine resulting in the formation of a thioester fatty-acid acyl enzyme intermediate and a lysophospholipid by-product. In the second step, the acyl group is transferred to apolipoprotein resulting in mature triacylated lipoprotein. We recently solved the X-ray crystal structure of Lnt of *Escherichia coli* and *Pseudomonas aeruginosa* that allowed us to obtain more insight into the molecular mechanism of the reaction. The structures of the two proteins are very similar, however, the enzymes have different substrate specificities for both the phospholipid and the diacylated peptide. Implications of our results will be presented.

P.14-028-Mon
Characterization of encystation-specific UDP-N-acetylglucosamine transporter of *Entamoeba*

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Chitin, a homopolymer of β-(1,4) linked N-acetylglucosamine (GlcNAc) is a major component of cyst wall in the protozoan parasites *Entamoeba histolytica* and *Entamoeba invadens*. Chitin is synthesized by a β-glycosyltransferase enzyme known as chitin synthase that utilizes cytosolic UDP-GlcNAc as activated sugar to synthesize chitin polymer inside the organelle lumen. However, nucleotide sugar transporters (NSTs), Type-II multi-transmembrane proteins that are involved in the transport of UDP-GlcNAc across the membrane for the synthesis of cyst wall chitin are not known yet. Here, we are reporting the role of *E. histolytica* UDP-GlcNAc transporter (EhNST3) and its ortholog in *E. invadens* (EiNST5) in chitin synthesis using yeast complementation assays. Both the EhNST3 and EiNST5 contain putative sequences for endoplasmic reticulum localization and UAA superfamily domains specific to UDP-GlcNAc transport as similar to that of an ER-localized UDP-GlcNAc transporter *YEA4* in *S. cerevisiae*. RT-PCR data reveals EiNST5 mRNA expression increases during *in vitro* encystations in *E. invadens*. Heterologous expression of both EhNST3 and EiNST5 in yeast model (*YEA4* mutant *S. cerevisiae*) results in increased chitin content in this chitin deficient yeast mutant. This suggests that EhNST3 and EiNST5 have a possible role in chitin synthesis which can be further investigated in encysting *Entamoeba*.

P.14-029-Tue
Rab3GAP-Rab18 module: a novel regulator of autolysosome maturation

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Autophagy is an evolutionarily conserved lysosomal degradation pathway in eukaryotes. During this process, dispensable or aberrant organelles are sequestered into double-membrane vesicles, called autophagosomes that eventually fuse with lysosomes. Inside the newly formed autolysosomal lumen, the cargo is degraded and the resulting monomers are subsequently recycled. Thus, this process is essential for cellular homeostasis and autophagy dysfunction is linked to several diseases such as neurodegeneration, cancer and aging. Autophagosome-lysosome fusion is

a key step of autophagy, however our knowledge about its genetic regulation is still limited. During the autophagic process, the cellular endomembrane system undergoes extensive transformation. Small GTPases of the Rab protein family are major regulators of membrane dynamics. The nucleotide bound state of Rab proteins is dependent of several cofactors, including the activator GEFs (guanine nucleotide exchange factor) and inactivator GAPs (GTPase-activating protein). Our research focuses on the Rab3GAP2 protein that forms a heterodimeric complex with Rap3GAP1. Rab3GAP1/2 complex is multi-modal Rab cofactor as on one hand it can function as a GAP for Rab3, but on the other, it can also act as a GEF for Rab18. In our study using *Drosophila melanogaster,* we found that the loss of Rab3GAP2 inhibits autophagic degradation. In addition, we showed that loss of Rab3GAP2 leads to the severe alteration of lysosome morphology and function leading to autophagosome accumulation. We also found that knocking-down Rab18 inhibits autophagy in a similar way to Rab3GAP2. Finally, we found that aged Rab3-GAP2 mutants show neuromuscular disorders, which is a well-known hallmark of autophagy defect. Taken together our results suggest that Rab3GAP-Rab18 module acts as a novel regulator of autolysosome maturation.

P.14-031-Mon
The genotoxic and antioxidant effects of environmental pollutant sodium omadine on zebra fish (*Danio rerio*)

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Growing of living organisms such as bacteria, protozoans, algae, and crustaceans can accumulate in large numbers on surfaces like pipes, tanks, and ships' hulls, resulting in corrosion, clogging and contamination, known as biocides. The control of biofouling in water systems is achieved through the avoidance of natural recruitment, physical removal and the use of antifoulants. Sodium omadine (NaoM) is a commonly used disintegrant and biocide for micro-organisms. The aim of the present study is to determine the effect of environmental pollutants (NaoM) on aquatic toxicology and the possible negative effects on humans by evaluating DNA/RNA oxidative damage, and free radicals. To generate tissue damage six groups of zebrafish (length 3–4.5 cm) were exposed to NaoM 1 μg/l and 5 μg/l for the planned time intervals as 24, 72 and 96 h. To evaluate the DNA/RNA oxidative damage a total zebra fish was homogenised for DNA isolation, hydrolysed and damage was measured by commercial kit as EIA. In addition, catalase (CAT) and superoxide dismutase (SOD) levels were measured by commercial spectrophotometric enzyme assay kits. DNA/RNA oxidative damage as 8-hydroxy-2′deoxyguanosine (pg/ml) and SOD activity (U/ml) was statistically significantly different compared to 24 h (1 μg/l) group ($P < 0.05$). Zebrafish (Danio rerio) is becoming a more widely used and increasingly powerful model organism for many fields of modern biomedical research. In conclusion, as marine pollution and deterioration of ecosystems are directly affect the humans. the results presents awareness among environmental pollution, marine pollution and health problems. The toxic effects of environmental pollutants on DNA damage and antioxidant systems on different stages of organisms on the food web provide basic data to understand and estimate the effects on the human beings.

P.14-032-Tue
Negative charge and membrane tethered viral 3B cooperate to recruit viral RNA-dependent RNA polymerase 3Dpol

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Most +RNA viruses replicate at replication organelles (ROs) known as replication factories that are derived from the host membranes (Golgi or ER most often). ROs provide microenvironment needed for efficient viral replication and also serve as a shelter from innate intracellular immunity. The key role in +RNA viral replication plays viral RNA dependent RNA polymerase $3D^{pol}$. This enzyme replicates RNA at (ROs) and the lipid hallmark of the Golgi – the phosphatidylinositol 4-phosphate (PI4P) lipid – was implied in recruitment of $3D^{pol}$ to ROs. However, +RNA viruses do not possess any phosphatidylinositol 4-kinase (PI4K), instead they hijack the human PI4Ks. Many picornaviruses use the Golgi resident acyl-CoA-binding domain-containing protein-3 (ACBD3) to hijack the lipid kinase PI4KB[1]. Here, we show using biomimetic model membranes, hijacking of PI4KB by the nonstructural viral 3A protein via the ACBD3 protein. Upon hijacking, PI4KB hyperphosphorylates membranes overproducing the PI4P. However, we found that not PI4P but rather the negative charge is responsible for the recruitment of $3D^{pol}$ to the membrane as PS is as efficient as PI4P[2]. Additionally, we show that membrane tethered 3B protein cooperates with the negative charge to increase the efficiency of $3D^{pol}$ recruitment.

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Signalling through membranes and receptors

P.15-001-Mon
Molecular mechanisms of vitamin D3 action on bone resorption/formation balance in Type 1 diabetes

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Type 1 diabetes (T1D) is endocrine disorder, accompanied by a number of side effects, one of which is secondary osteoporosis. T1D-induced osteoporosis can be attributable to significant disturbances in osteoblast to osteoclast balance caused, in particular, by changes in the activity of such proinflammatory transcription factors as the nuclear factor kB (NF-kB) and the

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nuclear factor of activated T-cells c1 (NFATc1). It is assumed that these anomalies are closely related to alterations in the functioning of the vitamin D para/autocrine system in bone tissue. The study was carried out to establish possible contribution of D-deficiency and impaired expression of key elements of the vitamin D para/autocrine system to osteoblast/osteoclast imbalance associated with experimental T1D; and to evaluate whether vitamin D3 administration can be effective in correcting diabetes-evoked changes. Diabetes was induced by single i.p. injection of STZ at dose 55 mg/kg b.w. After 2 weeks, diabetic animals were treated with or without vitamin D3 (600 IU/kg b.w., per os) for 30 days. It was shown that blood 25OHD content in T1D decreased by 40%. Vitamin D insufficiency was accompanied by the down-regulation of VDR expression in bone tissue, suggesting abnormal osteoblast-mediated osteosynthesis. The increase, most likely compensatory, in CYP27B1 mRNA was not accompanied by statistically significant changes in its protein level. In line with the assumption of osteoclastogenesis activation in T1D, the levels of both total and activated (phosphorylated at Ser 311) NF-κB/p65 were significantly increased (1.4- and 2.3-fold respectively vs. control). In turn, this led to 2-fold elevation of osteoclasts differentiation marker – NFATc1 in diabetes vs. control. Vitamin D3 treatment resulted in partial normalization of all investigated parameters in diabetic rats. Thus, restored vitamin D bioavailability can improve bone osteoblast/osteoclast balance and prevent secondary osteoporosis in T1D.

P.15-002-Tue
The variety of the regulatory mechanisms of the pore-forming activity of syringomycin E

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The effects of dipole modifiers, 50 μM thyroid hormones (thyroxine and triiodothyronine), 2.5 μM xanthene dyes (Rose Bengal, phloxineB, erythrosin, eosinY and fluorescein), and 1 mM local anesthetics (tetracaine and procaine) on the pore-forming activity of the lipopeptide produced by *Pseudomonas syringae* syringomycin E (SRE) were studied in a lipid bilayers. Triiodothyronine leads to 6-fold decrease of the steady-state membrane conductance induced by SRE (I) at −50 mV. Rose Bengal, phloxine B, erythrosin and tetracaine significantly increase I by 170, 50, 40 and 3 times, respectively. Thyroxine, eosin Y, fluorescein and procaine do not practically affect the pore-forming activity of SRE. We have showed that both thyroid hormones decrease the dipole potential of bilayers on about 60 mV, while Rose Bengal, phloxine B and erythrosin reduce the membrane dipole potential on 120, 80 and 50 mV, respectively. Tetracaine substantially increases the bilayer dipole potential on about 80 mV. Eosin Y, fluorescein and procaine do not significantly affect the magnitude of the membrane dipole potential. Using differential scanning microcalorimetry we show that triiodothyronine and tetracaine leads to a significant decrease in the temperature and cooperativity of the main transition of DPPC. In contrast, thyroxine and procaine slightly decrease the temperature of the main transition of DPPC. Xanthene dyes do not influence the transition of DPPC. It has been concluded that thyroid hormones and local anesthetics modulate SRE channels predominantly via the elastic properties of the membrane, whereas the xanthene dyes Rose Bengal, phloxine B and erythrosine influence SRE channels through the bilayer electrostatics. The study was supported by RFS (14-14-00565). S. Efimova was awarded by scholarship SP-484.2018.4.

P.15-003-Wed
PIP5K-PIP2 and PLD-PA in plasma membrane and exosomal sorting, respectively

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Lipid membranes act as selective barriers for signal transduction pathways and also constitute a source of lipids participating in such signaling events. Signaling molecules outside the membrane can change the lipid composition of the membrane via the activation of lipid modifying enzymes. Thereby, the signal is transmitted inside the cellular compartments. We have shown that the production of phosphatidylinositol 4,5-bisphosphate (PIP_2) on endosomes, under the control of ADP ribosylation factor 6 (ARF6) and its effector phosphatidylinositol 4-phosphate 5-kinase (PIP5K), mediates the recycling of endocytosed proteins to the plasma membrane. This process includes the sequential peptide and lipid binding by the PDZ domains of syntenin. ARF6 is also controlling the budding of endosomes to form multivesicular bodies (MVBs) that will liberate exosomes. That alternative pathway involves the ARF6 effector phospholipase D2 (PLD2), implying that the production of phosphatidic acid (PA) on endosomes might control the budding of cargo inside the lumen of endosomes and the formation of intraluminal vesicles. Further work now provides evidence for also a direct interaction between the PDZ protein syntenin and PA, pointing to a possible role of syntenin in the loading of protein cargo into ILVs, in a PLD2 dependent manner. This work illustrates how the combination of protein-lipid interactions, by the PDZ adaptor protein syntenin, can act as a driving force in membrane compartmentalization and cell signaling.

P.15-004-Mon
Regulation of mitochondrial large conductance calcium-activated potassium channel (mitoBKCa) by hydrogen sulfide and hemin

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Several potassium channels have been found in the inner mitochondrial membranes of various cells. One of them is mitochondrial large conductance calcium activated potassium channel ($mitoBK_{Ca}$), which is formed by so called DEC splice variant of KCNMA1 gene. $MitoBK_{Ca}$ is a tetramer of α subunit which contains seven transmembrane segments, a short N-terminus in the intermembrane space, and a large cytosolic C-terminus composed of two RCK (regulating conductance of K^+) domains. In addition to calcium ions numerous other molecules are known to regulate $mitoBK_{Ca}$ channels including activator NS1619, inhibitors paxilline and heme or its oxidised form hemin. It is also known that gaseous signaling molecules can regulate the activity of $mitoBK_{Ca}$, for example carbon monoxide (CO) activates $mitoBK_{Ca}$ channel. However, little is known about regulation of $mitoBK_{Ca}$ channel by hydrogen sulfide (H_2S). Mechanism of H_2S action is complex and includes redox reactions, persulfide formation with -SH groups of cysteines (S-sulfhydration) and sulfide-metal interactions in heme proteins. In this study, we applied biotin-switch method to show S-sulfhydration of $mitoBK_{Ca}$ channels after application of sodium hydrosulfide (NaHS) as a H_2S donor. We recorded the activity of single $mitoBK_{Ca}$ channels

after application of NaHS in different concentrations. In addition, we also investigated the impact of NaHS on mitoBK$_{Ca}$ channels inhibited by hemin and discovered that NaHS activates channels under this condition. Therefore, H_2S is an important molecule for regulation of the mitoBK$_{Ca}$ channel activity. This work was supported by Polish National Science Center, grant no. 2015/17/B/NZ1/02496.

P.15-005-Tue
Extracellular ATP induces activation and degranulation of meningeal mast cells through P2X7 receptor: a possible mechanism for migraine pain

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Expression and functional properties of P2X7 receptors in meningeal mast cells (MCs) which are involved in development of migraine pain have not been previously explored. Here we studied the mechanisms of ATP-mediated activation of murine native and cultured meningeal MCs. Experiments were performed on freshly isolated meningeal MCs and meningeal cell-derived cultured MCs (this is the first time that possibility of prolonged culturing of meningeal MCs was shown). MCs were treated with 1 mM ATP. To inhibit the function P2X7 receptors, MCs were pretreated with P2X7 antagonist A839977 followed by 1 mM ATP stimulation. As an independent approach, whole mount meninges were pretreated with or without 1 mM ATP, and the percentage of degranulated MCs in meninges was calculated after toluidine blue staining. For estimation of MC activation with ATP, we used the fluorescent dye YO-PRO1, which enters the cells through the dilated P2X7 receptor ion channel. Using flow cytometry we demonstrated that in control conditions, in a population of freshly isolated meningeal MCs, the percentage of YO-PRO1 positive cells was $14.4 \pm 1.9\%$ ($n = 10$ experiments) whereas the incubation with 1 mM ATP significantly augmented this number up to $22.3 \pm 3.4\%$ ($n = 8$, $P = 0.027$). Treatment with P2X7 antagonist A839977 prevented the ATP induced YO-PRO1 signal ($16.1 \pm 2.9\%$, $n = 5$, $P = 0.19$). We also verified the ability of 1 mM ATP to activate meningeal MCs using toluidine blue staining ($n = 4$, $P < 0.001$) and demonstrated that incubation of whole mount meninges in 1 mM ATP leads to degranulation of mast cells in dura mater. Taken together, these data demonstrate important contribution of P2X7 receptors to ATP-driven activation of meningeal MCs, suggesting these purinergic mechanisms as potential triggers of neuroinflammation and pain sensitization in migraine.

P.15-006-Wed
Sigma-1 receptor agonist amitriptyline attenuates Ca^{2+} responses induced by glutoxim and molixan in macrophages

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Sigma-1 receptors are unique multitasking molecular chaperones at the endoplasmic reticulum with a unique amino acid sequence and a unique pharmacological profile. These receptors can bind a wide spectrum of ligands with very diverse structures and with different pharmacological applications, such as antidepressants, neuroleptics, anticonvulsants, antitussives and analgesics. Sigma-1 receptors integrate many signalling pathways and in particular modulate Ca^{2+} signalling processes in cells. Earlier, we have shown that disulfide-containing immunomodulators glutoxim® (disodium salt of oxidized glutathione with d-metal at nanoconcentration, PHARMA VAM, Saint-Petersburg) and molixan® (complex of glutoxim with nucleoside inosine) cause biphasic intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increase due to Ca^{2+} mobilization from thapsigargin-sensitive Ca^{2+} stores and subsequent store-dependent Ca^{2+} entry in rat peritoneal macrophages. To elucidate the possible involvement of sigma-1 receptors in glutoxim and molixan effect on $[Ca^{2+}]_i$ in macrophages we used sigma-1 receptor agonist tricyclic antidepressant amitriptyline. Using Fura-2AM microfluorimetry we have found that macrophage preincubation with 20 μg/ml amitriptyline for 20 min before 100 μg/ml glutoxim addition leads to a significant suppression of both Ca^{2+} mobilization (by $39.6 \pm 9.2\%$) and subsequent Ca^{2+} entry (by $46.3 \pm 10.1\%$), induced by glutoxim. Similar results were obtained in experiments with molixan. Thus, we have demonstrated for the first time that sigma-1 receptor agonist amitriptyline inhibits both phases of the Ca^{2+} response induced by glutoxim or molixan, which indicates the possible involvement of sigma-1 receptors in signalling cascade triggered by these immunomodulators in macrophages. Our results also indicate that it is inadvisable to use glutoxim or molixan in combination with antidepressant amitriptyline in clinical practice.

P.15-007-Mon
Potassium homeostasis and calcium signalling in yeast cells

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Calcium signalling has a crucial role in various processes such a metabolic signalling or maintenance of intracellular pH in yeast cells. The cytosolic concentration of calcium cations is maintained at very low levels by vacuolar Ca^{2+} ATPases and exchangers which sequester more than 90% of all calcium cations in the vacuole. Upon an environmental stresses, e.g. an osmotic shock, Ca^{2+} cytosolic levels transiently increase due to a quick channel-mediated release from vacuole. To maintain cell cation and pH homeostasis, various transporters tightly cooperate. We investigated the Ca^{2+} signalling in yeast cells with disturbed K^+ homeostasis. We determined the concentration of Ca^{2+} cations in *S. cerevisiae* lacking K^+ influx (Trk1, Trk2) or efflux (Tok1, Nha1 and Ena1-5) systems. Mutant strains lacking K^+ exporters had the same basal cytosolic Ca^{2+} concentration as the wild-type. On the other hand, strains lacking K^+ importers

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maintained significantly higher (Trk2-less strain) or lower (Trk1-less strain) Ca^{2+} concentration. We also measured the transient increase of cytosolic Ca^{2+} during an osmotic shock and found that the strain lacking both Trk systems had the highest amount of Ca^{2+} released into the cytosol after the osmotic shock. On the other hand, a strain lacking all K^+ importers and exporters exhibited the lowest amount of released Ca^{2+} in the cytosol and the slowest reabsorption of Ca^{2+} back to the vacuole. Altogether, our data revealed mutual interconnection between potassium and calcium homeostasis in yeast cells. This work was supported by GACR (17-01953S).

P.15-008-Tue
Octarphin, a nonopioid peptide of opioid origin

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The peptide octarphin (TPLVTLFK) corresponding to the sequence 12–19 of beta-endorphin, was synthesized. It has been shown that it is a selective agonist of nonopioid (insensitive to the opioid antagonist naloxone) beta-endorphin receptor, Using tritium labeled octarphin the distribution of the receptor in the body was studied. It was found that it is available on immune cells (peritoneal macrophages, T and B lymphocytes of spleen and blood), endocrine (adrenal cortex, pituitary), and cardiovascular (cardiomyocytes) systems. Characterization of the binding specificity showed that only unlabeled beta-endorphin was able to compete with the labeled octarphin for the binding to receptor, tested in parallel alpha-endorphin, gamma-endorphin, enkephalins and a number of peptide hormones were inactive. Investigation of the octarphin effect on the target cells was showed that it increases the mitogen-induced proliferation of human and mouse T and B lymphocytes *in vitro*, activates murine peritoneal macrophages *in vitro* and *in vivo*, stimulates growth of human T-lymphoblast cell lines *Jurkat* and *MT*-4, inhibits adenylate cyclase activity of rat adrenal cortex membranes and suppresses the secretion of glucocorticoids from the adrenal gland into the blood. Data on the molecular mechanism of octarphin action were obtained. It was shown that in a concentration range of 1–1000 nM the peptide increases the activity of inducible NO-synthase (iNOS), and the content of NO and cGMP in lipopolysaccharide-activated murine peritoneal macrophages. The octarphin activity was depended on the dose and was maximal at a concentration of 100 nM. Taking into account that NO acts as a primary activator of soluble guanylate cyclase (sGC), it can be assumed that the activating effect of octarphin on macrophages is realized in the following way: increase in th iNOS expression → increase in the NO production → increase in the sGC activity → increase in intracellular levels of cGMP.

P.15-009-Wed
Tag7-activated lymphocytes induce necroptosis via FasL-Fas interaction

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A lot of tumor cells can avoid immune attack by depletion MHC complex on their surface. These cells are invisible for effector immune cells. Tag7 (PGLYRP1) is innate immune protein that can activate classical cytotoxic T cells after 6 days of treatment in 10^{-9} M concentration. Tag7-activated cytotoxic lymphocytes (Tag7-LA) express membrane FasL, which interacts with the Fas receptor on the surface of the target cell and triggers the mechanisms of cell death in HLA-negative tumor cells. It was shown that Tag7-LA induce in tumor cells 2 types of programmed cell death (PCD): apoptosis and necroptosis. The apoptosis was induced by Tag7-LA after short time of incubation through caspases activation. However, some tumor cells inhibit caspases and prevent development of caspase-depended apoptosis. We have detected necroptosis in target cells after long-time incubation Tag7-LA with tumor cells. Using the methods of inhibitory assay, western blot analysis and flow cytometry, we have demonstrated the main stages of necroptosis. We have approved that at the initiation of Fas-mediated necroptosis, a necrosome consisting of RIP1 kinase and RIP3 kinase is assembled. We have shown the participation of lysosomes enzymes cathepsin B and D, ROS-generation and P-STAT3 translocation in mitochondria. The results of this study confirm the fact that induction of necroptosis occurs after the activation of the Fas-receptor by a FasL and it's characterized by recruitment of necrosome and involvement of lysosomes and mitochondria and it's accompanied by ROS accumulation. This work was supported by Russian Science Foundation grant N 15–14–00031.

P.15-010-Mon
L-type voltage-gated calcium channels hyperactivity as a result of calcium store overload in neuronal AD models

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Today there is still no effective treatment for Alzheimer's disease (AD) and scientific society is in intense search for novel therapeutic targets. Nearly a half of Familiar AD cases are connected with mutation in presenilin-1 (PS1) gene. It has long been known that calcium homeostasis disturbances can mark a starting point for neurodegeneration. Numerous studies have demonstrated changes in activity of different calcium channels in plasma membrane or endoplasmic reticulum (ER). It is also known that activity of L-type voltage-gated calcium channels (L-VGCC) is suppressed by STIMs (calcium ER sensors). This study is aimed to investigate the activity of voltage-gated calcium channels in connection with ER calcium store content in 5xFAD hippocampal neurons (HNs) and HNs expressing M146V PS1. Calcium imaging, fluorescent microscopy, HNs from 5xFAD/C3HA mice, lipofectamine/lentiviral transfections. We have previously shown that SH-SY5Y cells bearing M146V PS1 were able to overload calcium stores and disturb translocation of STIM1 calcium sensors. Here we show that HNs bearing PS1 M146V mutant and HNs from 5xFAD mice had higher calcium ER content, abated store-operated entry, and elevated L-VGCC entry. L-VGCC entry in neurons after calcium store depletion equals to controls. Additionally, in M146V PS1 expressing cells nuclear factor of activated T-cells (NFAT1) tended to have nuclear localization, STIMs knock-down converted this changes. L-VGCC channels are a possible source of pathological neuronal calcium in FAD models. These channels can serve as therapeutic targets for future drug development. This work was supported by the RSF № 14-14-00720.

P.15-011-Tue
CXC chemokine receptor 4 (CXCR4) functions as a lactoferrin receptor in macrophages

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CXC chemokine receptor 4 (CXCR4) is a ubiquitously expressed G-protein coupled receptor that is expressed in many types of cells and tissues and has a role in multiple types of cellular events. CXCR4 is involved in infection of human immunodeficiency virus (HIV) into the target cells, including T-cells and macrophages. This CXCR4-mediated HIV-1 infection is inhibited by an endogenous ligand for CXCR4 (stromal derived factor-1; SDF-1/CXCL12). Lactoferrin is an iron-binding glycoprotein belonging to the transferrin family, particularly abundant in mammalian milk, colostrum and external secretions. Lactoferrin contributes to host-defense by anti-infective and immunomodulatory properties. As well as SDF-1/CXCL12, lactoferrin has an ability to inhibit the HIV-1 attachment to T-cells, suggesting that lactoferrin prevents the HIV-entry by interacting with CXCR4 at cell surface. In this study, we addressed whether CXCR4 acts as a lactoferrin receptor in THP-1-derived macrophages. We found that CXCR4-containing lipoparticles specifically interacts with bovine lactoferrin immobilized to polystyrene wells. The binding of SDF-1 to CXCR4 induces dimerization, tyrosine phosphorylation and ubiquitination of the receptor and activates multiple signaling pathways. We found that lactoferrin can mimic the ligand induced modifications of CXCR4. In addition, lactoferrin-induced activation of Akt was abrogated by silencing of CXCR4 expression and antagonist for CXCR4, suggesting that CXCR4 plays as a role as lactoferrin receptor mediating the activation of Akt signaling pathway.

P.15-012-Wed
Functional expression of Piezo1 channels in normal and transformed fibroblasts

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In the present study, mechanosensitive (MS) channel activity in normal (BALB/3T3) and transformed (3T3B-SV40) mouse fibroblasts have been analysed with the use of single current patch clamp technique. Our previous data suggest that MS calcium-permeable Piezo1 channels are the main candidates for plasma membrane mechanosensors in 3T3B-SV40 cells. Here, cell-attached patch-clamp experiments with the inclusion of Yoda1, a novel selective Piezo1 activator, to the patch pipette reveal the activity of Yoda1-induced currents with single channel conductance of 24.4 ± 0.9 pS, that is consistent with the conductance of the channels activated by membrane stretch. Electrophysiological assays together with immunofluorescent labeling indicate higher expression of Piezo1 channels in transformed 3T3B-SV40 fibroblasts comparing to their non-transformed BALB/3T3 counterpart. Particularly, stretch-induced channel activity was recorded in 70% (n = 28) of stable cell-attached patches on transformed cells and in 30% (n = 27) patches on normal fibroblasts. Importantly, we found that in both cell lines, stretch-induced calcium entry was coupled with the activity of KCa potassium channels of small conductance (SK1-4/IK channels). The molecular identity of SK channels was tested in patch experiments with apamine, selective blocker of SK1-SK3 channels. Apamine (200 nM) failed to block calcium-dependent potassium channel activation induced by local calcium entry. In sum, our results reveal functional coupling of Ca-transporting and Ca-operated channel proteins in cellular mechanotransduction in normal and transformed fibroblasts. The differences in Piezo1 activity may have significant impact on membrane signaling and motility in transformed cells comparing to their normal counterparts. The work was supported by RSCF grant 17-74-10123.

P.15-013-Mon
The structural model of the binding of mefenamic acid to GABAA receptors predicts an enhancement of fenamates action as positive allosteric modulators by endogenous neurosteroid allopregnanolone

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Mefenamic acid (MFA) belongs to the class of fenamates which are used in medical practice as nonsteroidal anti-inflammatory drugs. However fenamates may also modulate a variety of ion channels including receptors of γ-aminobutyric acid type A ($GABA_AR$). We have shown earlier that MFA strongly potentiates GABA-induced currents with $EC_{50} \cong 15$ μM in rat Purkinje cells. Previously we found also that MFA and general anesthetic etomidate (ETM) share the same binding site in the transmembrane β(+)/α(−) interface. It is known that endogenous neurosteroid allopregnanolone (ALP) enhances the action of ETM as a positive allosteric modulator of $GABA_AR$. Thus our structural model of MFA binding predicts that ALP should also enhance MFA potentiating action. Patch-clamp recordings support this prediction and showed that co-application with ALP (100 nM) reduced the EC_{50} for MFA potentiation of GABA-induced currents by about three times in acutely isolated rat Purkinje cells. We found also that co-application of ALP and MFA (100 μM) caused the concentration-dependent increase of the amplitude of MFA-induced currents with EC50 ≅ 130 nM. We built the homology model of open $\alpha_1\beta_2\gamma_2$ $GABA_AR$ based on the cryo-EM structure of α_1 GlyR (3JAE) and used Monte-Carlo energy minimization to optimize the geometry of ALP-receptor complex. Data from mutational studies indicate that neurosteroids bind in the cytoplasmic part of the β(+)/α(−) interface. We found that ALP binds between M1 and M3 helixes and forms a hydrogen bond with α_1 Q242 and strong van-der-Waals contacts with α_1 W246, I239 and β_2 L297, L301 hydrophobic residues. ALP binding site is located near the receptor desensitization gate and may enhance MFA potentiation affecting the probability of the channel transition from an open to a desensitized state. Supported by RFBR grant 18-015-00038.

P.15-014-Tue
Different types of store-operated calcium channels in patient-specific iPSCs-based model of Huntington's disease

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Neurodegenerative pathologies are one of the most serious and socially significant problems of the modern medicine along with cardiovascular and oncological diseases. The attention of the researchers is now focused on the models of hereditary neurodegenerative pathologies based on endogenous expression of mutant proteins in neurons differentiated from patient-specific induced pluripotent stem cells (iPSCs). Balanced regulation of calcium homeostasis is extremely important for maintaining physiological processes in the cell such as proliferation, gene expression, programmed cell death, etc. A large body of modern scientific publications suggests that disruption of calcium signaling plays a central role in the development of neurodegenerative

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pathologies, including Huntington's disease (HD). Store-operated calcium entry (SOCE) is an important part of intracellular calcium signaling. It has been shown to be upregulated in different HD models. We have shown that in iPSCs-based human neurons SOCE is maintained by currents through at least two different channel groups termed I_{CRAC} and I_{SOC} and both of them are upregulated in HD-specific neurons compared to wild type neurons. Notably that thapsigargin-induced intracellular calcium store depletion in most cases results in predominant activation of either I_{CRAC} or I_{SOC} in iPSCs-based human neurons. Further we have found that potential anti-HD drug EVP4593 that was previously shown to be an effective SOCE attenuator affects both I_{CRAC} and I_{SOC}. In general the iPSCs-based HD model can be used to study the fundamental mechanisms of neuronal calcium signaling and its disturbances in pathological states associated with neurodegeneration, and search for new molecular targets and test promising targeted drugs. The study was supported by the RSF grant № 17-74-20068 and the fellowship of the President of RF.

P.15-015-Wed
Renal prolactin signaling and its termination differ in normal female rat and under condition of cholestasis of pregnancy

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Prolactin (Prl) is a multifunctional protein, it takes part in pregnancy maintenance and water-salt balance. During pregnancy hyperprolactinemia (HPRL) can promote cholestasis of pregnancy (CP) – pathology connected with the disturbance of a bile outflow and kidney functional failure. Prl receptor signaling in the kidney under this condition is not investigated. There are long (LR) and short (SR) isoforms of Prl receptors in the kidney. STAT5s are the key proteins of LR signaling, FOXO3 and GALT are downstream components of SR signaling. Prl binding with LR induces STAT5 phosphorylation, its translocation to the nucleus and the modulation of target genes expression. Inhibition of STAT5-pathway may induced by dimers of LR and SR and transcription factors SOCS3 and PIAS3. PIAS3 also inhibits SR signaling. The aim of our study is to assess the contribution of the PRLR isoforms and key proteins of the PRL signaling and its termination in the CP. Experiments were performed on outbred mature female rats of 4 experimental groups: intact (normal), with HPRL, with cholestasis, and with cholestasis and HPRL (the model of CP). We analyzed protein expression of STAT5 and its phosphorylated form (pSTAT5), mRNA expression of STAT5A, STAT5B, LR, LS, FOXO3, GALT, SOCS3 and PIAS3 in outer medulla of kidney. In the CP pSTAT5 protein expression increases compared with intact rats. Protein expression of STAT5 and mRNA expression of STAT5A, STAT5B, FOXO3 don't change in all groups. The level of expression of LR and SR increase in cholestasis compared with the normal rats and decrease in CP compared with cholestasis. mRNA expression of SOCS3, PIAS3 elevate in CP compared with normal. The level of GALT increases in cholestasis compared with intact rats. Thus HPRL causes a disproportional increase of LR and SR expression in cholestasis, while CP leads to the reversal change in this ratio and a decrease the expression of both isoforms. In the CP the main contribution of LR is shown.

P.15-016-Mon
Gαq regulates mitochondrial trafficking in neurons and interacts with Alex3, Miro1 and Trak proteins

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G proteins are probably the most important signaling proteins through receptors at the plasma membrane. However, recent reports also indicate a novel localization of G proteins at the mitochondria and other endomembranes, where they regulate the physiology of these organelles. In particular, the Gq subfamily is required to keep the proper balance between mitochondria fusion and fission acting at both outer and inner membrane, among other functions. In order to unveil the putative effectors of Gαq that mediate those effects at the mitochondria, our group has undertaken a mass-spectrometry analysis based on Gαq immunoprecipitates from cellular endomembranes using different cell lines. The "mito-interactome" study has provided further evidence that G proteins regulate the mitochondrial dynamic process through the interaction with the mitochondrial armadillo domain proteins (Alex3 and 10). Subsequent immunoprecipitation and pull-down studies demonstrated a specific interaction of Gαq with the mitochondrial Rho GTPase 1 (Miro1) and the adaptor proteins TRAK1 and TRAK2, that couple mitochondria to Kinesin and Dynein motor proteins. Together, they constitute the main regulators of mitochondrial transport in neurons. To analyze the physiological role of those interactions, we have performed tracking analysis of mitochondria along the axons of mouse hippocampal neurons overexpressing Gαq or its constitutive-active mutant, GαqR183C. The results of these studies reveal a significant arrest of mitochondrial motility upon Gαq activation, which is absent in the presence of inactive Gαq. In summary, our group postulates a new non-canonical mitochondrial function of Gαq acting as a molecular switch in neurons that would stop mitochondria during its GTP-bound active state at the point of the synapsis and would allow mitochondrial movement when Gαq is bound to GDP.

P.15-017-Tue
Role of chymase on the disease progression of diabetic nephropathy might via STAT3 signaling pathway

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Diabetic nephropathy (DN) is the leading cause of end-stage renal disease. Angiotensin converting enzyme (ACE) and chymase are the key enzymes in renal renin-angiotensin system (RAS) that converted angiotensin I (Ang I) to angiotensin II (Ang II) via different pathways. We aimed to investigate the effects of ACE and chymase on DN pathogenesis in this study. The diabetic db/db mice fed with 4 weeks high fat diet (HFD) to accelerate DN progression and treated with captopril (ACE inhibitor) and chymostatin (chymase inhibitor). Urine, blood and kidney tissues were sampled after the mice sacrificed for biochemical and molecular assays. The activities of renal RAS, cellular

signaling pathways and histological assays were also evaluated. The results show that blood glucose, serum triglycerides and total cholesterol of HFD-fed db/db mice were higher than those in Control group (the mice fed with normal diet), but the levels were not reduced by chymostatin and captopril treatment. Urinary albumin level was significantly increased in HFD-fed db/db mice and significantly decreased after chymostatin treatment. After HFD feeding, renal ACE, chymase, Ang II, AT1R levels were significantly increased, and only ACE level was significantly decreased after captopril treatment. Chymase, Ang II and AT1R levels show a reduction in the mice treated with chymostatin. Increased p-STAT3 level was observed in HFD-fed groups and were significantly decreased after chymostatin treatment. Pathological results display severe leukocyte infiltration in HFD-fed groups and chymostatin could significantly ameliorate the leukocyte infiltration. By PAS staining, HFD-fed groups show slight mesangial matrix expansion compare with that in Control group, but mesangial matrix expansion were significantly reduced by chymostatin treatment. In conclusion, chymase might play a role in DN pathogenesis, and chymase-Ang II-AT1R axis exacerbating DN might via STAT3 signaling pathway in db/db mice.

P.15-018-Wed
Inhibitory effect of tricyclic antidepressant desipramine on voltage-dependent K^+ current in rabbit coronary arterial smooth muscle cells

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We describe the effect of a tricyclic antidepressant drug, desipramine on voltage-dependent K^+ (Kv) currents in freshly isolated rabbit coronary arterial smooth muscle cells using a conventional whole-cell patch clamp technique. Application of desipramine rapidly decreased the Kv current amplitude in a concentration-dependent manner, with an IC_{50} value of 5.91 ± 0.18 μM and a Hill coefficient of 0.61 ± 0.09. The steady-state inactivation curves of the Kv channels were not affected by desipramine. However, desipramine shifted the steady-state inactivation curves toward a more negative potential. Application of train pulses (1 or 2 Hz) slightly reduced the Kv current amplitude. Such reduction of the Kv current amplitude by train pulses increased in the presence of desipramine. Furthermore, the inactivation recovery time constant was also increased in the presence of desipramine, suggesting that desipramine-induced inhibition of the Kv current was use-dependent. Application of a Kv1.5 inhibitor (DPO-1) and/or a Kv2.1 inhibitor (guangxitoxin) did not change the inhibitory effect of desipramine on Kv currents. Based on these results, we concluded that desipramine directly inhibited the Kv channels in a dose- and state-dependent manner, but the effect was independent of norepinephrine/serotonin reuptake inhibition.

P.15-019-Mon
Alterations of ATP-sensitive K^+ channels in human umbilical arterial smooth muscle during gestational diabetes mellitus

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We investigated the alterations of ATP-sensitive K^+ (K_{ATP}) channels in human umbilical arterial smooth muscle cells during gestational diabetes mellitus (GDM). The amplitude of the K_{ATP} current induced by application of the K_{ATP} channel opener pinacidil (10 μM) was reduced in the GDM group than in the control group. Pinacidil-induced vasorelaxation was also predominant in the normal group compared with the GDM group. Reverse transcription polymerase chain reaction and Western blot analysis suggested that the expression of K_{ATP} channel subunits such as Kir6.1, Kir6.2, and SUR2B, were decreased in the GDM group relative to the normal group. The application of forskolin and adenosine, which activate protein kinase A (PKA) and thereby K_{ATP} channels, elicited K_{ATP} current in both the normal and GDM groups. However, the current amplitudes were not different between the normal and GDM groups. In addition, the expression levels of PKA subunits were not altered between the two groups. These results suggest that the reduction of K_{ATP} current and K_{ATP} channel-induced vasorelaxation are due to the decreased expression of K_{ATP} channels, not to the impairment of K_{ATP}-related signaling pathways.

P.15-020-Tue
Blockade of voltage-dependent K^+ current in rabbit coronary arterial smooth muscle cells by the tricyclic antidepressant clomipramine

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We investigated the effect of the tricyclic antidepressant clomipramine on voltage-dependent K^+ (Kv) channels in native rabbit coronary arterial smooth muscle cells. Our results showed that clomipramine inhibited vascular Kv channels in a concentration-dependent manner, with an IC_{50} value of 8.61 ± 4.86 μM and a Hill coefficient (n) of 0.58 ± 0.07. The application of 10 μM clomipramine did not affect the activation curves of the Kv channels; however, the inactivation curves of the Kv channels were shifted toward a more negative potential. The clomipramine-induced inhibition of Kv currents was not changed by the application of train pulses (1 or 2 Hz), which demonstrated that clomipramine inhibited Kv current in a state (use)-independent manner. Pretreatment with the Kv1.5 and Kv2.1 inhibitors, DPO-1 and guangxitoxin, respectively, did not affect clomipramine-induced inhibition of Kv currents. Therefore, we concluded that clomipramine inhibited vascular Kv channels in a concentration-dependent, but state (use)-independent manner, regardless of its own function.

P.15-021-Wed
Vildagliptin, an anti-diabetic drug of the dipeptidyl peptidase-4 inhibitor class, induces vasodilation via Kv channel and SERCA pump activation in aortic smooth muscle

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This study investigated vildagliptin-induced vasodilation and its related mechanisms using phenylephrine induced precontracted rabbit aortic rings. Vildagliptin induced vasodilation in a concentration-dependent manner. Pretreatment with the large-conductance Ca^{2+}-activated K^+ channel blocker paxilline, ATP-sensitive K^+ channel blocker glibenclamide and inwardly rectifying K^+ channel blocker Ba^{2+} did not affect the vasodilatory effects of vildagliptin. However, application of the voltage-dependent K+ (Kv) channel inhibitor 4-aminopyridine significantly reduced the vasodilatory effects of vildagliptin. In addition, application of either of two sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) inhibitors, thapsigargin or cyclopiazonic acid, effectively inhibited the vasodilatory effects of vildagliptin. These vasodilatory effects were not affected by pretreatment with adenylyl cyclase, protein kinase A (PKA), guanylyl cyclase, or protein kinase G (PKG) inhibitors, or by removal of the endothelium. From these results, we concluded that vildagliptin induced

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vasodilation via activation of Kv channels and the SERCA pump. However, other K^+ channels, PKA/PKG-related signaling cascades associated with vascular dilation, and the endothelium were not involved in vildagliptin-induced vasodilation.

P.15-022-Mon
Site-directed mutagenesis of the TRPV1 reveals amino acid residues crucial for receptor gating

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Pain is an universal protective neural response to noxious stimuli. Painful stimuli activate different receptors on the membrane of sensory neurons. One of the most important is nonselective cation channel TRPV1. An improper functioning of TRPV1 leads to pain syndromes with quite challenging treatment. Understanding of the relationship between the structure and function of TRPV1 may promote new therapies development. Existing cryo-EM 3D models do not provide a complete picture of the receptor gating mechanism as well as contradict each other in many ways. We identified several amino acid residues crucial for pore domain functioning by site-directed mutagenesis. "Hot spot" residues were proposed by molecular dynamics. TRPV1 mutants were tested in two-microelectrode voltage-clamp experiments of *Xenopus laevis* oocytes. We found two residues critical for rTRPV1 gating. Mutations of Ile679, Ala680 within the inner-pore region were found to inactivate the channel. Molecular dynamics model suggests Asn676 being involved in channel desensitization by calcium ions. We replaced Asn by Ser because of similar physical properties. This mutant appeared to be nonfunctional that proves the importance of this residue. In addition, replacing conservative Lys688 with Gly increases the flexibility of the TRP domain collocated with the pore region making the whole receptor more sensitive to activation. Identification of concrete amino acid residues involved in the pore domain functioning may allow us to take a completely different look at the present approach of development of new TRPV1-targeted drugs for the inflammation and pain syndromes treatment. This work was supported by Russian Science Foundation Grant 16-15-00167.

P.15-023-Tue
p53-E2F pathway plays different roles in benzo (a)pyrene induced transformed and normal human embryo lung fibroblasts

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B(a)P is one of the polycyclic hydrocarbon environment pollutants, existed in cigarette smoke and diesel. B(a)P is an indirect carcinogen which transformed into in the carcinogen BPDE under action of the cytochrome p450 enzyme system. BPDE can form DNA adducts, then cause DNA damage, lead to the disorder of cell cycle. In mammalian cells, proliferation is controlled by a series of positive and negative regulatory factors. p53 is the most important tumor suppressor genes. In about 50% of human tumors, it can be observed in the occurrence of mutations. The most common mutations is missense mutations and base substitution. In this study, roles of p53, AP-1, cyclin D1 cyclin-dependent kinase 4 (CDK4) and E2F-1 were investigated in B(a)P transformed human embryo lung fibroblast (T-cells). The stable transfectants, HELF AP-1 (H-AP), HELF p53 siRNA (H-p53), T-cell AP-1 (T-AP) and T-cell p53 siRNA (T-p53) were established. Our current analysis showed that in T-AP cells expressions of cyclin D1 and p53 were higher than H-AP, but not CDK4 and E2F-1/4. AP-1 can regulate the expression of cyclin D1 in T-AP, but not in p53. p53 can regulate the expression of p21 and E2F-1, but not the import of p21 into nucleus, the AP-1 activities and cyclin D1 in T-AP. The combinations of cyclin D1-p21 and cyclin D1-CDK 4 were significantly higher than H-AP, and p53 effects the combination of cyclin D1-p21 in T-AP. In summary, present studies demonstrate that in T-AP cells expressions of cyclin D1 and p53 were higher than H-AP, but not CDK4 and E2F-1/4. AP-1 can regulate the expression of cyclin D1 in T-AP, but not in p53. p53 can regulate the expression of p21 and E2F-1, but not the import of p21 into nucleus, the AP-1 activities and cyclin D1 in T-P. The combinations of cyclin D1-p21 and cyclin D1-CDK 4 were significantly higher than H-AP, and p53 effects the combination of cyclin D1-p21 in T-AP.

P.15-024-Wed
Cav1.2 mediated nuclear signaling via the H-Ras-ERK-CREB pathway

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Voltage-gated calcium channels are transmembrane cell surface proteins responsible for multifunctional signals. The mechanisms linking neuronal activity to the excitation-transcription (ET) coupling is not fully understood. The L-type Cav1.2 channel has been shown to activate a large number of different neuronal-specific genes. The prevailing view of the L-type Ca^{2+} channel activation of the downstream signaling is via mobilization of calmodulin (CaM) and the CaM-dependent protein kinase kinase (CaMKK) cascade responsible for the CREB phosphorylation. Other proposed pathways involve the calcium-regulated phosphatase calcineurin and the activation of the Ras/MAPK signaling pathways. To investigate the underlying mechanism of ET coupling, we transiently transfected HEK293 cells with wild-type (wt) Cav1.2 channel and monitored phosphorylation of ERK1/2, RSK, and CREB. We found that expressing dominant-negative H-Ras^{S17N} mutant together with wt Cav1.2 channel significantly inhibited ERK phosphorylation, while the dominant negative Ras^{S17N} only partially inhibited CREB. This negative impact suggests a functional role of H-Ras in mediating nuclear signaling. We tested the role of Ca^{2+} and showed that Ca^{2+}-impermeable mutant channel $\alpha_1 1.2^{L745P}/\alpha 2\delta/\beta 2b$ expressed in HEK293 cells triggered ERK1/2 and CREB phosphorylation, albeit smaller compared to wt channel. These results indicate that there is an additional ERK-independent nuclear activation pathway. The Fluo-4 fluorescence assay results showed elevated intracellular Ca^{2+} ($[Ca^{2+}]_i$) under depolarizing conditions in HEK293 cells expressing wt channel, while cells expressing the Ca^{2+}-impermeable mutant channel exhibited no $[Ca^{2+}]_i$ rise. Taken together, our data suggest the existence of a specific mechanism by which Cav1.2 can activate nuclear signaling independently of Ca^{2+} entry, and provide insight into the role of the Cav1.2 Ca^{2+}-bound pore in triggering ET coupling.

P.15-025-Mon
A novel regulatory role of RGS4 in neuronal cell proliferation and sprouting mediated via STAT5B transcriptional responses

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The regulator of G protein signaling 4 (RGS4) is a multitask protein highly expressed in developing neurons associated with various neurological disorders. Despite its initial role as GTPase activating protein, we have previously demonstrated that RGS4 interacts directly with opioid receptors (ORs) conferring selectivity for G protein coupling, interfering in ORs signaling and accelerating receptor endocytosis. It was also shown that δ-opioid receptor (δ-OR) forms a signaling complex, consisted of RGS4 and the Signal Transducer and Activator of Transcription 5B (STAT5B) that leads to neurite outgrowth and neuronal differentiation upon δ-OR activation. Based on these findings we wondered whether RGS4-STAT5B interplay is involved in cellular mechanisms controlling neurogenesis through STAT5B transcriptional regulation. Our data demonstrated that primary cortical neuronal cultures from RGS4−/− mice, lacking a functional RGS4, exhibit differential neuronal sprouting compared to controls in absence or presence of DSLET administration. Moreover, RGS4−/− adult brain extracts, displayed increased levels of p-STAT5B and the expression levels of STAT5B target genes Snx9 and Ptk2, implicated in axonogenesis, were also altered indicating that RGS4 is involved in neurite outgrowth possibly via STAT5B mediated transcriptional responses. In contrast, Neuro2A cells expressing RGS4 exhibited a significant reduction in cell number and proliferation rate. Additionally, isolated neural stem cells showed elevated proliferative properties with concomitant increases of the mRNA levels of the STAT5B Bcl-2 and Bcl-xl anti-apoptotic target genes. Collectively, these results demonstrate for the first time a novel, non-canonical regulatory role of RGS4 in STAT5B mediated-transcription thus providing insights into the involvement of RGS4 in neuronal signaling and synaptic plasticity.

P.15-026-Tue
Activation of TLR3 and TLR4 in long- and short-term cells incubation of astrocytes in high-glucose medium

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Bacterial and viral agents have different effects on the inflammatory responses in hyperglycemia. Accumulating studies have shown that hyperglycemia show inflammation in the central nervous system (CNS). Astrocytes play an important role in the development of inflammation responses in CNS. However, changes in inflammatory responses at different glucose concentrations are not well characterized. The aim of our research was to investigate the influence of long- and short-term high glucose (HG) cultivation medium on the response of astroglial cells to inflammatory stimuli. We compare inflammatory responses in course of the activation of Toll-like receptors (TLR) with a bacterial-like (TLR4) and viral-like (TLR3) stimuli. Primary astrocytes from newborn rats were obtained from brain cortices by conventional procedures. Cells were cultured 12 days before experiments in either DMEM with 5 mM glucose (normal glucose level, NG) or in media with 22.5 mM glucose (long-term high glucose level). In short-term HG model cells, 5 mM glucose DMEM was switched to 22.5 mM glucose for 48 h before stimulations. Astrocytes were stimulated with the lipopolysaccharide (LPS, a TLR4 agonist) or the poly I:C (PIC, a TLR3 agonist). Analysis of mRNA expression of cyclooxygenase 2 (COX-2), TNF-alpha and IL-10 was performed by qPCR, protein levels of COX-2 were determined by Western blot, levels of released cytokines IL-10, TNF-alpha and prostaglandin E2 (PGE2) were determined using ELISA kits. We obtained that inflammatory response to LPS reduced in long-term HG and induced in short-term HG (in comparison with NG). Both types of HG treatments induced inflammatory response to PIC. Effects were observed on mRNA levels of IL-10, TNF-alpha and both protein and mRNA of COX-2. Alterations in concentrations of released mediators IL-10, TNF-alpha and PGE2 were detected. The difference between PIC and LPS action was observed in a time-dependence manner. Supported by RSF (№ 16-15-10298).

P.15-027-Wed
Effective TRPA1 modulator from sea anemone venom appeared due to a mutation of a processing site

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Pain is a physiological condition that affects the being quality of life. The transient receptor potential ankyrin-repeat 1 (TRPA1) receptors play the significant role in initiation and propagation of pain signals. TRPA1 expressed in sensory neurons that innervate different tissues and organs. Receptors activate upon noxious chemical, mechanical and thermal (low temperatures) conditions, while some TRPA1 agonists expressed in mammalians during inflammation. So TRPA1 is an attractive target for the medical drug development. Animal venoms contain a dozen of bioactive compounds which includes modulators of different ion channels and receptors. During the search for TRPA1 modulators among sea anemone toxins, we identified a novel peptide named Ms 9a-1, which potentiates receptor activity. The bioactive molecule was isolated from *Metridium senile* venom by using chromatography methods. In electrophysiology experiments on *Xenopus laevis* oocytes expressing TRPA1 peptide increased agonist-induced activation of the receptor. However, pretreatment of mice by intravenous injection of Ms 9a-1 (0.3 mg/kg) significantly reduced nocifensive behavior and paw edema induced by TRPA1 agonist allyl isothiocyanate (AITC), as well as decreased non-specific inflammation in Freund's Complete Adjuvant test. Most probably Ms 9a-1 could potentiate TRPA1 activation by endogenous agonists and produce a depolarization block of sensory neurons. Peptide Ms 9a-1 evidently differs from homologous sea anemone peptides by extended C termini that probably appeared due to a mutation of a processing site. Such a mutation could be responsible for the appearance of TRPA1-potentiating activity. To determine the active site of the peptide we analyzed the effects of the N-terminal and C-terminal domains of Ms 9a-1. As a result, both parts of Ms 9a-1 were able to potentiate TRPA1, but with different level of efficiency. This work was supported by Russian Science Foundation Grant 16-15-00167.

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P.15-028-Mon
Peptidoglycan can induce expression of CD44 on lymphocytes during inflammatory response of bovine mammary gland

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CD44 is a proteoglycan that is expressed by different cell types. CD44 plays a role in leukocyte trafficking to extra lymphoid sites of inflammation or as a non-specific accessory adhesion molecule. The aim of this study was to inquire development over time of the surface expression of CD44 on lymphocytes during an inflammatory response of bovine mammary gland induced by peptidoglycan. Intramammary instillation of peptidoglycan resulted in an increase in the proportion of CD44-positive lymphocytes after 24 and 48 h. During resolution of the inflammatory response, there was observed a decrease in the proportion of CD44-positive lymphocytes. The results suggest that the cell surface receptor CD44 can play an important role in the inflammatory response of bovine mammary gland to bacteria and their components.

P.15-029-Tue
Expression and functional activity of specific membrane transport of serotonin in the mouse ovary

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Along with the classical function of serotonin as the neurotransmitter, it performs the regulation of female reproductive function. Serotonin is present in the female reproductive system and affects the maturation of oocytes, the synthesis of sex steroid hormones, as well as the processes of early embryonic development, which is shown in a variety of animals, including mammals. However, the mechanisms of serotonergic regulation of the female reproductive function have been poorly studied. The serotonin transporter *Sert* (*Sls6a4*) is of particular interest in this respect, which carries out the uptake of the serotonin from the extracellular medium. The mRNA of the *Sert* gene is expressed in the ovary, including follicular cells and oocytes at different stages of folliculogenesis. A quantitative study have shown that the expression level of the transporter is constant and does not depend on the development stage of the ovarian follicle. Immunostaining revealed that the transporter is localized in ovarian follicles, with immunoreactivity much more pronounced in the oocytes. There was an increase of the immunostaining intensity in all compartments of the ovary, including mouse follicles and oocytes after subcutaneous injections of serotonin during five days. According to the data obtained by HPLC, the concentration of serotonin in the ovaries of mice in the experimental group increased more than 4-fold. Thus, the ovary actively seizes exogenous serotonin from the bloodstream, probably through the membrane transporter Sert. The transmitter was accumulated in oocytes during cultivation of isolated ovarian follicles in vitro in the presence of serotonin but the addition of a selective serotonin reuptake inhibitor fluoxetine reverses the effect, which indicates the specificity of membrane transport. Thus, expression of the specific membrane serotonin transporter Sert in the ovary, and its specific functional activity in the oocytes of preantral follicles are revealed.

P.15-030-Wed
Protein sorting upon exit from the endoplasmic reticulum

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Protein sorting upon vesicular transport through the secretory pathway is essential to dynamically maintain functional compartmentalization and homeostasis in eukaryotic cells. During many years it was generally thought that all newly synthetized secretory proteins travel together in the same COPII vesicles from the endoplasmic reticulum (ER) to the Golgi, where they are sorted to different destinations. However, we found in yeast that glycosylphosphatidylinositol (GPI)-anchored proteins (GPI-APs), a special category of lipid-anchored secretory proteins, are segregated from transmembrane cargos at the level of the ER and then incorporated into distinct COPII vesicles. We have developed in yeast a genetic assay that allows visualization of cargo sorting in ER exit sites (ERES) by using super-resolution confocal live imaging microscopy. Our data suggest that GPI-AP sorting into specific ERES is driven by the acquisition of ceramide C:26 (a very long and saturated lipid) by the GPI anchor. GPI-ceramide remodeling might induce a strong hydrophobic mismatch in the ER membrane leading to the clustering of GPI-APs into specific lipid domains. We also show that the specific ER export machinery is recruited after GPI-AP sorting at ERES to produce specialized COPII vesicles.

P.15-031-Mon
Study of the PFNA cluster in bifidobacteria: structure, evolution and possible functions

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Signaling through STPK appears to be the dominant prokaryotic signaling system. STPK Pkb2 is the receptor-type kinase, with an extracytoplasmic sensor domain and an intracellular kinase domain, that indicates this kinase to transduce the external signals. Using the phylogenetic profiling method, we found an evolutionarily stable cluster of genes linked to pkb2 gene in the Bifidobacterium genus. The operon organization of the cluster in B. longum GT15 was confirmed with transcriptional analysis. The transcription start site was determined by 5′-RACE method. The cluster named PFNA was found in most bifidobacterial species obtained from various sources. The cluster contains genes that encode, mainly, membrane proteins, among which a protein with a cytokine receptor motif is annotated. Genes in the PFNA cluster show high sequence divergence between species which may be an indicator of rapid evolution in response to the rapid evolution of host's cytokine genes. To investigate the molecular evolution of the cluster we tested in silico hypotheses concerning positive and relaxed negative selection. The molecular evolution analysis of the cluster showed the presence of episodic positive selection as well as the relaxed negative selection in some phylogenetic clades. This allowed us to determine the structurally and functionally significant sites under the pressure of selection. The presence of episodic positive selection can indirectly indicate a possible role of the cluster in interacting with the host's immune

system. We analyzed the expression level of PFNA genes in different species by real-time PCR and correlated it with the effects of the molecular evolution. In order to compare the expression levels between different Bifidobacterium species, we selected housekeeping genes that have a stable level of expression under normal conditions in several bifidobacterial species.

P.15-032-Tue
THE cell-free dna gc-rich ribosomal repeat sequence activates the expression of tlr9 receptors in the neurons and cancer cells of the MCF7 line

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During chronic pathology, leading to the increased level of cellular death, the cell-free DNA (cfDNA) is accumulating in the bloodstream. The cfDNA is characterized with GC-enriched sequence of ribosomal repeat (rDNA) resistant to the nuclease hydrolysis. Previously, we showed the rDNA accumulated in the cfDNA influences the gene expression and the functional activity of human cells. rDNA causes the development of an adaptive response at the concentration of 5–100 ng/ml, inhibiting apoptosis and activating the NF-kB pathway through the TLR9 in lymphocytes, endothelial cells and MSC. We have suggested rDNA in the cfDNA may induce an adaptive response in cancer cells, may penetrate the blood-brain barrier when its permeability increases and activate the TLR9 receptors of brain neurons. In our in vitro experiment the effect of rDNA at the concentrations of 20–50 ng/ml on the expression of the TLR9 gene in granular neurons of cerebellum of 9 day-old rats and in the MCF7 cancer cells has been studied. We created a genetic construct – (prDNA) containing the rDNA with a promoter. As the "base" vector a pEGFP-C1 plasmid containing the fluorescent protein gene EGFP was selected. We have shown the introduction of the easy-oxidizing GC-rich rDNA into the plasmid leads to its penetration into cultured neurons and MCF7 cells while the vector without insertion penetrates into the cells weakly. We have shown rDNA contains TLR9 binding sites. In both cultured cells, the level of the TLR9 gene expression increases by 2–4 times ($P < 0.01$ for both) after 3–24 h. The plasmid without insertion did not cause an increase. The TLR9 expression increase may lead to the development of an adaptive response. It may be favorable for neurons, while adaptive response in cancer cells may contribute to their survival and it may be important during the cancer therapy. This work is supported by the Basic Research Program of the RAS "Fundamental Research for Biomedical Technologies" (1.42).

P.15-033-Wed
Action mode peptide ligands of TRPV1 is bimodal and depended on the activation stimuli strength

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The TRPV1 receptor is an extremely promising target for novel drug development for the treatment of a variety of pathological states associated with inflammation. Activation of TRPV1 leads to the pain and decrease the body temperature, while inhibition of this receptor can result in an increase of the body temperature. Peptides from sea anemone *Heteractis crispa* (APHC1 and APHC3) have been previously characterized as molecules, which inhibited TRPV1 and produced a pronounced analgesic effect and a decrease in the body temperature in experimental animals. We found that APHC1 and APHC3 have a bimodal action which depended on the activation stimuli strength. They produced potentiation of low-amplitude responses and no effect/inhibition of high-amplitude responses. Thus the action of these peptides depends not only on the nature of activation stimuli but also on the strength of the stimuli. These peptides can potentiate weak endogenous agonists of TRPV1 and decrease of body temperature of experimental animals. But in the sites of inflammation peptides can inhibit strong activation of TRPV1 and produce the analgesic effect. We produced a hybrid peptide that contained all residues important for the binding of the peptides APHC1 and APHC3 to the TRPV1. Biological tests on animals showed that the hybrid molecule not only combined the analgesic properties of both peptides but, unlike the parent molecules, also increase the body temperature of experimental animals. Therefore enhancement of inhibition power provided the peptide molecule properties of highly efficient small-molecule antagonists of TRPV1. We can conclude that APHC peptides have unique properties; they don't inhibit normal receptor function and don't cause hyperthermia. Only in the case of pathologically strong activation, the desired therapeutic effect will occur. These results are important for further development of TRPV1 ligands for therapeutic use. This work was supported by RSF Grant 16-15-00167.

P.15-034-Mon
Molecular genetic analysis of CFTR gene in 952 Turkish cystic fibrosis patients and identification of novel mutations: 25-years experience

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Cystic fibrosis (CF) is one of the most common autosomal recessive disorders with a frequency of 1/3000. It is caused by mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) gene which encodes a cAMP/protein kinase A regulated chloride channel. CF is mainly characterized with respiratory tract infections, pancreatic insufficiency, intestinal obstruction and infertility. So far more than 2000 mutations have been identified in the CFTR gene. Mutations are classified according to their effect on

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CFTR protein synthesis, folding, transport and correct functioning at the membrane. CFTR gene mutations vary depending on ethnicity of the patients. Mutations in Turkey are heterogeneous and F508del frequency is much more lower than Central European populations. This molecular heterogeneity makes it difficult to establish a genotype phenotype correlation. In this study we report CFTR mutation frequencies and identification of novel mutations in a large cohort of 952 CF patients diagnosed between the years 1993- 2018. Patients were screened initially for F508del, 1677delTA, G85E, 2789+5G-A, 3849+10 kbC-T, N1303K, CFTRdele2, CFTRdele2,3. Then they were subjected to CF strip assay followed by DNA sequencing of the CFTR coding region and exon/intron boundaries. We identified 94 different mutations on 60% of the alleles. The most common mutations were as follows: F508del (19.4%), 1677delTA (4.4%), N1303K (3.3%), G85E (3%), 2789+5G-A (2.7%), 2183AA-G (2.7%), G542X (2.5%), CFTRdele2 (1.4%), W1282X (1.2%), 3849+10kb C-T (1.2%), R334W (1.1%). Five mutations were described for the first time. The heterogeneous mutation spectrum of CF in Turkey demonstrated that already commercially available mutation panels were not suitable for screening therefore population specific panels should be developed. Also detection of rare CFTR mutations at frequencies less than 1%, suggested that improving a model system for personalized medicine in these patients will have invaluable significance.

P.15-035-Tue
Multireceptor complexes regulate cis-Golgi assembly by facilitating vesicle tethering

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Endoplasmic reticulum (ER) cargo receptors are major transmembrane constituents of the early secretory pathway that continuously cycle between ER and Golgi apparatus. They function individually to mediate selective incorporation of specific cargo proteins into ER-derived vesicles by linking the cargo with the COPII coat. Here, we show that yeast cargo receptors associate at the ER exit sites to form large multiprotein complexes. We also found that the combined absence of two of these cargo receptors compromises the cell growth and the dynamic structure of the cis-Golgi. Both phenotypes are restored by the overexpression of SLY1-20, a dominant allele that suppress COPII vesicles tethering deficiencies. Furthermore, we show that COPII binding by these receptors is a prerequisite for Golgi assembly. Our results imply that cargo receptors cooperate to promote vesicle tethering events required to maintain the cis-Golgi homeostasis. We propose that cargo receptors collectively stabilize coats on the vesicle membrane to ensure correct functioning of tethering factors ant thus coupling cargo selection to vesicle delivery.

P.15-036-Wed
VEGFR2 transactivation by formyl-peptide receptor 1 requires NADPH oxidase-dependent superoxide generation

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G protein-coupled receptors (GPCRs) belong to a large family of cell-surface receptors involved in many intracellular signaling pathways. Despite GPCRs lack intrinsic tyrosine kinase activity, tyrosine phosphorylation of a tyrosine kinase receptor (TKR) occurs in response to binding of specific agonists of several such receptors. Crosstalk between GPCRs and TKRs may occur by different molecular mechanism such as metalloproteases activation, non-receptor tyrosine kinases involvement, or reactive oxygen species (ROS) production, a process mediated by the NADPH oxidase enzymatic complex. Formyl-peptide receptors (FPRs) are seven transmembrane domain receptors, coupled to heterotrimeric Gi proteins and sensible to pertussis toxin (PTX). FPR1, FPR2 and FPR3 expression was observed in a wide variety of tissues and it has been implicated in tissue repair, angiogenesis and cancer. We investigated FPR1-mediated VEGFR2 transactivation and its involvement in angiogenesis processes in ECV304 endothelial cells. We demonstrated, by RT-PCR, that ECV304 cell line expresses $p47^{phox}$, $p67^{phox}$ and $p22^{phox}$ subunits of NADPH oxidase, as well as FPR1, FPR3, VEGFR1 and VEGFR2 receptors. Stimulation of ECV304 with the FPR1 agonist N-fMLP induces $p47^{phox}$ phosphorylation, which is considered the key event of NADPH oxidase activation. Preincubation with PTX prevents $p47^{phox}$ phosphorylation. FPR1 stimulation by N-fMLP also triggers Y951, Y996 and Y1175 phosphorylation of cytosolic residues of VEGFR2, which elicits intracellular signaling cascades responsible of angiogenesis. Preincubation with PTX or FPR1 siRNA, or the inhibition of NADPH oxidase functions by apocynin or $p22^{phox}$ siRNA, prevents VEGFR2 tyrosine phosphorylation and, in turn, downstream signaling cascades.

Extracellular matrix

P.16-001-Mon
Functional analysis of novel compound heterozygous ACP4 mutations causing hypoplastic amelogenesis imperfecta

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Amelogenesis imperfecta (AI) is a rare hereditary condition affecting tooth enamel. The affected enamel can be hypomineralized and/or hypoplastic. Recently, recessive mutations in *ACP4* (acid phosphatase 4) have been identified to cause hypoplastic AI. In this study, we identified novel heterozygous compound *ACP4* mutations in a hypoplastic AI family using whole exome sequencing and Sanger sequencing: c.262C>A (p.Arg88Ser) and c.419C>T (p.Pro140Leu). Western blot revealed that the mutant proteins were expressed in a greatly reduced level compared to the wild type protein. To further explicate the effect of the altered protein sequence on ACP4 homodimerization, Co-IP was done. Moreover, to compare AP activity levels of wild type and mutant forms, acid phosphatase assay was performed. The aim of this study was to elucidate how the compound heterozygous ACP4 mutations, located in extracellular part of ACP4 protein, affect protein structure and function of ACP4.

P.16-002-Tue
Connective tissue growth factor (CTGF) and total protein concentrations in tear fluid and aqueous humour in pseudoexfoliation syndrome and pseudoexfoliative glaucoma

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Pseudoexfoliation syndrome (PES) is a disease of extracellular matrix featured by accumulation of abnormal fibrillary material in ocular tissues. Pseudoexfoliative material may cause glaucoma (PEG) raising intraocular pressure by obstructing the drainage canals of aqueous humor. Fibrillar components are overproduced in PES, so growth factors may have roles in pathophysiology. CTGF is expressed in many tissues, including anterior chamber of eye. Tear and aqueous humor proteomes are thought to be related with ocular diseases. This study aims to investigate total protein and CTGF concentrations in tear and aqueous humor for PES and PEG. Aqueous humor from 23 controls, 8 PEG and 17 PES patients and tear fluid from 78 controls, 78 PEG and 77 PES patients were collected by Gulhane Education and Research Hospital, Ophthalmology Unit, Ankara, Turkey. Total protein levels were determined with Bradford and CTGF concentrations were assigned by ELISA. In aqueous humor, total protein was found as 388.65 ± 161.52 μg/ml in PEG, 270.17 ± 199.12 μg/ml in PES and 196.85 ± 91.63 μg/ml in controls ($P = 0.012$), while CTGF was found as 5.40 ± 2.87 ng/ml in PEG, 3.62 ± 2.16 ng/ml in PES and 4.10 ± 2.90 ng/ml in controls ($P = 0.190$). In tear fluid, total protein was found as 18.91 ± 8.91 mg/ml in PEG, 16.47 ± 8.94 mg/ml in PES, and 16.34 ± 10.29 mg/ml in controls ($P = 0.011$), while CTGF was found as 12.15 ± 15.69 ng/ml in PEG, 10.08 ± 12.11 ng/ml in PES and 11.91 ± 26.59 ng/ml in controls ($P = 0.004$). This was the first study to investigate CTGF in tear fluid of PES and PEG patients. This study shows that in tear fluid, total protein of PEG patients was significantly higher than PES and controls; CTGF of controls was significantly higher than PES and lower than PEG. Moreover, total protein concentration in aqueous humor of PEG patients is higher than PES and controls. Acknowledgment: This study was supported by TUBITAK (115S360).

P.16-003-Wed
Fibronectin fragment regulates catabolic responses via modulation of HMGB1-dependent autophagy pathway in human articular chondrocytes

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Fibronectin fragments found in synovial fluid of patients with osteoarthritis (OA) induce the catabolic responses in cartilage. Nuclear high mobility group protein Box 1 (HMGB1), a damage-associated molecular pattern (DAMP), is responsible for the regulation of signaling pathways related with cell death and survival in response to various stimuli. In this study, we investigated whether 29-kDa amino-terminal fibronectin fragment (29-kDa FN-f)-induced change in HMGB1 expression influences the pathogenesis of OA through HMGB1-modulated autophagy signaling pathway. The HMGB1 level was significantly reduced in human OA cartilage compared to normal cartilage. Although 29-kDa FN-f significantly reduced the HMGB1 expression at the mRNA and protein levels 6 h after treatment, the cytoplasmic level of HMGB1 was increased in 29-kDa FN-f-treated chondrocytes. 29-kDa FN-f significantly inhibited the interaction of HMGB1 with Beclin-1 but increased the interaction of Bcl-2 with Beclin-1, together with decreased levels of Beclin-1 and phosphorylated Bcl-2. In addition, the level of microtubule associated protein 1 light chain 3-II (LC3-II), an autophagy marker, was down-regulated in 29-kDa FN-f-treated chondrocytes, whereas the effect was antagonized by mTOR knockdown. Furthermore, prolonged treatment with 29-kDa FN-f significantly increased the release of HMGB1 into the culture medium. These results demonstrated that 29-kDa FN-f has a harmful impact on chondrocyte through suppressing HMGB1-dependent autophagy pathway.

Glycans

P.17-001-Mon
Galectin-3-mediated NETosis in systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the generation of circulating autoantibodies that target multiple organs. NETosis is a cell death pathway characterized by extrusion of chromatin bound to cytosolic and granular contents called neutrophil extracellular traps (NETs). Aberrant NETs and NET degradation play roles in the pathogenesis of SLE. Galectin-3, a β-galactoside-binding animal lectin, is involved in many inflammatory responses. However, whether galectin-3 contributes to the SLE pathogenesis remains unclear. The aim of this study was to investigate whether galectin-3 impacts NETosis and thereby contributes to the pathogenesis of SLE. We used clinical samples of SLE patients and a pristane-induced lupus-like mouse model to examine the potential correlation of galectin-3 with NETosis in SLE. Our results showed that patients with SLE expressed higher levels of galectin-3 in the peripheral blood mononuclear cells than normal individuals. Galectin-3 was detected in NETs of human neutrophils treated with phorbol 12-myristate 13-acetate (PMA) for inducing NETosis. Treatment with lactose, a galectin-3 inhibitor, reduced NETosis in a dose-dependent manner. In animal studies, we found that pristane-treated C57BL/6 mice exhibited immune complex deposition and had elevated galectin-3 expression in the hemorrhagic lung. Compared to wild-type mice, galectin-3 KO mice had lower levels of proteinuria, reduced pulmonary hemorrhage, decreased inflammatory cell infiltration, and enhanced survival time following pristane treatment. While NETosis and the NET marker citrullinated histone 3 were detectable in lipopolysaccharide (LPS)-treated neutrophils of wild-type mice, they were absent in their galactin-3 KO counterparts. Taken together, our results suggest that galectin-3 may be involved in the pathogenesis of SLE through mediating NETosis.

P.17-002-Tue
Xylosyltransferase invalidation produced defects in skeletal development in mice

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Proteoglycans play an essential role in several major physiological processes such as cell signaling, proliferation and migration; this is mainly due to interactions between their glyco-

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saminoglycan (GAG) chains with soluble mediators and their receptors. The initiation of the synthesis of GAG chains of PGs is catalyzed by xylosyltransferase I (XT-I). To study the role of XT-I in vivo, we generated XT-I knock-out mice. Embryo analysis shows that XT-I knock-out mice exhibit pronounced dwarfism and apparent frontonasal hypoplasia, thus reflecting abnormalities in skeletal development. Analysis of PG content revealed a strong decrease in PGs synthesis in XT-I knock-out mice. Examination of chondrocytes zones in growth plate of long bones revealed extended proliferative zone and short hypertrophic zone. To identify the factors and mechanisms underlying skeletal abnormalities in XT-I KO mice, the expression of several genes involved in skeletal development and in the regulation of chondrogenesis were analyzed by in situ hybridization using RNAscope technique. The results showed a strong expression of hypertrophic chondrocyte markers in XT-I KO mice compared to wild-type alternates, thus suggesting an early maturation of chondrocytes in the XT-I knock-out mice leading to premature ossification and hence to dwarfism.

P.17-003-Wed
Importance of CH-π stacking interaction in carbohydrate-protein complexes

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CH-π stacking, a special type of hydrogen bonding interaction, plays an important role in carbohydrate-biomacromolecule interactions involved in cell recognition, growth, and differentiation, as well as in many pathological processes. To better understand this particular type of hydrogen bonding between a CH group and an aromatic ring, we examined the CH-π stacking interaction in carbohydrate-protein complexes in a computational structure-based study. We searched the Protein Data Bank database to examine complexes with carbohydrates in a close proximity of aromatic amino acid (tryptophan, tyrosine, phenylalanine, and histidine). CH-π stacking interaction was found in 61% of these complexes, where the complexes between a carbohydrate and tryptophan were the most populated. Each aromatic amino acid showed a unique CH-π stacking pattern, demonstrated by a characteristic orientation, bond distances, and bond angles between the carbohydrate and a particular amino acid. These results provide critical insight into the importance of CH-π stacking in carbohydrate-protein interactions. This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601).

P.17-004-Mon
Marine bivalve lectins induce tumor cells death

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Glycosylation plays an important role in determining protein function, and can play a role in disease development. An alteration in the glycosylation patterns of cell surface proteins is associated with tumor progression. Binding of lectins to glycans on tumor cells can activate cell death by apoptosis. Previously we isolated two GalNAc/Gal-specific lectins from marine mussels *Crenomytilus grayanus* (CGL) and *Mytilus trossulus* (MTL). The establishment of the amino acid sequence showed that CGL and MTL have a high degree of homology and represent a new class of lectins. Glycan array assay revealed that CGL and MTL were able to bind both α and β anomer of galactose. CGL interaction with the αGal-terminated glycans was stronger. As for MTL, preferable binding with β anomers was observed. CGL showed high affinity to Galα1-4Galβ1-4GlcNAc motif similar to globotriose structure (Gb3: Galα1-4Galβ1-4Glc). CGL recognized Gb3 on the surface of Burkitt's lymphoma Raji cells (high Gb3 expression), leading to dose-dependent cytotoxic effect, G2/M phase cell cycle arrest and apoptosis. Lectin had no effect on erythroleukemia K562 cells (no Gb3 expression). The activity of CGL was specifically blocked by alpha-galactoside. MTL, on the other hand, had dose-dependent cytotoxic effect on K562 cells, but no activity was observed on Raji cells. TF-antigen (Galβ1-3GalNAc) expressed on the membrane of K562 cells was significant for MTL effect. Both lectins effectively inhibited the tumor cells proliferation. Thus despite the high degree of homology, CGL and MTL have different ligand specificity as lectins interacted with different glycoconjugates expressed on the surface of tumor cells. Overall, our data indicate that CGL and MTL have the potential to be used in cancer diagnosis and treatment. The investigation was partially supported by the Program of FEB RAS "Far East" (project 18-4-007).

P.17-005-Tue
Arginase is an enzyme with real tricks in antitumor treatment of 7,12-dimethylbenz(a) anthracene induced breast cancer in rats

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Polyamines are vital for cell proliferation and the increased level of ornithine, due to the elevated arginase activity, linked to the development of carcinogenesis. The main question was addressed in this work, would treating the rats with arginase inhibitor N^G-hydroxy-nor-L-arginine (nor-NOHA) affect cancer progression and the oxidative responses (malondialdehyde (MDA), nitrite and ammonia ions)? A total of 35 adult female Wistar rats weighing 90-120 g were used. Rats were divided into five groups (control, saline, nor-NOHA; 7,12-dimethylbenz(a)anthracene (DMBA) and DMBA+nor-NOHA, 7 rats per group). Rats in DMBA and DMBA+nor-NOHA groups were administrated intragastrically each with a single dose of 20 mg/ml DMBA dissolved in 0.5 ml olive oil and 0.5 ml saline. Rats in nor-NOHA and DMBA+nor-NOHA groups were injected by nor-NOHA intraperitoneally for 5 weeks (after 10 days of DMBA administration, every 3rd day) in dose of 3 mg/kg body-weight in 0.25 ml saline. Rats blood plasma arginase activity, MDA, total nitrite anions and ammonia quantities were determined by corresponding methods and with the rules of the Ethical Committee. At the end of 20th week, rats were sacrificed by decapitation under anesthesia. Histopathological alteration in DMBA group (2–4 tumors, 0.3–0.8 cm) in the 20th week has revealed the invasive ductal and lobular carcinoma, and in DMBA+nor-NOHA group (0–2 tumors, 0.1–0.3 cm) – only lobular carcinoma *in situ*.

In DMBA group increased blood arginase activity, MDA, NO_2^- and NH_4^+ levels by 98.7%, 115.1%, 78.2% and 40.8%, respectively in the 13th and 87.4%, 96.8%, 68.5% and 32.4% in the 20th week comparing to the control groups. Co-treatment with the nor-NOHA blocked these increases, resulting in mean values similar to those of the control groups for MDA, NH_4^+ and leading to an even lower mean for arginase activity and NO_2^-. We conclude that nor-NOHA inhibits DMBA-induced mammary cancer progression and protect against oxidative stress.

P.17-006-Wed
The dose-dependent PAMPs binding activity of lectins from sea invertebrates

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Invertebrates lack the typical adaptive immune responses of vertebrates but are endowed with effective defense mechanisms against infectious challenge. They comprise both cellular and humoral responses mediated by multiple soluble factors and cell-associated receptors that can recognize pathogens and lead to effectors functions, including opsonization, phagocytosis, coagulation cascades and expression of antimicrobial peptides. The interaction between pathogen-associated molecular patterns (PAMPs) and pattern recognition receptors (PRRs) is an important stage in the immune response. Lectins are some of the most important PRR families widely distributed in almost all organisms, including marine invertebrates. We have identified and characterized some lectins from sea invertebrates: GalNAc/Gal-specific lectins from the sea mussels *Crenomytilus grayanus* (CGL) and *Mytilus trossulus* (MTL), GalNAc/Gal-specific lectin from the scallop *Patinopecten yessoensis* (PYL) and two lectins from the ascidian *Didemnum ternatanum*: GlcNAc-specific lectin (DTL) and GlcNAc/GalNAc specific lectin (DTL-A). The enzyme-linked immunosorbent assay (ELISA) was performed to detect whether these lectins bound to PAMP, such as lipopolysaccharide (LPS), peptidoglycan (PGN) and β-1,3-glucan. The results showed that DTL-A, CGL and MTL strongly bound to polysaccharides, including LPS and PGN in dose-dependent manner, but showed a relatively weaker binding activity to β-1,3-glucan. The DTL and PYL have shown insignificant activity compared to the others lectins. Thus our results are in good agreement with a statement that during the past few years it has become increasingly clear that lectins from marine invertebrates also bind glycans on the surface of potentially pathogenic microbes and can function as PRRs.

P.17-007-Mon
Heavy-oxygen assisted autohydrolysis of sulfated polysaccharides: a technique for partial degradation and selective labeling of polysaccharide fragments

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The interest to the water-soluble polysaccharides from brown algae – laminarans and fucoidans remains strong through decades: they possess diverse biological activities, especially important is anticancer activity of fucoidans. However, it was recently shown that sulfation of the laminarans also showed good results. Since it is essential to know exact chemical structure of the active agent and polysaccharides are structurally complex, it is important to develop methods that could both simplify the structure and save or even increase the activity. Autohydrolysis is a form of mild acid hydrolysis using polysaccharide's own sulfate groups as the source of acid. This method is compatible to the tandem electrospray ionization mass spectrometry, ESIMS/MS, which is a great tool for the analysis of anionic carbohydrates. The labeling of the reducing end (during autohydrolysis in $H_2{}^{18}O$) allowed us to precisely distinguishing between the different by nature fragment ions but having the same m/z values. We examined molecular weights (MW) of polysaccharide parts of fucoidan (FeF, MW = 160 kDa from Fucus evanescens) and sulfated laminaran (ScLS, MW = 20 kDa, from S. cichorioides) during autohydrolysis at 37°C for 24 (MW = 65 kDa), 48 (MW = 51 kDa) and 72 h (MW = 46 kDa) of reaction, while low-molecular mixtures were analyzed by ESIMS/MS. ScLS was readily decomposed only at 60°C. The most interesting results of ESIMS/MS: a detection of minor sulfation at C-3 of ScLS sample due to ^{0,3}X-type signals; the simultaneous presence of ^{0,2}A and ^{0,3}A-type of fragment ions indicated 1,6-type of linkage, while ^{0,2}A-type ion indicated only 1,4-type of linkages. The in-source elimination of a sulfate group at C-2 involved the ^{18}O at the reducing end. The estimation of MWs of polysaccharides was performed in the center for collective use of scientific equipment "Far Eastern Center of Structural Studies". The study was supported by the Grant No. 16-13-10185 from the RSF.

P.17-008-Tue
Enzymatically stable galectin inhibitors: C-disaccharides as novel glycomimetics

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Over the last two decades, human galectins (particularly galectin-1, 3, 7 and 9) have become attractive targets for anti-cancer and anti-inflammatory drug development. In addition, galectin-1 enhances the binding affinity of human immunodeficiency virus type-1 (HIV-1) glycoprotein gp120 to host cells and increases viral infectivity. Recently, we have introduced the modular stereoselective synthesis of *C*-disaccharides that is based on sp3-sp2 cross-coupling reactions followed by stereoselective oxidative-reductive transformations. Since lactose and TDG are known scaffolds in preparation of galectin-1 and galectin-3 inhibitors, the initial goal was therefore preparation of their carba-analogues. We have found that CDG (β-d-Gal*p*-*C*-(1→1)-β-d-Gal*p*)

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occupies in free unbound state predominantly one conformation, which is very similar to the conformation of TDG, and therefore perfectly arranged to fit into the binding site of galectin-1. However, the determination of bidding affinity of CDG (K_d = 416 mM) to galectin-1 by isothermal titration calorimetry showed repeatedly K_d worse than for lactose (K_d = 327 mM). Moreover, TDG binds to galectin-1 about 6 times stronger than lactose (K_d = 57 mM). This difference in binding affinities of CDG and TDG might be therefore attributed to special geometric arrangement in proximity of sulfide bridge. We have tested prepared glycomimetics in our optimized viral entry assay using LuSIV cells infected with HIV-1 and in red blood cells hemagglutination assay. Both assays show, that CDG has comparable effect as TDG. These biochemical and biological findings, together with molecular modeling, will serve as a basis for further synthetizing novel glycomimetics with improved efficiency, stability and bioavailability.

P.17-009-Wed
Withdrawn

P.17-010-Mon
Investigating mycobacterial cell wall synthesis inhibitors: galactan biosynthesis as a potential antituberculosis drug target?

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The synthesis of the cell wall of *Mycobacterium tuberculosis*, the causative agent of tuberculosis, is a target for several antibiotics. However, none of them targets the synthesis of the galactan polymer, a part of the mycolyl-arabinogalactan-peptidoglycan cell wall core. The three main enzymes participating in the synthesis of the galactan polymer, UDP-galactopyranose mutase UGM, galactosyltransferase GlfT1 and galactosyltransferase GlfT2, can be regarded as potential drug targets. They are essential for the growth of mycobacteria and they catalyze reactions involving UDP-galactofuranose, a rare sugar nucleotide, which is not present in humans. We have developed cell-free enzyme assays, and a whole cell labeling assay that can determine whether inhibitors of these enzymes successfully block the synthesis of mycobacterial galactan polymer. For the cell-free assays, a fraction of mycobacterial cell walls and membranes is used as a source of the enzymes for galactan synthesis, and UDP-[14C]Gal*p* is used to monitor the synthesis of galactan intermediates. The addition of an active inhibitor of any of the enzymes mentioned, should significantly reduce the incorporation of the radiolabeled Gal*f* into the growing polymers. The whole cell labeling using [14C]-glucose or [14C]-acetate allows us to monitor the effects of the inhibitors on the synthesis of the cell wall in growing mycobacteria. We will present evaluation of the selected compounds described in the literature as inhibitors of the enzymes involved in galactan biosynthesis by these assays in order to assess their potential for further development. This work was supported by Slovak Research and Development Agency (project no.: APVV-15-0515).

P.17-011-Tue
Heparitinase I and II from *Pedobacter heparinus* and their use in the study of heparan sulfate proteoglycan-dependent cytoskeleton dynamics

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The use of heparitinases has been limited by difficulties in isolation and separation of sufficient quantities of the enzymes and by contaminating polysaccharide degrading and modifying enzymes, such as chondroitinases and sulfatases. We cloned and expressed heparitinases I and II genes from *Pedobacter heparinus* in *Escherichia coli* BL21. Expression was induced in the log phase by addition of 0.5, 1 or 2 mM of IPTG. Three temperatures, 22, 29 and 37°C, and three different times for induction, 4, 12 and 18 h, were tested. Soluble fraction was purified by Ni^{2+} affinity column chromatography. Heparan sulfate and heparin were used as substrates to the recombinant enzymes. The digestion products of heparan sulfate and heparin were analyzed by HPLC and agarose gel electrophoresis in PDA buffer. We found that the ratios of soluble fraction to inclusion body were influenced by the high temperatures to heparitinase I and high concentration of IPTG for both heparitinases I and II. Optimum expression conditions for heparitinase I was 29°C for 12 h and 1 mM IPTG, whereas heparitinase II was 37°C for 18 h and 1 mM IPTG. Both enzymes were active and stable at 37°C. Owing to the fact that both enzymes target different regions of heparan sulfate chains that decorate heparan sulfate proteoglycans (HSPGs), they were used to study cytoskeleton dynamics. Vascular endothelial cells from rabbit aorta were transfected with vectors encoding actin fused with GFP and treated for 6 h with both enzymes separately. Cytoskeleton parameters such as fiber organization, coupling of actin-associated proteins and focal adhesion points distribution were analyzed using confocal microscopy and the results compared to cells in which HSPGs had been knocked down. The gathered data shows the pivotal role of HSPGs glycosaminoglycan chains and further pinpoint their importance for cell behavior.

Protein – folding, dynamics, interaction and degradation

P.18-001-Mon
Cytochrome *c* is revealed as a double activator of apoptosis through its interaction with 14-3-3ε

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Apoptosis is a widely regulated kind of cell death, given its relevance in the proper development and homeostasis of multicellular organisms. Cytochrome *c* (C*c*) is a key piece in the activation of the apoptotic intrinsic pathway, since its well-known interaction with Apaf-1 allows the apoptosome assembly – therefore triggering the caspase cascade activation. Herein, we explore the recently discovered interaction between cytosolic C*c* and 14-3-3ε, a direct inhibitor of Apaf-1. Our studies expose the ability of the metalloprotein to block 14-3-3ε-mediated Apaf-1 inhibition, thereby unveiling a novel role for C*c* as an indirect activator of caspase-9/3. This mechanism allows to speed up the caspase cascade activation, by blocking 14-3-3ε-mediated pro-survival pathways. With the aim to provide details of the structural features of C*c*/14-3-3ε complex, a combination of Isothermal Titration Calorimetry (ITC) and Nuclear Magnetic Resonance (NMR) has been used. Calorimetry measurements evinced C*c* binds 14-3-3ε dimer at two different binding sites, with similar binding affinities in the μM range. The use of site directed mutagenesis on 14-3-3ε and ITC assays lets to identify the concave and the convex faces of the dimer as C*c*-binding sites. In addition, the C*c* NMR perturbation map shows how the metallo-protein uses the rim of its heme group to bind 14-3-3ε; both ITC and ^{19}F NMR titrations displayed that the same surface is also used to recognize the C-terminal tails of the dimeric protein. Finally, Molecular Dynamics (MD) and Docking methods allow to generate restrain-based structural models of such interaction. To sum up, our findings indicate a Cc-mediated double mechanism to modulate the apoptosome formation, therefore expediting the caspase cascade activation.

P.18-002-Tue
Thermodynamic and kinetic analysis of specific lesion recognition by wild-type endonuclease III and its catalytic mutants

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Endonuclease III is a bifunctional DNA-glycosylase from *E. coli* that shows both N-glycosylase and AP-lyase activity. While interacting with the enzyme the DNA substrate proceed through several conformational rearrangements in its structure. Several mutant forms (K120A, D138A) were constructed to determine the functional role of each amino acid residue in the catalytic reaction. Here the stopped-flow method is used to analyze structural transitions in several DNA substrates that take place during Endo III catalytic cycle. Structural changes in DNA substrates were registered using fluorescent analog of DNA base incorporated opposite the specific base. The DNA duplexes contained 5,6-dihydrouracil base, non-cleavable analog of abasic site and native guanosine as non-damaged DNA-duplex. Changes in fluorescence intensity were registered in 5–37°C temperature range. To calculate the rate constants of DNA conformational transitions, a number of kinetic curves for each DNA substrate were obtained. It was shown that mutant forms Endo III K120A and D138A lack of both N-glycosylase and AP-lyase activity. Understanding the mechanism of the catalytic complex formation was improved by thermodynamic analysis of the binding and cleavage steps. The dependences of equilibrium constant on temperature were analyzed according to the van't Hoff equation and allowed to calculate Gibbs free energy, enthalpy and entropy of individual interaction stages. The analysis of the temperature dependence of the reaction rate constant k_{cat} using the Eyring equation provided the standard activation enthalpy and standard activation entropy of the transition state. This method made it possible to define the thermodynamic parameters of specific stages of interaction between Endo III and DNA duplexes. This work was supported by Russian Science Foundation (Grant No 16-14-10038).

P.18-003-Wed
Unravelling the mitochondrial cytochrome-C maturation

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C-type cytochromes are metalloproteins that contain heme(s) covalently bound to the polypeptide chain via two thioether bonds to cysteine side chains. These proteins play fundamental roles in biological processes across all domains of life, including in respiratory processes, catalysis, and apoptosis. Given their importance, the process that leads to their formation in nature is equally important. The covalent attachment of the heme to the apo-protein requires a maturation machinery, a process that is far from fully understood. System III (CcHL) is the maturation system present in most eukaryotes including humans. This is the least understood maturation system and its characterization can have important health implications as the deficiency in cytochrome maturation is associated with diseases such as MLS (Microphthalmia with linear skin defects). A consensus sequence that is recognized by System III and guides the attachment of the heme *c* has been described. However, this system has not been shown to be able to recognize multi-heme apo-cytochromes-*c*. In this study, the consensus sequence was inserted in the multiheme cytochrome STC (small tetraheme cytochrome) to understand the ability of system III to recognize and mature this type of proteins. Results show that CcHL is able to recognize the apo-cytochrome, but the folding of the protein is not correct, leaving the hemes in a non-native high-spin state. This work reveals for the first time that this protein is able to recognize a multiheme cytochrome and suggests that the correct folding of the cytochrome is not spontaneously induced by covalent attachment of the heme, as described in the literature. This work opens the door for further characterization of System III, a starting point for development of cytochrome *c*-related therapies, including those for the treatment of MLS.

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P.18-004-Mon
The calpain-calpastatin system in human central nervous system tumours: new insight on its prognostic value

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The calpain/calpastatin proteolytic system regulates several physiopathological processes and can play opposing roles in cancer. New strategies to control alterations of this system for therapeutic purposes require a full understanding of the functional role played by the single protein members. For this purpose we have evaluated the involvement of calpain-1 in the proliferation of human meningioma and glioblastoma cells obtained from brain surgery. We demonstrate that tumour samples with an altered intracellular homeostasis of Ca^{2+} show increased conversion of the 80 kD inactive calpain-1 to the autolysed 75 kD form, catalytically active at mM Ca^{2+} concentrations *in vitro*. Intracellular calpain-1 targets, such as spectrin and the natural calpain inhibitor calpastatin, result degraded *in vivo* in these tumour samples. Specifically, calpastatin undergoes a limited digestion to discrete fragments that maintain the inhibitory activity. Tumour samples with mean or high amounts of 75 kD calpain-1 show a wide cytosolic diffusion of the inhibitor, while samples characterized by low levels of active calpain-1, retain calpastatin in cytosolic aggregates. Since higher calpain-1 activation does not correspond to a detectable decrease in the Ki67 cell proliferation index, the activity of calpain-1 seems to have a low prognostic significance in the progression of the brain tumours analysed in this study. However, the perturbation of Ca^{2+} homeostasis promotes an increased synthesis of Matrix Metalloproteinase-9 (MMP-9), known to be involved in tumour invasiveness, suggesting that other Ca^{2+}-dependent processes could be related to the malignancy of these cells. These evidences suggest that the potentially harmful activity of autolysed 75 kD calpain-1 can be efficiently controlled by the low Mr calpastatin produced by the protease itself *in vivo*, allowing cell proliferation.

P.18-005-Tue
Effects of melatonin and resveratrol on renal expression of sodium-glucose cotransporters SGLT1 and SGLT2 in rat model of aging

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Mechanisms of aging are poorly understood. Aging is associated with loss of renal function and structure. Elevated tissue concentrations of reactive oxidative species, known to be present in old humans and experimental animals, may affect the expression and/or activity of various renal transporters, including those that mediate reabsorption of glucose, such as SGLT1 and SGLT2. SGLT2 in the proximal tubule S1/S2 segments mediates a bulk (65–90%) glucose reabsorption, whereas SGLT1 in the S3 segment mediates reabsorption of the remains. To test hypothesis that the expression of SGLT1 and SGLT2 could be changed in old age, and corrected with antioxidants, we treated male and female Wistar rats with melatonin and resveratrol. Starting from their age of 3 months, for the next 21 months the rats were drinking antioxidants in water (~1 mg/kg b.w./day), whereas the control animals were drinking water with vehicle (0.01% ethanol). The expression of renal SGLT1 and SGLT2 was analysed by Western blotting of isolated total cell membranes and by immunohistochemistry of tissue cryosections using specific antibodies. Melatonin and resveratrol did not notably change the expression of renal SGLT1 in both sexes, but in melatonin-treated males, a slight tendency to SGLT1 upregulation was observed. Also, melatonin treatment did not affect the SGLT2 expression in both sexes. However, resveratrol significantly upregulated the SGLT2 expression in male, but not in female rats. We conclude that in old rats, the melatonin treatment has a negligible effect on renal SGLTs, whereas the resveratrol effect on SGLT2 is sex-related, being restricted to males. (Croatian Science Foundation project IP-2013-11-1481).

P.18-006-Wed
Comparative analysis of PARP1 and PARP2 in base excision repair context

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The genome stability in eukaryotes is provided by functioning of DNA repair systems. Base excision repair (BER) is one of the

main DNA repair systems in eukaryotes. For correct functioning these systems require precise regulation. PARP1 and PARP2 are widely considered as regulators of DNA repair processes, including BER. PARP in response to the DNA damage synthesize polymer of ADP-ribose (PAR) covalently attached to the acceptor proteins including PARP itself. PAR formation can directly influence the acceptor properties and also it is an intracellular signal about DNA damage. PARP1 is well characterized protein but both proteins are under careful attention of researchers. In our work we studied interaction of PARP2 with model DNAs containing several key BER intermediates including AP sites. Moreover AP-site is one of the most frequent types of DNA damages. Using reconstituted systems we made a comparative study of PARP1/PARP2 interaction with several key BER proteins (Pol-beta, FEN1 and APE1) demonstrating inhibitory effect of both PARPs. Wherein in contrast to PARP1 the inhibition caused by PARP2 is hardly regulated by PAR synthesis. We next demonstrated the ability of PARP2 to cross-link with AP site via Schiff base formation like PARP1 does. For both PARPs we determined major role of the N-terminal domains in formation of cross-links with AP DNA. We have also confirmed that DNA binding by PARP2, in contrast to PARP1, is not modulated by autopoly(ADP-ribosyl)ation. Taken together, our results testify to the complicated multilevel regulation of short- and long-patch pathways of BER under coordinated action of PARP1 and PARP2. This work was supported by RSCF project № 17-74-20075.

P.18-007-Mon
Studying of DNA binding proteins using crosslinking reaction with the participation of the cysteine residues

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The correction of the errors occurred in the process of DNA replication is performed by DNA mismatch repair (MMR) system. The process of DNA repair is initiated when MutS protein binds to the mismatch. At the next step MutS binds ATP, releases the mismatch and starts moving along DNA as a 'sliding clamp'. This process results in complex formation of MutS with other MMR protein – MutL. The existence of this ternary complex activates DNA repair. This multistage process has been thoroughly studied for *E. coli* MMR system. We suggest investigating the process of DNA repair by MMR system taking place in bacterium *Neisseria gonorrhoeae,* a parasite of mammals. The MutL protein in *N. gonorrhoeae* possesses endonuclease activity contrary to MutL from *E. coli* and initiates MMR by itself. Taking into account the conformation variability and dynamic nature of the MMR proteins themselves and in the complex with DNA, one of the ways to study the interaction of constituents of this complex is their covalent binding. For this purpose, the crosslinking reaction between the MutL cysteine and amino groups of MutS is performed using N-β-maleimidopropyl-oxysuccinimide ester in the presence of DNA. The crosslinked biomolecules are planned to be analyzed by mass spectrometric method. The crosslinking reaction involving cysteine residues was also performed in the case of nicking endonuclease (NE) BspD6I. This enzyme recognizes a specific sequence in DNA and cuts only one strand nearby the recognition site. No crystal data for the complex of NE BspD6I with DNA is available to date. To elucidate the possible contacts of the protein with DNA, the crosslinking reaction was performed. In this case, DNA contained disulfide group in 2′-position of nucleoside that reacted only with cysteine residues. The obtained data allow suggesting which cysteine residues of NE BspD6I can locate in close proximity to DNA during complex formation. The work was funded by RFBR grant № 17-54-45126.

P.18-008-Tue
Low homology FRPs from different cyanobacteria functionally interact with Synechocystis OCP

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Photosynthesis requires a balance between efficient light harvesting and protection against photodamage. The cyanobacterial photoprotection system uniquely relies on the functioning of the photoactive orange carotenoid protein (OCP) that under intense illumination provides fluorescence quenching of the light-harvesting antenna complexes, phycobilisomes. The recently identified fluorescence recovery protein (FRP) binds to the photoactivated OCP and accelerates its relaxation into the basal form, completing the regulatory circle. Lately, it was shown that, in addition to the function mentioned above, FRP is able to detach photoactivated OCP from phycobilisomes. At the same time, the molecular mechanism of FRP functioning is largely controversial. Moreover, since the available knowledge has mainly been gained from studying *Synechocystis* proteins, the cross-species conservation of the FRP mechanism remains unexplored. Besides the phylogenetic analysis of FRP sequences, we performed a detailed structural-functional analysis of two selected low-homology FRPs by comparing them with *Synechocystis* FRP (*Syn*FRP). While adopting similar dimeric conformations in solution according to the SAXS data and preserving binding preferences of *Syn*FRP toward various OCP variants, the low-homology FRPs demonstrated distinct binding stoichiometries and differentially accentuated features of this functional interaction. By providing clues to understand the FRP mechanism universally, our results also establish foundations for upcoming structural investigations necessary to elucidate the FRP-dependent regulatory mechanism.

P.18-009-Wed
Analysis of the interaction between 14-3-3 and steroidogenic acute regulatory protein (StAR)

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14-3-3 proteins participate in different cellular processes through a multitude of interactions with phosphorylated partner proteins. Recently, 14-3-3 proteins were found as components of the multi-

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protein complex, transduceosome, that mediates cholesterol transfer to mitochondria and were also suggested to be direct partners of the StAR protein. Disturbance of cholesterol transport impairs steroidogenesis and leads to the fatal disorder called lipoid congenital adrenal hyperplasia, however, the detailed mechanism of transduceosome functioning and the role for 14-3-3 in this process remain unclear. As an important component of transduceosome, StAR contains two sites phosphorylated by PKA (S57 and S195), from which S195 was reported to play a role in interactions with 14-3-3. Since the structural features suggest that both sites might be relevant, in this work we dissected their role in the 14-3-3/StAR interaction. The binding ability of 14-3-3 proteins with three forms of StAR protein containing one phosphosite (pS195), two sites (pS57/pS195) or an engineered StAR with pS195 and pS57(L59P) (i.e., an optimized 14-3-3 motif) was tested by gel-filtration. Under conditions used, only the 14-3-3/StAR.pS57(L59P)pS195 complexes (~80 kDa, 2:1 stoichiometry) were detected. According to the SPR data, the apparent Kd of the 14-3-3/StAR.pS57(L59P) pS195 complex was ~50 μM, indicating a rather weak direct interaction between StAR and 14-3-3. Therefore, we used a new approach to study transient interactions and designed chimeric structures containing the 14-3-3 protein core connected to a short phosphopeptide from StAR. Our preliminary data on the 14-3-3/peptStAR chimeras showed clear perspectives for understanding the features of the primary 14-3-3/StAR interaction, but further optimization of the method is required, including stabilization of interaction by low-molecular compounds including the natural stabilizer fusicoccin. Supported by the RSF grant № 17-74-10053.

P.18-010-Mon
Polymer-driven control of aggregation of different amyloid proteins and enzymes

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Since the enzymes are increasingly used for creation of drugs, biosensors, and bioreactors, they should be stabilized at elevated temperature and adjusted for the practical use. Besides, stabilization of amyloidogenic proteins can help for treatment of neurodegenerative diseases. Recently we demonstrated antiaggregation activity of synthetic polyanions including the capability of release the enzyme from the aggregates accompanied with partial reactivation. In the present work, we investigated interaction of model enzymes with thermoresponsive polymers. Chaperone-like activity based on recognition of the unfolded state of the enzyme was observed. Then we tested chaperone-like activity of different polymers, including synthetic sulfated polymers, sulfated polysaccharides, and polycations on amyloidogenic proteins such as ovine prion protein and alpha-synuclein. It was shown that amphipathic sulfated polymers suppress formation of amyloid fibrils of both proteins in contrast to sulfated polysaccharides which activate amyloid aggregation. According to electron microscopy data, relatively small particles were formed in the presence of the polyanions instead of long fibrils. Furthermore, treatment with these polymers resulted in significant decrease of the toxicity of prefibrillar oligomers. The obtained results suggest a new approach for the control of protein aggregation prospective for a practical use of enzymes as well as for treatment of amyloid aggregation.

P.18-011-Tue
A *Streptococcus pneumoniae* key player in ribosome assembly and the impact in translation

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Ribosomes are macromolecular machines that carry out protein synthesis, through a process termed translation. There are present in both prokaryotic and eukaryotic cells and their function are crucial for cell survival since cell growth requires a continues synthesis of new proteins. In bacteria, the 70S ribosome is composed of two subunits, a small 30S subunit and a large 50S subunit. Each subunit is composed of ribosomal RNA and ribosomal proteins. A proper and coordinated assembly of these players is crucial to form an active ribosomal particle. Ribonucleases (RNases) are enzymes that ensure maturation, degradation and quality control of RNA thus, contributing to the maintenance of the optimal amount of each transcript in the cells. The RNB family of enzymes is present in all domains of life and usually includes RNase R, RNase II and the eukaryotic Rrp44/Dis3, Dis3L1 and Dis3L2 proteins. In *Streptococcus pneumoniae* only RNase R is present. RNase R level is increased in several stress conditions such as heat shock, stationary phase or cold shock, conditions in which most of the proteins translation is blocked. Here, we explored the relevance of RNase R in translation by comparing the wild type strain with an *rnr* mutant strain. For this purpose, we compared the ribosomal profile between the two strains using sucrose gradient polysome separation and Western blots. We have also performed Northern blots analysis of transcripts involved in translation and ribosome assembly. We show that RNase R interacts with ribosomes mostly with the 50S subunit. Furthermore, in the absence of this enzyme we observed a decrease in the amount of the 70S ribosomal subunit. We hypothesized that RNase R has an important role in translation through the control of several key players in ribosomal dissociation. This investigation highlights the importance of *S. pneumoniae* RNase R in translation.

P.18-012-Wed
Retrieving the patterns of selectivity to small molecular inhibitors from amino acid sequences of protein kinases

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The protein kinases are the enzymes, which catalyze the protein phosphorylation mediating the regulation of different biological processes. Disorders associated with the malfunction of protein kinases result in several pathological conditions. The search of selective kinase inhibitors is one of promising direction in the drug design. This task is sophisticated since the targeted binding area reveals the conserved structural features in different protein kinases. However, the inhibitors display the paradoxical selectivity to the separate kinase types. Different methods are used to recognize the patterns of inhibitor specificity to protein kinases including the 3D modeling and study of the combinatorial protein-ligand space. The most methods use the multiple sequence alignment, but they have limitations in prediction accuracy due to sequence divergence within the investigated protein family. We applied the original algorithm (SPrOS), estimating the specificity of the single amino acid positions regarding to the certain

ligands. SPrOS calculates the scores for single positions based of the similarity of position surroundings in the studied amino acid sequence and training sequences, classified in accordance with the protein inhibitor interactions. We used the training sets based on publications presenting different experimental studies. The significant results were reproduced with the different training sets. Validation with 3D structures and molecular dynamics of protein kinase complexes with their inhibitors was performed, allowing to confirm the interaction between predicted positions and the ligand molecule. We also revealed structural characteristics, which conditioned the interaction of the same proteins with different ligands. This work is supported by Russian Foundation of Basic Research, Grant no. 16-04-00491.

P.18-013-Mon
Structural and functional characterization of PMGL3 esterase from Siberian permafrost microbial community

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The Siberian permafrost is a unique environment for a variety of microorganisms surviving at low temperatures. The elucidation of the structural and molecular mechanisms of adaptation of microorganisms provides understanding of the possible limits of existence of life and the basis for the development of new biocatalysts. As a result of the construction and screening of fosmid libraries from permafrost metagenomic DNA, we obtained genes coding for new esterases belonging to Hormone Sensitive Lipase (HSL) Family. To determine the molecular basis of microbial adaptation to permafrost conditions, we studied the functional characteristics of the enzyme PMGL3 and determined the 3D structure of a protein with a resolution of 2.1 Á. It was demonstrated that PMGL3 has a typical α/β-hydrolase fold and forms dimers in crystals and solution. We have shown that PMGL3 is an esterase with maximum activity at 30°C toward p-nitrophenyl octanoate (C8) as a substrate. The half-life time of the protein at 40°C was 6 min, and it was completely inactivated by incubation for 6 min at 50°C. Thus PMGL3 is a typical cold-adapted enzyme with decreased stability. To increase the thermal stability of the enzyme, we performed site-directed mutagenesis aimed to enhance the cross-domain hydrophobic interactions in the protein molecule. For the resulting mutants with increased stability thermodynamic parameters were measured and compared with wild type PMGL3. For the mutant C207F which demonstrated 4-fold increased stability after incubation at 40°C thermodynamic parameters were measured and compared with wild type PMGL3. The work is supported by RFBR grant 18-04-00491.

P.18-014-Tue
Arginyltransferase Ate1 is a target of apoptosis-inducing effect of amyloid-beta peptides

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Accumulation of amyloid-beta (Aβ) in the form of insoluble amyloid plaques in specific brain regions is one of the main neuromorphological signs of Alzheimer's disease (AD). However, long before the formation of amyloid plaques intracellular Aβ accumulation occurs in the pyramidal neurons of the hippocampus and entorhinal cortex, that points to the important role of intracellular proteolytic systems in AD pathogenesis. Up to 90% of proteins in mammalian cells degrade through ubiquitin-proteasome system (UPS). We have shown that Aβ inhibits the activity of the N-end rule proteolytic pathway, which is the part of UPS and involved in the proteins degradation prior proteasome. Aβ impairs the process of proteins arginylation – second step of the N-end rule pathway. Arginylation regulates a lot of key physiological events, in particular, degradation of proteins with Asp, Glu, and Cys at N-terminus through UPS. Aβ bearing "English" H6R familial mutation (H6R-Aβ) that causes early-onset AD has greater inhibition effect on the proteins degradation through the N-end rule pathway than Aβ. We have shown that Aβ peptides interact with both isoforms of arginyltransferase (Ate1-1, Ate1-3). Aβ reduces the catalytic rate of Ate1-1. H6R-Aβ impairs the activity of both Ate1-1 and Ate1-3 significantly more efficient than unmodified Aβ. In contrast, Aβ peptides do not affect the catalytic power of arginyl-tRNA synthetase. Using flow cytometry, it was found that H6R-Aβ induces apoptosis and necrosis of mouse neuroblastoma Neuro-2a cells more efficient than Aβ. Ate1 knockout in Neuro-2a cells completely eliminates the apoptotic effect of both Aβ peptides. Thus, suppression of Ate1 activity by amyloid peptides leads to inhibition of proteins degradation and apoptosis that play the important role in AD development. Supported by the Russian Scientific Foundation (grant #14-24-00100).

P.18-015-Wed
Biochemical and functional characterization of the yeast dead-box protein Dbp7

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The RNA helicases are DEAD box proteins involved in ATP-dependent double stranded-RNA unwinding and strand annealing. They perform these activities during RNA metabolism ensuring the correct folding occurs and maintaining the RNA structure. One of the processes in which they are involved is the biogenesis of rRNA. Nineteen RNA helicases have been identified that are involved in the synthesis of ribosomal RNA in yeast. These include *DBP6*, *DBP7*, *DBP9* and *SPB4*. These RNA helicases are required for the correct maturation and assembly of pre-60S ribosomal particles. To develop their functions in rRNA biogenesis, the RNA helicases are often regulated by protein partners. Until now, not too much is known about the function of *DBP6*, *DBP7*, *DBP9* and *SPB4* in rRNA biogenesis and the

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proteins that can modulate their activities in this process. Consequently, the objective of this project is to characterize the RNA helicases *DBP6, DBP7, DBP9* and *SPB4* including its cofactors and substrates, determining the role that these proteins carry out in rRNA biogenesis. To accomplish this goal the recombinant proteins *DBP6, DBP7, DBP9* were overexpressed in *E. coli* BL21 Codon Plus and purified by affinity and size exclusion chromatography. The ATPase activity of these helicases were measured by a colorimetric assay. The essential kinetic parameters were determined in Dbp7 including Km and V_{max}. In parallel, we are analysing the *in vivo* relevance of different domains of the Dbp7 protein by site-directed mutagenesis, including specific substitutions of single conserved residues and truncations at its N- and C-terminal extensions. Progress on this approach will be presented.

P.18-016-Mon
Properties of MutL protein from the *Rhodobacter sphaeroides* mismatch repair system

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The DNA mismatch repair (MMR) pathway removes errors that appear during genome replication. MMR corrects DNA by excising an extended single-stranded fragment of the newly synthesized DNA and then filling the resulting gap. The signal for the excision is hydrolysis of the phosphodiester bond in one of the DNA strands. In γ-proteobacteria this break is introduced by the MutH. However, most prokaryotes and all eukaryotes lack a *mutH* gene. Presumably, in these organisms MutL homologs cut the nascent strand of the DNA. Conserved endonuclease motifs were identified in these MutL. In the alignment of 1792 sequences of bacterial MutL homologs we identified five endonuclease motifs comprising the catalytic site responsible for DNA cleavage in 1390 sequences. MutL proteins possessing the endonuclease motifs have been purified only from five bacterial species. Mechanism of searching of cleavage site in DNA by MutL is still unclear. We have purified the MutL protein from *Rhodobacter sphaeroides* (rsMutL) for the first time. We demonstrated the influence of metal ions on specificity and efficiency of DNA hydrolysis by rsMutL. The protein showed the highest activity in the presence of Mn^{2+}. The extent of plasmid DNA hydrolysis declined in the row $Mn^{2+} > Co^{2+} > Mg^{2+} > Cd^{2+}$; Ni^{2+} and Ca^{2+} did not activate rsMutL. Zn^{2+} inhibited rsMutL endonuclease activity in the presence of Mn^{2+} excess. Apparently, Zn^{2+} is not involved in DNA cleavage by rsMutL and could perform a structural function. Mn^{2+} and Mg^{2+} together induced double strand break followed by an increase in the amount of linear DNA form that could be a result of rsMutL second subunit activation. Analysis of amino acid sequences and biochemical properties of studied bacterial MutL homologs with endonuclease activity revealed that rsMutL is similar the MutL proteins from *N. gonorrhoeae* and *P. aeruginosa*. The work was funded by RFBR grant 16-04-00575 and RSF grant 16-14-10319.

P.18-017-Tue
Characterization of membrane complexes involved in CFTR stabilization by EPAC1 activation

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Cystic Fibrosis is caused by mutations in the gene encoding the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) protein, a cAMP-regulated chloride channel expressed at the apical surface of epithelial cells. At the plasma membrane (PM), CFTR membrane stability is regulated by cAMP through activation of the exchange protein directly activated by cAMP 1 (EPAC1), a process that involves binding to the PDZ-domain protein NHERF1. However, the mechanism by which EPAC1 interacts with NHERF1 and consequently regulates CFTR is still poorly understood. Here, we aim at identifying the proteins involved in CFTR stabilization at the PM under EPAC1 activation to identify putative therapeutic targets to CFTR PM lifetime. Methods: Cystic Fibrosis Bronchial Epithelial (CFBE) cells expressing wt-CFTR were treated with the adenylyl cyclase activator forskolin or with the specific EPAC1 agonist 007-AM. CFTR Immunoprecipitation followed by NanoLC-Triple TOF was performed and proteins showing differential interactions were selected for validation. Specific siRNAs for 19 genes were used to determine their impact in CFTR trafficking. Results and Discussion: More than 1000 interacting proteins were identified. The largest number of specific CFTR interactors was detected in cells treated with the EPAC1 agonist (>100 proteins) with 15% corresponding to proteins involved in cytoskeleton regulation. Several of these interactors were not previously directly associated with CFTR. Knock-down of 12 out of the 19 genes selected for validation impacted significantly on the efficiency of CFTR trafficking to the PM. Further validation and characterization of such CFTR interactors will clarify their mechanism of action and contribute to the identification of targets to specifically increase CFTR PM stability. Work supported by PEst-OE/BIA/UI4046/2011 – BioISI (from FCT – Portugal). JD Santos supported by: PD/BD/106084/2015 (from FCT – Portugal).

P.18-018-Wed
Mapping of plasma membrane proteins interacting with Arabidopsis flotillin-like protein 2

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Flotillins form a group of SPFH (stomatin/prohibitin/flotillin/HflK/C) domain containing proteins localized in plasma membrane microdomains, which act as signalling hub mediating the cell response to stress. Such microdomains and flotillin homologues were detected also in plants. Moreover, the similarity of plant and animal flotillins, whose function in signalling pathways most likely lies in the affection of other proteins via protein-protein interaction, suggests the similar role of plant flotillins. However, the mechanisms of plant response to various stress cues differ from those in metazoans. Therefore, the determination of

plant flotillins interacting partners could contribute to the revelation of their role in signalling pathways and deepen knowledge of plants defence. To provide the reliable determination of plasma membrane proteins interacting with *A. thaliana* flotillin-like protein 2, we used approach based on the immunoaffinity purification of enriched plasma membrane proteins with mass spectrometry detection. Hereby, the proteins playing role in the plant response to various biotic and abiotic stress factors were identified as potential interacting partners of flotillin-like protein 2. Additionally, the employment of split-ubiquitin yeast two hybrid system helped us to confirm that proteins such as plasma membrane-type ATPase 1 (AHA1), harpin-induced protein-like (NHL3), hypersensitive-induced response protein 3 (HIR3) or probable aquaporin PIP2-6 (PIP2-6) interacts directly with flotillin-like protein 2. Those proteins have already been connected with the mediation of the response to bacteria, viruses, oomycetes or nematodes as well as the response to water deprivation and abscisic or salicylic acid signalling.

P.18-019-Mon
Cancer metabolism and the human hexokinase 2 as an anticancer target

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Cancer utilizes glucose at elevated levels to support its growth and proliferation, historically known as " Warburg effect". Targeting glucose metabolism in cancer cells to limit its growth will enhance patients' survival rate. Hexokinase catalyzes glucose phosphorylation, and is a major step in regulation of glycolysis. One of four isozymes found in humans, hexokinase 2 (HK2), the first enzyme of the glycolytic pathway, catalyzes the phosphorylation of glucose for its activation and further metabolism. Moreover, gene expression profiling experiments of different types of cancer showed high expression levels of HK2, and various biological studies highlighted the importance of HK2 in tumor metastasis making it an ideal target to characterize cancer metabolism and for the development of new class of cancer therapeutics. The crystal structure of human HK2 was determined in complex with glucose and glucose-6-phosphate (PDB code: 2NZT), where it is a homodimer with catalytically active N- and C-terminal domains linked by a seven-turn helix. Through biochemical and biophysical characterization of HK2, we found that the N-terminal domain not only catalyzes a reaction but it thermodynamically stabilizes the entire enzyme, where deletion of the N-terminal helix altered the stability and catalytic activity of the full-length enzyme. Also, conformation of the N-terminal active site but not C-terminal is important in stabilizing the enzyme. Understanding the structural and molecular mechanisms of human HK2 in cancer metabolism and apoptosis will accelerate the design and development of new class of cancer therapeutics.

P.18-020-Tue
Structural properties of a C1q-like domain of otolin-1 from human and zebrafish

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Otolin-1 is a collagen-like protein expressed in the inner ear of vertebrates. It provides an organic scaffold for otoliths of fish and otoconia of land vertebrates, which are calcium carbonate biominerals involved in sensing of balance. Collagenous fibers and globular heads of otolin-1 may bind other proteins from biomineral matrix, possibly enabling the spatiotemporal control of biomineralization. Despite that, little is known about the structure of otolin-1 and its globular C1q-like domain. We developed expression and purification procedures of C1q-like domain of otolin-1 from human and zebrafish and examined their structures. The results show that the C1q-like domain forms trimers in solution in the presence of calcium ions. Without calcium ions, C1q-like domain exists in a monomer-trimer equilibrium with high association and dissociation rates. Calcium ions strongly influenced the secondary structure and stability of the proteins. The human variant was particularly sensitive to the calcium ions. Fluorescent properties of the C1q-like domain indicate that the protein has hydrophobic regions exposed to the solvent, which may participate in binding of macromolecular ligands from biomineral matrix. These observations indicate that C1q-like domain may facilitate formation of otolin-1 trimers. Influence of calcium ions on the structure and stability of C1q-like domain indicates the importance of the C1q-like domain for the assembly of the organic matrix of otoliths and otoconia. Destabilisation of the organic matrix induced by changes resulting from aging or disease may facilitate dissolution and release of otoconia and contribute to benign paroxysmal positional vertigo (BPPV). This project was funded by the National Science Centre (Poland) grants UMO-2015/19/B/ST10/02148 and DEC-2012/06/M/ST4/00036, and a statutory activity subsidy from the Polish Ministry of Science and High Education for the Faculty of Chemistry of Wroclaw University of Science and Technology.

P.18-021-Wed
Amyloid properties of the *Escherichia coli* YghJ protein

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Amyloids are protein fibrils with a characteristic spatial structure. In previous studies, using a method for the proteomic screening and identification of amyloids, we identified 61 proteins of *Escherichia coli* that formed detergent-resistant aggregates *in vivo* and without overproduction. Among these proteins, YghJ was the most enriched with bioinformatically predicted amyloidogenic regions. YghJ is a lipoprotein with the zinc metalloprotease M60-like domain ($YghJ_M$) that is involved in the mucin degradation in the intestine as well as in proinflammatory responses. We detected detergent-resistant aggregates of $YghJ_M$ by SDS-PAGE and SDD-AGE and confirmed that these aggregates are resistant to α-chymotrypsin. Next, we demonstrated that the $YghJ_M$ aggregates bind Thioflavin-T amyloid-specific dye and exhibit CD-spectra typical for protein aggregates rich in β-sheets. The Congo red stained $YghJ_M$ fibrils demonstrated bright apple-green birefringence which is considered to be the "gold standard" for verification of the amyloid structure. Finally, we showed that $YghJ_M$ forms amyloid fibrils on the surface of the *Escherichia coli* cells using the curli-dependent amyloid generator system (C-DAG). Overall, we demonstrated that $YghJ_M$ forms amyloid fibrils *in vitro* and *in vivo*. Our data on the amyloid properties of the YghJ protein contribute to the knowledge of bacterial amyloids and is useful for investigations of the relationship between amyloidogenesis and virulence of bacteria. This study was

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P.18-022-Mon
Lipids inhibit aggregation of α-synuclein

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α-Synuclein (aSyn) is a small neuronal protein that exists in equilibrium between unstructured cytosolic and helical membrane bound forms. In neurons affected by Parkinson's disease aSyn can aggregate forming pathological amyloid fibrils composed of thousands of monomers. aSyn fibril formation is an autocatalytic multistage process. Slow spontaneous formation of first fibrils (lag phase) is followed by fast autocatalytic fibril elongation by binding monomers to the ends (elongation phase). Interaction of aSyn with lipid membranes can inhibit fibril formation. We aimed to investigate the role of lipids in aSyn fibril formation studying separately the influence on lag phase and on elongation phase. We measured aSyn fibrillization kinetics in the presence of various concentration of model membranes (vesicles) quantifying amount of formed fibrils using Thioflavin T fluorescence. To measure the fibril elongation rate selectively, we added to solution some amount of pre-formed short aSyn fibrils serving as growth centers. We found that decrease in fibril elongation rate in the presence of lipids correlates with the fraction of membrane-bound aSyn monomer. It indicates that lipids inhibit fibril elongation by depletion aSyn monomer from solution. The influence of lipids on the initial fibril formation (lag phase) was much stronger. It was observed at lipid concentrations not sufficient for binding significant fraction of aSyn monomer. So we propose the model where lipids can preferentially bind rare intermediate species (oligomers) in primary nucleation process.

P.18-023-Tue
Effect of chromate(VI), magnesium and calcium on the proteome of *Arthrobacter globiformis 151B*

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In our study, we investigated bacterial cell culture of *Arthrobacter globiformis 151B* isolated from a most contaminated site of Georgia (Kazreti). According to our previous research, *Arthrobacter* species are characterised with chromium(VI) reduction capability from Cr(VI) to Cr(III). Fe and Mg are among the most abundant elements in earth's crust and play an important role in all living organisms. Natural habitats are often characterized by the coexistence of Cr, Mg and Fe. *A. globiformis* 151B cells were grown in a TS Broth medium, containing up to 50 ug/ml Cr(VI), Mg and Ca. The exposure of bacterial cells in metal containing growth medium continued up to 40 h (late stationary phase). The metal accumulation ability of bacteria and the influence of Mg^{2+}, Ca^{2+} ions, on the reduction process of Cr^{6+} was determined by atomic absorption spectroscopy. According to the same experimental design we have investigated the influence of Cr(VI), Mg, Ca ions on bacterial proteome changes by 2-D electrophoresis. Differentially expressed bands were identified by Mass-Spectrometry. Differentially expressed proteins included: Carboxy muconolactone decarboxylase, DNA-directed RNA polymerase subunit beta, Capsular glucan synthase, Aldehyde dehydrogenase, ABC transporter, ABC transporter substrate-binding protein, FAD-linked oxidoreductase and many others. This work was supported by Shota Rustaveli National Science Foundation (SRNSF) FR/218 018/16 project title: "The influence of different metal ions on the Cr(VI) reduction process ongoing in bacteria such as Arthrobacter species".

P.18-024-Wed
Deepening into the modulation of human D-amino acid oxidase

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Human D-amino acid oxidase (hDAAO, EC 1.4.3.3) is a peroxisomal FAD-dependent enzyme that catalyzes the degradation of D-amino acids, taking part into different physiological processes: in the brain, the favorite substrate is D-serine (D-Ser), the main endogenous co-agonist of the N-methyl-D-aspartate receptors (NMDAr). Converging lines of evidence suggest that a dysregulation in processes tuning D-Ser concentration, and thus in NMDAr transmission, is involved in the mechanism of predisposition in various pathologies. The evidence of the important role played by hDAAO in modulating D-Ser levels has resulted in an increased interest for this flavoenzyme. Although its structural and biochemical properties have been extensively investigated, several aspects in the modulation of the enzymatic activity and stability remain elusive. hDAAO is known to be regulated by small size ligands and cofactor binding as well as by the interaction with the proteins pLG72 and bassoon. Furthermore, hDAAO has been proposed to be regulated by nitrosylation and phosphorylation [NetPhos 2.0 server], but the consequences of these modifications on the enzyme's properties were poorly investigated. Moreover, it has been suggested that the flavoenzyme could be mistargeted (to cytosol and nuclei) or secreted. Here, we investigated hDAAO post-translational modifications (PTMs) in several tissue lysates by immunoprecipitation and Western blot analysis. Moreover, the enzyme modifications and their effects on the structural and functional properties of hDAAO were studied *in vitro* using the recombinant enzyme. Finally, hDAAO PTMs were also investigated by cellular studies. The identification and the comprehension of the mechanisms of hDAAO modulation might allow the identification of new targets for the treatment of pathologies in which this flavoenzyme is involved.

P.18-025-Mon
β-sheet breaker NABi reduces Aβ aggregates and prevents neuronal cell death

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Amyloid beta (Aβ) aggregates are an important therapeutic target for Alzheimer's disease (AD), a fatal neurodegenerative disease. Previously, we found that intracellular Aβ specifically

interacts with misfolded mutant Cu/Zn superoxide dismutase (SOD1). Based on the property that Aβ aggregates have the cross-β-structure, a common structural feature in amyloids, we designed an Aβ-aggregation inhibitor and identified NABi (Natural Aβ Binder and Aβ-aggregation inhibitor) composed of β2-3 strands, a novel breaker of Aβ aggregation, which does not self-aggregate and has no cytotoxicity at all. The NABi blocks Aβ-fibril formation in vitro and prevents neuronal cell death, a hallmark of AD pathogenesis. Experiments of whether this effect works in AD model mice are on progress. Various mutant peptides will be investigated for optimization of effective peptide sequences. This study will provide molecular insights into the design of amyloidogenic inhibitors to cure AD.

P.18-026-Tue
Engineering of enzyme gating driven by molecular dynamics, transient kinetics, and single molecule spectroscopy

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Enzymes are efficient biocatalysts with outstanding specificity and selectivity. Enzymatic reactions take place in the active sites, frequently hidden inside the protein core. The active sites are connected to the bulk solvent through one or more transport tunnels. In some cases, tunnels link two or more active sites within the same enzyme molecule. Beyond classical complementarity between the substrate and the binding site (Fisher's lock and key model), tunnels offer another level of resolution for the molecules entering and leaving the enzyme active cavity. The tunnels discriminate preferred substrates or cofactors, reduce the access of solvents which may disturb chemical reactions, or prevent the escape of reactive intermediates, potentially harmful to the cells. In orchestration of enzymatic reactions that commonly require a precise order of several individual steps, the character of transport tunnels and their dynamical behavior can play a key role in the catalytic function [1]. Dynamical tunnel gates adopting the open or closed states have been described for a large number of enzymes [2]. The presented work is focused on the analysis of conformational dynamics of a molecular gate introduced by protein engineering [3]. The molecular modeling provided the design and a molecular picture of the novel protein variants, the single molecule spectroscopy enabled the direct experimental observation of the introduced molecular gating in excellent quantitative agreement with the rate constants obtained by transient kinetics. The introduction of a molecular gate resulted in the most catalytically efficient variant ever reported within the enzyme family of haloalkane dehalogenases. Designing of dynamical elements in proteins is a new and highly challenging area of protein engineering research.

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P.18-027-Wed
N-terminal acetylation of actin by NAA80 impacts cell migration

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N-terminal acetylation (Nt-acetylation) is emerging as a widespread and essential protein modification with functional roles in countless cellular activities. Depending on the targeted protein, the Nt-acetyl group may have a substantial impact on the regulation of protein lifetime, protein-protein interaction or protein folding. Actin is known to be Nt-acetylated, however the functional consequences of this modification have remained unexplored due to the unknown identity of the N-terminal acetyltransferase (NAT) that catalyses the transfer of an acetyl group onto actin's N-terminus. We have established NAA80 (Nα-acetyltransferase 80) as actin's NAT and demonstrate that Nt-acetylation of actin plays crucial roles in cytoskeleton function in cells and *in vitro*. NAA80 knockout cells display accelerated cell migration along with altered cytoskeletal organization such as increased formation of filopodia and lamellipodia as well as an increased ratio of filamentous to globular actin. Together, the results reveal a crucial role for Nt-acetylation of actin in cytoskeletal control with impact on cellular motility.

P.18-028-Mon
Understanding the interaction between main *Bothrops jararaca* venom serinoproteases and serinoproteases inhibitors, using surface plasmon resonance and affinity chromatography data

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Serine proteases are one of the main components of the *Bothrops jararaca* snake venom (BJSP), with a key role in the physiopathology observed in the *Bothrops* envenomation. These enzymes have a high specificity by binding to and cleaving different biomolecules, and are an interesting target for inhibitors that could modulate their functions. Here we address the possible correlation between the results of the interaction of distinct BJSP with two inhibitors of serine proteases, viz, sun flower trypsin inhibitor (SFTI) and p-aminobenzamidine (PAB), as determined by affinity chromatography and surface plasmon resonance (SPR), to reveal suitable inhibitors for each of these enzymes based on their selectivity and binding parameters. To approach this, we isolated different Nα-benzoyl-DL-arginine p-nitroanilide-hydrolyzing enzymes of the *B. jararaca* venom by a combination of affinity chromatography steps on both SFTI-agarose and PAB-agarose, followed by size exclusion chromatography on Sephacryl S100. We found that all BJSP were retained by the PAB-agarose resin but not on the SFTI-agarose resin. This is indicative of some degree of selectivity of the SFTI moiety

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towards BJSP as compared with that of other known trypsin inhibitors. The distinct BJSP preparations were then analyzed by SPR on a Biacore T200 equipment fitted with CM5 chip derivatized with SFTI and PAB. Sensorgrams obtained with the BJSP purified by affinity chromatography on SFTI-agarose showed a high affinity for SFTI inhibitors, reaching a steady state and displaying fast association (kon) and dissociation (koff) rates. Thus far we were unable to get unambiguous SPR results regarding interactions between all of the purified BJSP and PAB. Possible causes for these inconclusive results are being discussed. The overall results concerning the interactions between BJSP and SFTI provide useful insights for the development of new inhibitors of snake venom proteases.

P.18-029-Tue
Role of internal dynamics in ligand binding of the bile acid transporter protein gastrotropin

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The protein gastrotropin, also known as intestinal ileal bile acid-binding protein (I-BABP) or ileal intestinal lipid-binding protein (ILBP) or fatty acid binding protein 6 (FABP6), is a member of intracellular lipid binging binding protein family. Gastrotropin binds bile acids in the cytoplasm of intestinal cells and takes part in the enterohepatic circulation of bile salts. The protein adopts a beta clam structure, composed by two beta sheets having 10 beta strands altogether and a helical cap encompassing a helix-loop-helix motif. The protein binds glycocholate and glycochenodeoxycholate in a cooperative way. NMR investigations suggest that gastrotropin in its apo form is in slow exchange with a low-populated conformation reminiscent to the doubly-liganded holo structure. In this work we performed ensemble-based molecular dynamics simulations with and without NMR-derived experimental data (NOE distances and backbone S^2 order parameters) as restraints. The generated structural ensembles are in good agreement with NMR parameters not included in the simulations and reveal two kinds of internal motions that can be relevant for ligand binding. Extensive docking calculations as well as additional investigations in search for a structure similar to the low-populated form in exchange with the apo state allowed us to outline an initial atomic-level model on the role of internal dynamics in ligand binding in gastrotropin.

P.18-030-Wed
The relevance of ACBD3 protein in energy metabolism in various cell lines

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Acyl-CoA biding domain contain 3 (ACBD3) is a protein of Golgi apparatus which has many functions in the cell such as division of neuronal cells, in neurodegeneration, lipid homeostasis, in response of cell stress, apoptosis or in maintenance of Golgi. Moreover, ACBD3 has important role in replication of some plus RNA viruses. In steroidogenic cells ACBD3 is essential in transport of cholesterol into mitochondria. In this type of cells, ACBD3 coexists in multiprotein complex named transduceosome together with other proteins (VDAC1, TSPO, PKA regulatory subunits α (PKAR1α), ANT etc.) in the outer mitochondrial membrane. ACBD3 works as a scaffold protein of TSPO and PKAR1α. Knockdown of ACBD3 suppresses hormone-induced steroidogenesis (Li et al., 2003). In patient with homozygous mutation in ACBD3 gene, we observed different distribution of cholesterol in fibroblasts and increased and enlarged mitochondria in muscle together with decreased amount of OXPHOS complexes CI, CIII, CIV and ATP synthase. The aim of study was to characterize the impact of full absence of ACBD3 in HeLa cells. We created knockout cell lines of ACBD3 by CRISPR/CAS9 system and studied overall impact of absence of the gene on mitochondrial metabolism, using proteins and functional analysis of the OXPHOS system or by microscopy analysis. Supported by research projects: GAUK 542217, GAČR 14-36804G, RVO VFN64165/2012 and SVV 260367.

P.18-031-Mon
Structural ensembles of 3rd PDZ domain of PSD-95 describing a dynamic ligand-binding mechanism

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PDZ domain containing proteins in the postsynaptic density (PSD) of excitatory synapses play a fundamental role in synaptic plasticity. The third PDZ domain (PDZ3) of PSD-95, the most abundant scaffold protein in the PSD, has an additional alpha helix on its carboxy terminus besides the common PDZ architecture. Although this helix does not make direct contacts with the ligand, its phosphorylation/truncation is a potential regulator of ligand binding. Atomic-level details of this intermolecular allosteric process, involving conformational entropy, are not yet elucidated in detail. We performed a number of molecular dynamic simulations of different lengths incorporating NMR-derived backbone and side-chain mobility data as restraints using bound and unbound, as well as full and truncated PDZ3 structures. The obtained ensembles correspond well to experimental data and show characteristic differences near their ligand-binding regions. Extensive comparative analysis, including PCA, were used to decipher the motions and intramolecular interactions of the PDZ3 domain in its different states. Estimates of changes in conformational entropy were also calculated between the free and bound forms. Our results suggest that dynamic structural ensembles are capable of revealing atomic-level insights into the mechanistic action of PDZ domains beyond what can be deciphered based merely on the experimental observations.

P.18-032-Tue
Interactions of 17beta-hydroxysteroid dehydrogenase type 10 and cyclophilin D in Alzheimer disease

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Nucleus-encoded mitochondrial matrix protein 17beta-hydroxysteroid dehydrogenase type 10 (17beta-HSD10) binds to amyloid beta (Abeta). In people with Alzheimer disease (AD), 17beta-HSD10 brain overexpression was observed. It is assumed that cytosolic 17beta-HSD10 is imported into mitochondrial matrix where binds to cyclophilin D (CypD) and, by preventing its translocation to the inner mitochondrial membrane, regulates the

opening of the mitochondrial permeability transition pore mediated by CypD. Mitochondrial Abeta could influence the regulation of CypD by 17beta-HSD10. In this study, we proved the interaction between 17beta-HSD10 and CypD by using the surface plasmon resonance (SPR) biosensor method in which 17-beta-HSD10 immobilized on the surface of SPR sensor was interacting with CypD in solution. The formation of 17beta-HSD10 – CypD complex was further confirmed by indirect approach in which the 17beta-HSD10 – CypD complex previously formed in solution was determined by second antibody on the surface of SPR sensor. Then we evaluated the ability of 17-beta-HSD10 to regulate CypD in brain mitochondria isolated from 11-month old transgenic McGill-R-Thy1-APP rats, one of animal models of AD with accumulated Abeta. We observed no changes (levels of 17beta-HSD10, CypD, Abeta 1-42 and 17beta-HSD10 – Abeta complexes), the significant increase (Abeta) and the significant drop (17beta-HSD10 – CypD complexes) in a comparison with wild-type rats. We also estimated levels of 17-beta-HSD10 – Parkin complexes in cerebrospinal fluid of AD people but we did not find marked alterations compared to controls. Our results suggest that up-regulation of 17beta-HSD10 in AD does not have to be followed by its increased transport into mitochondrial matrix *via* the PINK1-Parkin-TOM/TIM pathway. Moreover, the ability of 17beta-HSD10 to regulate CypD in mitochondrial matrix, under conditions of accumulated Abeta, is weakened. Supported by GACR (P304-12-G069) and AZV (16-27611A) projects.

P.18-033-Wed
Yeast stress granule protein Pub1 is involved in translation termination and detoxification of the [*PSI*$^+$] prion

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Termination of translation in eukaryotes is controlled by interacting polypeptide chain release factors, eRF1 and eRF3 (known as Sup45 and Sup35 in yeast, respectively) and the ABCE1 (Rli1 in yeast) protein facilitating ribosome recycling. Here we describe a novel participant involved in termination. We show that Pub1, a yeast protein known to be involved in stress granule formation, regulation of gene expression, and organization of the tubulin cytoskeleton, also plays a role in translation termination. This protein was bound to ribosomes and was preferentially associated with heavy polysomes enriched with terminating ribosomes. Besides, it interacted with the N-terminal glutamine-/asparagine-rich prion-forming domain of eRF3, though its binding to ribosomes did not depend on interaction with Sup35. Lack of Pub1 decreased efficiency of nonsense readthrough at a majority but not all tetranucleotide stop signals. This distinguishes Pub1 from most other known binding partners of the release factors which were shown to modulate readthrough of all nonsense codons uniformly. Besides its effect on translation termination, Pub1 influenced properties of [*PSI*$^+$], a prion form of Sup35. Deletion of *PUB1* (Δ*PUB1*) caused approximately a 2-fold increase of Sup35 prion polymer levels in cells containing [*PSI*$^+$]. Importantly, combining Δ*PUB1* with deletion of *UPF1*, a protein involved both in nonsense mediated mRNA decay and translation termination, but does not affect Sup35 prion polymerization, was lethal. Analysis of this effect showed that lethality was caused by excessive sequestration of Sup45 into Sup35 prion aggregates. The obtained data show that Pub1 can act as an accessory translation factor involved in fine-tuning of translation termination and as an important factor of [*PSI*$^+$] prion detoxification. The work was supported by a grant from the Russian Foundation for Basic Research (# 17-04-00032).

P.18-034-Mon
Single-molecule FRET reveals Zn(II)-dependent switch in *S. cerevisiae* Rad50 dimer's quaternary structure

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Rad50 is an internal constituent of the MRN(X) complex, a complex responsible for double-stranded DNA damage sensing and repair, as well as triggering the protein network of DNA damage response pathway. Recent studies unveiling the structure of the central part of human Rad50 shows two Rad50 protomers with tetrathiolate Zn(II)-binding site in a rod-shaped assembly, with coiled-coil segments of both protomers arranged in a parallel fashion. Our preliminary data indicate that yeast ortholog may significantly differ from human one and presents itself as a dynamic entity able to adopt two distinct dimer assemblies – open and closed, depending on the Zn:Rad50 stoichiometric ratio of the complex, namely Zn(II)-mononuclear and Zn(II)-binuclear Rad50 dimers corresponding to 1:2 and 2:2 ratio, respectively. Using tetracysteine tag and hetero-biarsenical labeling (FlAsH-ReAsH) of yeast Rad50 zinc hook domain we were able to visualize resonance energy transfer between protomers inside Rad50 dimer with single-molecule resolution. Single-molecule FRET, anisotropy decay and cross-linking experiments indicate that additional metal ion could initiate zinc-hook domain refolding from open – *P. furiosus*-like to closed – *H. sapiens*-like, assembly. Such event would have to manifest itself in global conformational change of the entire MRN(X) complex and therefore change of its functional status as well, linking together three separate physiological pathways – DNA damage response, cell cycle and redox-intertwined Zn(II) distribution. Financial support by National Science Center (grant no. 2016/23/N/NZ1/00040) is gratefully acknowledged.

P.18-035-Tue
Cracking the code of N-myristoylation at structural and genome scales

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Comprehensive characterization of the entire repertoire of a protein modification of an organism is yet unachieved. N-Myristoylation (MYR) is a crucial eukaryotic N-terminal protein modification consisting on the covalent addition of a C:14 lipid by the N-Myristoyl Transferases (NMTs) to the amino-termini of a subset of proteins displaying unmasked N-terminal glycine. This modification is involved in major biological processes influencing membrane association of peripheral membrane proteins, protein targeting to membrane subdomains, protein-protein interaction, and protein trafficking. We assembled complementary strategies to tackle the complete MYR status in proteomes. A series of crystal structures of human NMT1 in complex with

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reactive and non-reactive peptide ligands mimicking true targets reveal unforeseen binding clefts enlightening the recognition pattern of the enzyme. With this information, a 2,048 macroarray derived from genuine protein sequences was designed and processed to diagnose the MYR modification space in both humans and the model plant *Arabidopsis thaliana*. The array encompasses all positive sequences of the studied organisms and a majority of negatives. The physiological relevance of the specificity observed in MYR positives was challenged using dedicated prediction algorithms, and *in vivo* mass spectrometry (MS). This first global profiling of MYR proteins allowed us to identify more than one thousand of new, unsuspected heterogeneous targets in both organisms. Furthermore, stable isotope labeling protein N-terminal a acetylation quantitation (SILProNAQ) MS approach was used to determine the N-terminal status of proteins at the proteomic scale revealing that N-acetylation involves a minor set of overlapping targets with MYR. Finally, we observed that the sequence signature marks for a third acylation *S*-palmitoylation, tightly associated with MYR, are imprinted in the genomes allowing visual recognition of sequences exhibiting both acylations.

P.18-036-Wed
N-terminal acetylation – a prevalent protein modification that likes to surprise

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N-terminal acetylation (NTA) is among the most common protein modifications in eukaryotes. Its frequency increases with organismal complexity and reaches up to 80% in the human cytosolic proteome. In yeast, NTA is tightly linked to ubiquitin-mediated proteasome degradation, but its significance in higher eukaryotes is still enigmatic. NTA has been shown to affect protein-protein interaction, subcellular localization and stability of N-terminal a-helices in a limited number of examples. Six N-terminal acetyltransferases (NatA-NatF) catalyze the transfer of the acetyl group from acetyl-CoA to the N-terminus of cytosolic proteins in higher eukaryotes. Here, we characterize the plant NatA complex and reveal evolutionary conservation of NatA biochemical properties within higher eukaryotes. Based on the identified substrate specificity, NatA targets up to 40% of the cytosolic plant proteome. The combined application of quantitative proteomics, transcriptomics, and metabolomics on NatA depleted plants uncovers specific and essential functions of NatA for development, biosynthetic pathways, and stress responses. Hitherto, NTA was believed to be constitutive in eukaryotes. We show that NTA decreases significantly after drought stress in plants and identify the phytohormone abscisic acid as an efficient and prompt regulator of NatA abundance *in planta*. Transgenic down-regulation of NatA promotes the drought stress response program and results in strikingly drought-resistant plants. In addition, NatA controls the plant immune response by regulating the stability of the Nod-like immune receptor SNC1. We propose that imprinting of the proteome by NatA is an important, hormone-regulated switch for control of abiotic and biotic stress responses.

P.18-037-Mon
Conformation and dynamics of intrinsically disordered microtubule associated protein 2c

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Microtubule associated protein 2c (MAP2c) regulates structure and dynamics (polymerization and degradation) of microtubules in developing neurons and other cells. MAP2c is a 49 kDa intrinsically disordered protein (IDP) consisting of several structural and functional regions. The N-terminal part contains two important regions: An N-terminal region with a high content of negatively charged amino acids and a proline-rich region. The former segment contains a binding site for the regulatory subunit of cAMP-dependent protein kinase (PKA) and proposed binding site for steroids, while the latter one contains several phosphorylation sites. The second important part of MAP2c is a highly-conserved C-terminal domain that binds to microtubules. In order to investigate relation between highly dynamic structural features of MAP2c and its functions, we studied dynamics of MAP2c using nuclear magnetic resonance (NMR) relaxation and performed quantitative conformational analysis of NMR chemical shifts, small angle X-ray scattering, and paramagnetic relaxation enhancement. Pools of possible chain conformations were generated by the program Flexible-Meccano, which builds consecutively a polypeptide chain. The ensemble of structures reproducing the experimental data was selected by the program ASTEROIDS which uses a genetic algorithm. We found out that the least flexible amino acids are involved in transient long-range contacts between the acidic N-terminal domain and the microtubule-binding domain and that more ordered regions correlate with the regions of known or proposed function. Our results thus indicate importance of the N-terminal regions for the specificity of regulatory roles of MAP2c and a close relation between biological functions and conformational behavior of this protein. This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II, project CEITEC 2020 (LQ1601).

P.18-038-Tue
The N-end rule pathway of proteolysis couples polycomb repressive complex 2 to environmental sensing in angiosperms

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The polycomb repressive complex 2 (PRC2) regulates epigenetic gene repression in eukaryotes. Although many functions for PRC2 are known, mechanisms regulating its signal-responsiveness and activity are poorly understood. Here we show that the stability of the plant Su(z)12-like PRC2 component VERNALIZATION(VRN)2 is controlled by the oxygen-sensing function of the Arg/N-end rule pathway, which restricts VRN2 abundance in normoxia. We show that vernalization induces a hypoxia-like cellular state that enhances the stability of VRN2 and other oxygen-sensing proteins, and also identify novel developmental and stress-related functions for VRN2 linked to its proteolytic regulation. VRN2 was coupled to N-end rule regulation following gene-duplication and N-terminal truncation early in angiosperm

evolution, revealing a latent internal N-degron that facilitated PRC2 neo-functionalisation through coupling su(z)12 stability to environmental signals.

P.18-039-Wed
A major protein Rv0341 in the membrane of dormant *Mycobacterium tuberculosis* binds DNA and reduces the rate of RNA synthesis

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Formation of dormant *Mycobacterium tuberculosis (Mtb)* cells is responsible for the phenomenon of latent tuberculosis. Molecular mechanisms of the transition of viable cells to dormant state are not fully elucidated. Recently we have shown that gradual acidification of the external medium results in the formation of morphologically distinct dormant *Mtb* cells *in vitro*. Comparative proteomic analysis between dormant and active *Mtb* cells resulted in accumulation of unknown Rv0341 as a major protein in membrane fraction of dormant forms. Using electrophoresis in native conditions with low concentration of acrylamide and MALDI-TOF analysis we found presence of large complexes (680 kDa and greater) contained of Rv0341 protein in membrane fragment of dormant *M. tuberculosis* cells. In early stage of dormant cell reactivation, destruction of these complexes to monomer form of protein begins and in logarithmic phase large complexes of Rv0341 disappeared. *M. tuberculosis* strain with overexpression of Rv0341 (*ovRv0341*) demonstrated reduction of growth rate on solid and liquid medium and the formation of large clumps during growth. The cells of this strain were characterized by lower metabolic activity vs cells of control strain and by increased floating density showing significant changes in structure and metabolism of *ovRv0341*. The recombinant Rv0341 protein bound to pDNA in a gel mobility shift assay. This study for the first time demonstrates that Rv0341 protein accumulates in significant amount in dormant mycobacteria, can bind to DNA and possibly participates in molecular events resulted in transition of viable cells to dormancy and maintenance of cell viability for long time in non-replicative conditions. This work was financially supported by the Russian Science Foundation grant 16-15-00245 (comparative proteomic analysis between dormant and active *Mtb)* and the Russian Foundation for Basic Research grant 18-015-00239a (experiments with Rv0341).

P.18-040-Mon
Modification of protein dynamics by directed evolution based on insertions and deletions

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The common ancestral enzyme between haloalkane dehalogenase (HLD, EC 3.8.1.5) and *Renilla*-luciferin 2-monooxygenase (RLuc, EC 1.13.12.5) was resurrected *in silico* based on the sequences of modern enzymes. This ancestral enzyme dubbed AncHLD-RLuc exhibits both activities, with dehalogenase as its main activity (10x lower than LinB), luciferase as secondary promiscuous activity (10000x lower than RLuc). Comparison between crystal structures of ancestral and extant enzymes uncovered high structural similarity between these proteins. The main difference lies in the arrangement of secondary structure elements of the cap domain and in the conformation of a loop connecting the main and the cap domain. The flexibility of this region is believed to contribute to the binding of the luciferase substrate, coelenterazine. Random insertion and deletion mutagenesis was then performed on the ancestral AncHLD-RLuc to investigate whether altering the protein backbone would lead to a change in specificity. The generated variant libraries were screened and the best hits were characterized. Interestingly, variants exhibiting up to 60x increase in luciferase activity carried alterations in the specific regions where the structure of the ancestral enzyme differs the most from the structure of RLuc. High throughput molecular dynamics was used to perform adaptive sampling of the Root mean square deviation of the Cα atoms. Total simulation time was more than 4000 ns and no less than 12 epochs. Molecular dynamic simulations as well as experimental analysis based on hydrogen-deuterium exchange connected with mass spectrometry are in progress to analyze changes in the flexibility of the hot-spot regions.

P.18-041-Tue
In vivo incorporation of photoproteins into GroEL tetradecameric quaternary structure

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To study protein location in a cell and protein-protein interactions *in vivo*, it is often necessary to introduce labels into protein structures. One of the promising approaches to mark proteins *in vivo* is expression of genes encoding fusion proteins consisting of a target protein and a photoprotein (such as various fluorescent proteins and luciferases). However, when the target protein is a subunit of a complicated oligomeric protein, there arises the problem of packing the fused subunits into the native oligomeric structure. Here, we report the insertion of the GroEL subunit fused with EGFP (M.w. 29 kDa) or with *Gaussia princeps* luciferase (M.w. 20 kDa) into the GroEL tetradecameric quaternary structure. Expression of the prepared plasmids in *E. coli* cells resulted in folded GroEL subunits and folded photoproteins within the fusion proteins, but the fused subunits failed to form the tetradecameric oligomeric structure and were purified as monomeric forms. However, when these plasmids encoding fusion proteins were co-expressed with plasmids encoding only GroEL (with an expression efficiency ratio of ~1:10), practically all subunits fused with photoproteins were incorporated into the tetradecameric quaternary structure. The purified GroEL with incorporated fusion subunits was characterized by size-exclusion chromatography, absorption and luminescence spectroscopy, as well as by urea transverse gradient electrophoresis, diffuse small-angle X-ray scattering, and electron transmission microscopy. The results show that only two subunits fused with photoproteins are incorporated into the tetradecameric GroEL structure (possibly one fused subunit per one GroEL heptameric ring). Packing and stability of the GroEL quaternary structure with incorporated fusion subunits are similar to those of the wild-type version, and the photoproteins are apparently packed within the

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GroEL inner cavity. This work was supported by the Russian Science Foundation grant 14-24-00157.

P.18-042-Wed
Characterization of Nα-acetyltransferase 60 (NAA60) isoforms

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In humans, 80–90% of all proteins are acetylated at their N-termini. This major and vital protein modification is catalyzed by N-terminal acetyltransferases (NATs). As of today, there are six known human NATs, alphabetically named NatA to NatF. Most NATs exist as a complex made up of different subunits, where catalytic activity is mediated by the Nα-acetyltransferase (NAA) subunits NAA10 to NAA60. All NAAs share the evolutionarily conserved GNAT (GCN5 (general control nonderepressed 5-related *N*-acetyltransferase)-domain. In the NAT family, NatF (NAA60) is in some ways different from the others. NatF consists solely of NAA60, and no auxiliary subunits are necessary for NAT-activity. NAA60 was the first NAT to be characterized as an organellar enzyme, when it was shown to localize at the Golgi. The part of NAA60 that is required for membrane interaction is a C-terminal region that is not present in other NATs. It has been demonstrated that NAA60, as opposed to other NATs, has a preference for transmembrane substrates. Interestingly, NAA60 knockdown studies indicated an important role for NAA60 in the maintenance of Golgi-structure integrity. There are six known isoforms (isoform a to f) of NAA60, resulting from the use of alternative promotors, start codons and splicing sites, where isoform a is the Golgi-associated isoform. Two of the other isoforms, b and d, contain an intact GNAT-domain, and thus could be presumed to retain catalytic activity. The difference between isoforms a, b and d, is restricted to their N-terminal region; Isoform b has a distinct and longer N-terminus, while the N-terminus of isoform d is 65 amino acids shorter than for isoform a. All previous studies of NAA60 have focused on isoform a. In this study, we characterize isoforms b and d in terms of cellular localization, catalytic activity, and mRNA level in various cell lines and pathological tissue.

P.18-043-Mon
Putative molecular mechanisms of anti-Alzheimer's action of the Y-box binding protein 1 (YB-1)

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YB-1 is a multifunctional DNA/RNA-binding protein of vertebrates. It is involved in many cellular events, including proliferation, differentiation, and stress response. Using animal models of sporadic and hereditary Alzheimer's disease, it was shown that intranasal administration of this protein results in inhibition of plaque formation in the brain of animals, thereby preventing cognitive impairment. Here we propose some molecular mechanisms of putative YB-1 effect on progression of Alzheimer's disease (AD). A key role in the process is played by Aβ-peptide plaques accumulated in the interneuron space. Thioflavin T fluorescence and electron microscopy were used to show that addition of YB-1 to the Aβ-peptide inhibits formation of amyloid fibrils of the latter *in vitro*. The most pronounced inhibitory effect of YB-1 was observed at the initial stages of polymerization of the Aβ-peptide. Another AD symptom is the formation of intracellular tangles consisting of hyperphosphorylated tau-protein that has lost its ability to bind to microtubules, thus provoking microtubule destruction and disruption of mRNA transport along axons. We have compared the effects of YB-1 and tau-protein on tubulin polymerization *in vitro* and found that the stimulating effect of YB-1 is even higher than that of tau-protein. We believe that YB-1 can functionally replace the inactivated hyperphosphorylated tau-protein, thus maintaining microtubule integrity and the active mRNA transport in AD patients. This study was supported by RSF (# 14-14-00879) and RFBR (#18-04-00595).

P.18-044-Tue
Proteomic features of dormant *Mycobacterium tuberculosis* cells with ovoid morphology

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Mycobacterium tuberculosis (*Mtb*) may form dormant cells as a result of adaptation to stress conditions causing latent form of tuberculosis. For obtaining dormant *Mtb* cells (DC), we applied a model based on the formation of "non-culturable" ovoid cells with reduced metabolic activity and resistance to antibiotics. We characterized protein composition of DC by 2D electrophoresis and MALDI-TOFF analysis followed by densitometry spots for identification and quantification of proteins isolated from active and DC. Despite the long storage (1 year) of cells without division, they retained a certain number of intact proteins, among of which 90 proteins are not present in the proteome of active cells ("unique" proteins). Such protein stability is possibly associated with the detection of significant number of enzymes involved in protection from oxidative stress, as well as chaperones. Among of "unique dormant" proteins those that bind to DNA with the possible role of chromosome compactization (Rv0341 and Rv2986c) are found. DC also contain as "unique" proteins enzymes involved in the main synthetic pathways of glutamine, histidine, pyridine and mycothiol. In addition, there were some enzymes involved in major energy pathways. Whether the active metabolism is maintained by the enzymes found in the DC or they are "frozen" and are necessary for the reactivation process remains unclear. The absence of respiration probably indicates a second assumption. However, the possibility of some basic metabolism at a low level could not be ruled out. In particular, the DC proteome was enriched by enzymes involved in degradation processes of lipids, proteins and polysaccharides that may allow to maintain some level of metabolism during dormancy. The observed differences in DC protein composition compared to active cells apparently reflect adaptation of bacteria to the transition and maintenance of dormant state. This work was supported by Russian Science Foundation grant 16-15-00245.

P.18-045-Wed
New functions of the PCID2 protein in the cytoplasm of the *Drosophila melanogaster* cells

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Transport of mRNA from nucleus to cytoplasm is a complex process. In *Drosophila*, TREX-2 (transcription-export complex) is responsible for transfer of mRNA from transcription site to the nuclear pore. It consists of 3 subunits: Xmas-2, ENY2, and

PCID2. To date, functions of PCID2 in *Drosophila* remain unexplored. We have shown that PCID2 in nucleus and cytoplasm exists in two different forms of different sizes. We show that ubiquitinylation of the cytoplasmic form accounts for this difference in size. Using immunoprecipitation, we show that Xmas-2 and ENY2, components of TREX-2, bind only to nuclear form. We hypothesized, that PCID2 performs similar functions both in cytoplasm and nucleus, but changes its partners. Using chromatography and Western blot analysis, we purified PCID2-containing complex from embryonic cytoplasmic fraction. Using affinity purified antibodies to PCID2 and MALDI-TOF MS, we found that cytoplasmic complex contains NudC. We generated antibodies to NudC and confirmed its interaction with PCID2 by immunoprecipitation. Based on assumption that PCID2-NudC complex is involved in cytoplasmic transport of mRNA, we showed by RIP-CHIP that only PCID2 binds mRNA. Also, we found that PCID2 interacts with cytoskeleton and motor proteins, such as alpha-tubulin and dynactin, using immunoprecipitation. We found that the cytoplasmic form of PCID2 exists only in the presence of NudC. In conclusion, we found that in *Drosophila*, PCID2 protein exists in two forms, the nuclear form, ca. 41 kDa, and cytoplasmic form, ca. 45 kDa. We detected interaction of PCID2 nuclear form with ENY2 and Xmas2. We have shown that in cytoplasm, PCID2 binds mRNA, and also no longer interacts with components of TREX-2, but instead interacts with NudC, motor proteins, and cytoskeleton components, which confirms our hypothesis about its mRNA transport role in cytoplasm of *Drosophila* cells. This work was supported by grant from RAS Presidium "Molecular and Cellular Biology".

P.18-046-Mon
Identification of amyloid-forming proteins in the proteome of *Rhizobium leguminosarum*

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Amyloids represent unbranched protein fibrils characterized by highly ordered spatial structure called cross-β. The formation of amyloid fibrils is associated with development of various incurable disorders such as type-2 diabetes and Alzheimer's disease. Nevertheless, the observations of the last decades demonstrated the ability of amyloids to perform diverse physiological functions. The majority of functional amyloids of bacteria were identified within *Gammaproteobacteria* species including *Escherichia coli* and *Salmonella enteritidis*. Functional amyloids of *Gammaproteobacteria* play important roles in the biofilm formation, act as a storage of toxins and cause hypersensitive response in plants. We performed screening for novel amyloid-forming proteins in the proteome of *Rhizobium leguminosarum*, an agriculturally important species of *Alphaproteobacteria*. Using previously developed Proteomic Screening and Identification of Amyloids (PSIA) approach, we identified 52 potentially amyloidogenic proteins in the detergent-resistant fraction of *Rhizobium leguminosarum*. For further analysis, we selected the outer membrane protein, RopA, which demonstrated the highest mass-spectrometry score during identification. Using Curli-Dependent Amyloid Generator (C-DAG) system we demonstrated that bacterial cells that export RopA protein exhibit apple-green birefringence upon binding with Congo Red, and RopA protein forms fibrils on the surface of the cells *in vivo*. Taking together, we may propose that the RopA of *Rhizobium leguminosarum* is able to form amyloid fibrils. This work was supported by the Russian Science Foundation (Grant No 17-16-01100).

P.18-047-Tue
Does the novel PA2504 protein function as a scaffold polypeptide in *Pseudomonas aeruginosa* cells?

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Pseudomonas aeruginosa is an ubiquitous, Gram-negative bacterium widely spread in the environment. It can cause severe opportunistic infections, especially in immunocompromised patients. Its high ability to accommodate to new conditions and resistance to many antibiotics makes *P. aeruginosa* difficult to eradicate. Thus, there is an urgent need to search for novel targets for antibacterial therapies. *In silico* analysis of PA2504 has shown that orthologs of this protein are present in some pathogenic bacteria such as *Bacillus anthracis* and *Bacillus cereus*, but not in non-pathogenic species of the same genus. Homologs of PA2504 are present in all *P. aeruginosa* strains including highly resistant NCGM2 and DK2 strains. This led us to an assumption that PA2504 might be associated with pathogenesis. Using bacterial two hybrid system (BACTH) and pull down assays we have shown that PA2504 interacts with a number of cellular proteins including negative regulator of exotoxin A – the PtxS protein, RhlB – a protein of bacterial degradosome, the SuhB protein of the Nus complex and with the Nudix-type RNA pyrophosphohydrolase. We have also established that PA2504 forms homodimers. The NCBI Conserved Domain Database shows that PA2504 comprises of two domains: DUF2314 – found in proteins containing putative ankyrin repeats (such repeats were identified in some scaffold proteins) and DUF2185 domain. Our observations indicate that the PA2504 protein functions as a scaffold protein, which can bind different factors depending on the cell requirements. This work was supported by grant no: UMO-2014/15/B/NZ6/02562 from the National Science Center.

P.18-048-Wed
Characterization of a putative amphipathic α-helix II of the adenylate cyclase toxin from *Bordetella* pertussis

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Bordetella adenylate cyclase toxin-hemolysin (CyaA, ACT or AC-Hly) is a multifunctional protein. It translocates an adenylate cyclase (AC) enzyme domain into host cells, causing uncontrolled conversion of cytosolic ATP to cAMP and thus intoxicating the cells. The toxin also forms small cation-selective (hemolytic) channels in host cell membranes. The algorithm of Eisenberg *et al*. predicts that the pore-forming domain of CyaA contains five transmembrane amphipathic a-helices. We have examined the function of the putative α-helix II by substituting the Gly531, Ala538 and Ala546 residues with proline, histidine and glutamate residues. The Gly530 and Gly537 residues were then substituted by alanines. We show that the region containing Gly531 is important for hemolytic activity, while the region containing residues Ala538 and Ala546 is crucial for AC domain translocation. Neutral substitutions of Gly530 and Gly537 had little to no effect on toxin activities on cells. Examination of CyaA and of its mutant variants on artificial asolectin lipid membranes revealed that the overall membrane activity of the G537A, A538p and

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A546p toxin variants on artificial lipid bilayers does not correlate with their hemolytic activities on erythrocytes. The molecular basis of this intriguing phenomenon is under investigation and will be presented.

P.18-049-Mon
A yeast model for genetic variants in human NAA15 associated with autism spectrum disorder

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According to WHO 1 in 160 children has an Autism Spectrum Disorder (ASD), which is a neurodevelopmental disorder manifesting in a range of symptoms such as impaired social skills, repetitive behaviors, and restricted interests. There is a broad consensus that ASD has a strong hereditary component and extensive research efforts have been undertaken to identify genetic risk factors of ASD. We have identified and phenotypically characterized 37 individuals from 32 unrelated families with 25 different and likely gene disruption variants in *NAA15*, which is emerging as a high impact ASD gene. All subjects have variable degrees of neurodevelopmental disabilities, including intellectual disability, delayed speech and motor milestones, and ASD. In most cases the mutation occurred *de novo*, while familial inheritance was observed in three families. The *NAA15* gene encodes the auxiliary subunit of a protein complex called NatA, which N-terminally acetylates proteins while they are being synthesized. To gain an insight into the genotype to phenotype relationships in ASD we turned to the budding yeast *Saccharomyces cerevisiae*. Functional assays in yeast confirmed a deleterious effect for two of the truncating variants in *NAA15*. We propose that defects in NatA-mediated protein N-terminal acetylation lead to variable levels of neurodevelopmental disorders in humans, supporting the importance of NatA activity in normal human development. This work gives new insight into the heredity of ASD and may lead to better treatment of patients with neurodegenerative disorders.

P.18-050-Tue
Crystal structures of the complex of kallikrein inhibitor BbKI with trypsin and a comparison of its inhibitory properties for various kallikreins

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Crystal structures of a recombinant Kunitz-type serine protease inhibitor from *Bauhinia bauhinioides* (BbKI) complexed with bovine trypsin were determined in two crystal forms. The crystal structure of a single-site mutant of BbKI (L55R) was determined in two space groups, $P6_4$ and in the monoclinic space group $P2_1$, at resolution 1.94 A and 3.95 A, respectively. Additionally, the structure of the native BbKI complexed with trypsin was determined at 2.0 A resolution in the isomorphous $P6_4$ form. Since BbKI has been previously found to be a potent inhibitor of plasma kallikrein, it was tested against several tissue kallikreins as well. We determined that BbKI is a potent inhibitor of the chymotrypsin-like human tissue kallikrein 7 (KLK-7). We have built models of its complex with the catalytic domain of human plasma kallikrein, as well as KLK-7, and analyzed the interactions responsible for its potency. Inhibition properties of BbKI towards other tested tissue kallikreins were rationalized in structural terms.

P.18-051-Wed
The loss of the tertiary structure of fibrinogen induced by the external factors

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Fibrinogen is a blood plasma protein responsible for blood coagulation. Its denaturation may be utilized in biomedical applications, e.g., for development of bioactive matrixes to favour hemostasis or to resist protein adsorption. In this work, using high resolution atomic force microscopy (AFM) we have investigated unfolding of single fibrinogen molecules induced by their heating and adsorption on highly oriented pyrolytic graphite (HOPG) surface modified with oligoglycine-hydrocarbon graphite modifier (GM). The morphology of heat denatured fibrinogen molecules was significantly different not only from their trinodular nativelike structure but also from the morphology of fibrinogen molecules unfolded by extended contact with a GM-HOPG surface. Thermal denaturation has led to formation of globular structures surrounded by short fibrils. Surface induced denaturation has resulted in appearance of fibrillar structures that resembled organization of the nativelike protein molecule. Moreover, a lot of fibrils have been formed after extended adsorption of heated molecules onto a GM- HOPG surface. The obtained results provide better understanding of fibrinogen denaturation at a single molecule level that may be important in biotechnological applications. Moreover, GM-HOPG surface may be used for AFM investigation of fibrinogen unfolding in real time. The authors acknowledge funding from the Russian Science Foundation [17-75-30064 to D.V.K.].

P.18-052-Mon
Cytoprotective effects of hydrogen sulfide and its influence on proteasome activity

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Active forms of sulfur (APS), in particular, hydrogen sulfide H_2S, display cytoprotective properties and generally play important role in maintaining of cellular homeostasis. APS mechanisms of action, leading to the adaptation of cells to unfavorable conditions, are currently under intense investigation. Lipopolysaccharide (LPS) is well-known inducer of inflammation and oxidative stress. To better understand how APS help to preserve homeostasis and decrease inflammatory response, we investigated effects of GYY4137, a novel slow H_2S-releasing molecule, on neuroblastoma SH-SY5Y cells alone and in combination with LPS treatment. We have shown that 100 and 200 μM of GYY4137 if added prior, or after LPS treatment, reduce reactive oxygen species production by 35% in SH-SY5Y cells. Moreover, TNFα production by LPS-stimulated neuroblastoma cells was also decreased from 3 to 6 times in the presence of GYY4137. Furthermore, it was previously demonstrated that APS affect ubiquitin proteasome system (UPS), which is responsible for the degradation of most intracellular proteins. It has been demonstrated that H_2S stimulate proteasome expression and activity and it is one of the mechanisms of cellular homeo/proteostasis maintenance. With this in mind, we tested if GYY4137 treatment influences proteasome activity. Surprisingly, we revealed that in the presence of GYY4137 chymotrypsin-like and caspase-like proteasome activities were decreased by up to 30%, while the amount of proteasomes was unchanged. Interestingly, no immunoproteasome subunit expression was detected in the studied cell line irrespective of LPS or GYY4137 presence. Taken together, our data indicate that observed cytoprotective effects of H_2S and its influence on the UPS are more complex and need to be further investigated in detail. The work was supported by Russian Science Foundation grant 17-74-30030 and by the Program of fundamental research for state academies for 2013–2020 years (№ 01201363822).

P.18-053-Tue
Nur77 phosphorylation by CK2α regulates its stability and cytokine production

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Nur77, also known as NGFI-B, TR3, or NR4A1, was the first member of the NR4A family to be identified as a gene induced by NGF. Nur77 is an immediate early response gene and plays a critical role in cellular processes in response to diverse stimuli including cytokines, stress, and apoptotic signals. Nur77 has been implicated in a many pathological processes, including cancer, immune alterations and metabolic or neurological diseases. Here we show that CK2α binds to and phosphorylates a serine 154 residue on Nur77. CK2α-mediated phosphorylation stimulates Nur77 degradation through ubiquitin-dependent proteasomal mechanism, thereby reducing Nur77 protein stability, which accelerates IL-6 production. Conversely, the inhibition of CK2α or introduction of CK2α inactive mutant into cells reduces Nur77 degradation and IL-6 production. Taken together, these results suggest that Ser154 phosphorylation of Nur77 by CK2α play an important role in its stability and cytokine level.

P.18-054-Wed
CalFitter: A web server for analysis of protein thermal denaturation data

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Principles that govern protein folding and unfolding have been explored by scientists for more than 50 years. Despite the great advances in experimental and *in silico* techniques that are used to study protein folding on a single molecule level, the analysis of experimental data generated by "low resolution" techniques such as calorimetry or spectroscopy is often limited to relatively simple models. Currently, only a few data analysis tools are available for specific types of experiments, often with a limited selection of unfolding models. To address this, we have developed the CalFitter software and corresponding web server which serve as unified platforms for comprehensive analysis and fitting of data from protein thermal denaturation experiments. The input includes data measured by differential scanning calorimetry, spectroscopic techniques (fluorescence, circular dichroism, absorbance) and temperature jump (kinetics). The server allows upload of data in various formats, data pre-treatment, visualization and simultaneous global fitting to one of the twelve models of protein unfolding that are available, including reversible and irreversible transitions and their combination. Data fitting provides quantitative description of unfolding pathway in terms of the number of steps, energy parameters with confidence intervals and statistical information that enables comparison between different models. The server offers interactive and easy-to-use interface that allows users to directly analyse input datasets and simulate expected signal based on the model parameters. The results of the analysis can be exported in well-defined format. CalFitter web server is freely available at https://loschmidt.chemi.muni.cz/calfitter/.

P.18-056-Tue
Extending the N-end rule concept for Gly-starting proteins from protein half-life to subcellular compartmentalization

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The N-end relies the nature of the N-terminal amino acid to protein half-life. For the past three decades, a comprehensive number of achievements has allowed to reveal the various pathways describing how 19 out of the 20 natural amino acids do contribute to the N-end rule. Glycine is the last residue for which a related mechanism has not been yet identified. Although N-terminal acetylation (NTA) is now recognized as an important feature

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related to the N-end rule, Gly is not found acetylated (NTAed) in yeast, remaining as a result with a free N-terminus (FNT). In addition, Gly appears to be unique among other amino acids as it lacks a side-chain, and may undergo N-Myristoylation (MYR). MYR corresponds to a C:14:0 lipid modification used by a number of proteins all starting with Gly. MYR acts as an anchor targeting the modified protein to various cell compartments, including plasma membrane, ER-Golgi and mitochondrion. We have characterized the modification state of this N-terminal residue in both plants and human proteomes by focusing on all protein isoforms starting with a Glycine. This set defines the Gly-ome. We show first that MYR involves as much as 20% of all proteoforms starting with an N-terminal Gly while acetylation is found on 30% of the proteins belonging to the Gly-ome. Interestingly, 10–20% of the proteins of the Gly-ome is made of proteoforms displaying mixed modifications at their respective amino termini (MYR/NTA, NTA/FNT, MYR/FNT or MYR/FNT/NTA). Next we carried out quantitative analysis of plant cell accumulation of the proteoforms of the Gly-ome in plant. This analysis revealed that proteins with NTA accumulate significantly more than those featuring MYR or FNT. Together our data suggest that the acylation state of an N-terminal Glycine residue plays not only a role in cell compartmentalization of the protein targets but also in protein half-life, resulting in differential accumulation.

P.18-057-Wed
The effect of chemical chaperones on aggregation of glutamate dehydrogenase and alpha-lactalbumin

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Test systems based on thermal and dithiothreitol (DTT)-induced aggregation of proteins are widely used for the estimation of the antiaggregation activity of various low molecular compounds called chemical chaperones. In this work DTT-induced aggregation of bovine alpha-lactalbumin (α-LA) and thermal aggregation of bovine liver glutamate dehydrogenase (GDH) from bovine liver were studied using the combination of dynamic light scattering and asymmetric flow field-flow fractionation. The data on the aggregation kinetics were analyzed using the quantitative approaches and aggregation pathway graphs proposed in our earlier works [1, 2]. It was shown that in the case of heat-induced aggregation of GDH (0.1 M Na-phosphate buffer, pH 7.6, 40°C and 50°C) arginine and its derivatives (argininamide and arginine ethyl ester) acted as aggregation suppressors. The antiaggregation effect of these chemical chaperones was more prominent at 50°C, with argininamide and arginine ethyl ester having the higher antiaggregation activity than arginine at both temperatures. In the case of DTT-induced α-LA aggregation (0.1 M Na-phosphate buffer, pH 6.8, 20 mM DTT, 37°C) polyamines (putrescine and spermidine) had a dual effect on the test system. Relatively low concentrations (up to 50 mM spermidine and 100 mM putrescine) caused the enhancement of aggregation by promoting the sticking of aggregates. The increase in the polyamines concentration suppressed protein aggregation. This study was funded by Russian Science Foundation (grant number 16-14-10055).

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P.18-058-Mon
Effect of pH on DNA binding and catalysis of human apurinic/apyrimidinic endonuclease 1

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Human apurinic/apyrimidinic endonuclease 1 (APE1) is a key participant in the cascade of base excision repair reactions. APE1 initiates the search and repair of such DNA damage, as AP-sites, which can lead not only to the cytotoxic effect, but also to mutagenesis, if left unrepaired. It is known from X-ray diffraction data that enzyme amino acid residues interact preferentially with one of the duplex strands to form usually hydrogen bonds and electrostatic contacts. The enzyme active site is formed by a large number of polar amino acids, which provide extensive contact of the DNA binding site with the phosphate groups of the DNA substrate. The phosphate residue located on the 5′-side of the AP-site is directly coordinated by amino acid residues Asn-174, Asn-212, and His-309. The catalytic reaction begins with an attack of the water molecule, presumably coordinated by Asp-210. Alternative mechanisms suggest that Tyr-171 in the phenolate form attacks the scissile phosphate or His-309 as the general base, generating the attacking nucleophile. In this case, the carboxyl group Asp-283 redistributes the electron density on the imidazole ring of histidine to stabilize the positive charge. Therefore, the aim of the present work was to elucidate the influence of pH on the efficiency of formation of the enzyme-substrate complex and catalytic reaction. Pre-steady-state kinetic analysis of mutual conformational changes of the enzyme and DNA-substrates in the course of their interactions was performed by the stopped-flow technique. It was shown that the activity of APE1 increases with increasing pH due to acceleration of the rate of catalytic complex formation as well as the rate of catalytic reaction. This work was supported by grant from the Russian Foundation for Basic Research (16-04-00037). The part of the work with FRET detection combined with stopped-flow kinetics was specifically funded by Russian Science Foundation grant 16-14-10038.

P.18-059-Tue
Impact of Rad50 phosphorylation in zinc hook domain on its stability and global architecture

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The Rad50 protein is a part of conserved Mre11-Rad50-Nbs1 complex (MRN) – required e.g. in initial DNA double-stranded breaks response pathway or DNA recombination. The Rad50 protein consists of two ATP-ase globular domains separated by coiled coils elements and zinc hook domain at the apex. Our structural study of the central part of human Rad50 protein shows two coiled-coil protomers in a rod-shaped arrangement with interprotein zinc hook. The role of the zinc hook is to connect two Rad50 monomers together, promoting the activation of ATM upon ATP binding in Rad50 catalytic domains. It has been shown that phosphorylation of Rad50 at S635 by ATM in response to DNA damage is essential in regulating downstream signaling. High throughput analysis has identified another phosphorylation site at T690 in human Rad50 protein. Our crystal structure of the fragment of human Rad50 showed that both phosphorylated residues are located at major loops constituting coiled-coil extrusions facing outwards hydrophobic core of the

protomer. However, our knowledge regarding the function of the second site remains unknown. Additionally, the mechanism of structural impact of S635 and T690 phosphorylation on Rad50 structure and global conformation of MRN complex is also elusive. In this study we produced a central 182-aa fragment of human Rad50 with phosphomimetic mutation T690E residue which was subjected to UV-Vis spectroscopy and spectropolarimetric studies with Zn(II) in order to determine the stability of non- and phosphomimetic states. N-terminally fluorescently labeled proteins, were subjected to examine conformational changes of Zn(II) complex under phosphorylated and dephosphorylated states. Our study indicates that phosphorylation of Rad50 at T690 decreases affinity of zinc hook to Zn(II) ion and promotes major conformational change in coiled coil region in the homodimer. This work was supported by the National Science Center of Poland under Opus grant no. 2014/13/B/NZ1/00935.

P.18-060-Wed
Effect of crowding on oligomeric state of sHsps at elevated temperatures

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Protein aggregation is a universal and unfavorable process for all cells leading to the production of non-native protein structures. It is known that protein aggregates are a hallmark of an increasing number of human diseases including neurodegenerative disorders. Small heat shock proteins (sHsps), as a class of molecular chaperones, form a large family of ubiquitous proteins, which act to prevent protein aggregation. As a rule, sHsps tend to form highly dynamic assemblies of different size and composition, which exchange subunits constantly. It is supposed that the polydispersity and quaternary structure dynamics play an important role in cellular sHsp chaperone function. The detailed mechanism of sHsps chaperone function remains debatable; however, it is often supposed that the large assembly of sHsps undergo reversible dissociation followed by interaction with unfolded proteins and subsequent reassociation to large chaperone-substrate complexes. Unfortunately, there are no data on oligomeric states of sHsps collected either directly in vivo or under conditions realistically mimicking the cell interior. Here, we present a few studies on assembly/disassembly and oligomeric distributions of several sHsps at elevated temperatures in vitro in the presence of agents that mimic crowded conditions. We showed by analytical ultracentrifugation that α-crystallin and αB-crystallin dissociated at elevated temperatures (40 and 48°C) in dilute buffer solutions. However, under crowded conditions sHsps tend to form large assemblies at elevated temperatures. For example, sedimentation coefficient, $s_{20,w}$, of HspB5 increases from 11 S in dilute solution to 20 S and 40 S in the presence of crowded agents and molecular mass of HspB5 increases from 480 kDa to 2 MDa. This study was funded by the Russian Foundation for Basic Research (grant 16-04-00560-a).

P.18-061-Mon
Physico-chemical properties of the chimeric tobamovirus coated with hordeivirus capsid protein with the deleted C-terminal region

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We replaced the CP gene of turnip vein cleaning tobamovirus (TVCV) by the mutant CP gene of Barley stripe mosaic hordeivirus (BSMV) with deletion of 22 C-terminal amino acid residues (TVCV ΔC-CP BSMV). Previously we demonstrated that the infectious cDNA clone of the mutant chimeric virus agroinfiltrated into N. benthamiana plants efficiently accumulated in infected and systemic leaves. Here we studied the physico-chemical characteristics of TVCV (ΔC-CP BSMV) isolated from the systemic symptomatic leaves. The virus preparation was characterized by transmission electron microscopy (TEM) and atomic force microscopy (AFM). The TEM data demonstrated that the preparation contained a heterogeneous set of filamentous structures with width of 5–7 nm, variable in length forming network clusters of a weak electron density. Similar images were obtained by AFM. Measurement of the hydrodynamic diameter of TVCV (ΔC-CP BSMV) particles by dynamic laser light scattering showed the presence of two peaks with sizes of about 100 nm and 800 nm indicating that the virus particles also form aggregates in the solution. However the removal of the C-terminal fragment of the CP BSMV does not significantly affect either the protein structure or its surface properties according the data of circular dichroism spectroscopy in the near ultraviolet range and the measurement of the surface zeta potential. It is known that the C-terminal disordered fragment of the CP is not involved in the formation of intersubunit interactions in the mature virion but we can not ruled out that it participates in the initial stages of the virion assembly. Our data indicate the importance of the integrity of the CP C-terminal region for the correct assembly of virions, and possibility of effective systemic transport of the tobamovirus genome in form of chimeric atypical virions (assumed ribonucleoprotein complexes). This work was supported by the Russian Science Foundation project No 14-24-00007.

P.18-062-Tue
Structural characterization of transferrin-bound ruthenium(II) terpyridine complexes

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Human serum transferrin (Tf) is 80 kDa protein that readily binds and transports Fe^{3+} throughout bloodstream and tissues. Transferrin contains two Fe^{3+} binding sites where two Tyr, one Asp and one His residues are included in Fe^{3+} binding. Also, Tf is believed to transport various metals and metal-based drugs, including Ru anticancer drugs. Since majority of tumor cells overexpress Tf receptor, delivery of Ru drugs via Tf cycle increases drug selectivity. Although some data on Ru(III) drug binding to Tf exists, data on Ru(II) drugs binding to this protein is scarce. In this work, binding of two Ru(II) drugs of general formula mer-[Ru(L3)(N-N)Cl][Cl] (where L3 = 4′-chloro-2,2′:6′,2″-terpyridine (Cl-tpy); N-N = 1,2-diaminoethane (en) or 1,2-diaminocyclohexane (dach)) to Tf has been confirmed using liquid chromatography (LC) and matrix-assisted laser desorption

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and ionization mass spectrometry (MALDI MS). For the purpose of determining exact binding sites, Tf was incubated with 10 fold molar excess of each complex for 24 h. Unbound portion of the complexes was removed by ultrafiltration and the obtained adducts were subjected to trypsin digestion. The resulting peptides were separated using LC, and Ru-containing fractions were collected for MALDI MS analysis. The obtained spectra revealed presence of five ruthenated peptides. Binding amino acids have been determined by MS/MS analysis of target sequences. According to the obtained results, both Ru(II) complexes bind five histidine residues, namely: His642, His300, His585, His289 and His273. Only His585 is included in Fe^{3+} binding site, while other His residues are mainly located on the protein surface. It can be concluded that both complexes show high affinity towards His residues and since the binding of the complexes does not cause changes in Tf structure, it can be suggested that these compounds can use Tf cycle to be actively transported into tumor cells.

P.18-063-Wed
The effect of arginine and ionic strength on aggregation of UV-irradiated glycogen phosphorylase *b*

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Arginine (Arg) is widely used not only because it stabilizes proteins and accelerates their folding, but also because of its ability to suppress protein aggregation. However, Arg is a charged molecule, and therefore, when studying the action of Arg on protein aggregation, the effects of ionic strength should be taken into account. In this work the effect of ionic strength and Arg on aggregation of UV-irradiated glycogen phosphorylase *b* (UV-Ph*b*) was studied. With this test system we can obtain information on the effects of different agents on aggregation of denatured protein molecules. The process of UV-Ph*b* aggregation includes the rate-limiting monomolecular stage of structural reorganization of the Ph*b* molecule, containing concealed damages, and a relatively fast aggregation stage. The process of structural reorganization of UV-Ph*b* is characterized by a first-order rate constant (k_1). The kinetics of UV-Ph*b* aggregation was studied using dynamic light scattering (DLS) at 37°C (0.03 M Hepes buffer, pH 6.8) at various ionic strength values (0.02–0.7 M NaCl or Arg). It was shown that an increase in NaCl concentration caused a decrease in the k_1 value, suggesting a slowdown of the UV-Ph*b* structural reorganization process. Circular dichroism data confirmed this conclusion. Analytical ultracentrifugation and DLS data have shown that an increase in ionic strength leads to the formation of smaller aggregates, testifying that the change in the aggregation pathway occurs. To evaluate the effect of Arg, we conducted experiments at fixed values of ionic strength (0.15 M and 0.5 M NaCl or Arg). It was shown that at a low ionic strength Arg accelerated the process of protein aggregation and induced changes in the aggregation pathway. At high concentrations Arg acts as a charged molecule, and its effect on protein aggregation is due solely to a change in ionic strength of the solution. The study was funded by the Russian Science Foundation (grant 16-14-10055).

P.18-064-Mon
Relationship of growth rate and muscle protein turnover in Atlantic salmon *Salmo salar* L. under natural and artificial photoperiods

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It is known that protein degradation in fish muscles depends on three proteolytic systems such as cathepsins, calcium-dependent proteases (or calpains), and the ubiquitin-proteasome system (UPS). Calcium-dependent proteolysis is a major pathway regulating muscle turnover in fish, while cathepsins and ubiquitin-targeted protein digestion by the proteasome are primarily responsible for bulk protein degradation. Calpain activity is considered as a marker of fish growth and health state at different life stages along with myofibrillar protein expression levels, key digestive and metabolic enzyme activities, lipid contents, etc. This study was conducted to evaluate the effects of natural and artificial (24:0 or 18:6 light:dark, L:D) photoperiods on the growth rate and calpain activity in the skeletal muscles in Atlantic salmon. Maximal growth rate was attained at 24L:0D, followed by 18L:6D, and a minimal was inducted by a natural photoperiod. The results suggested positive correlation between length-weight growth dynamics in individuals and calcium-dependent proteolysis level in their skeletal muscles as well as their orchestrated regulation by photoperiod variations. Stimulating effect of a continuous (24L:0D) light photoperiod on growth performance and muscle protein turnover in salmon was concluded. The work was supported by the Russian Science Foundation, project no. 14-24-00102.

P.18-065-Tue
Inhibition of the actin N-terminal acetyltransferase NAA80

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A common modification of proteins is the acetylation of their N-termini by N-terminal acetyltransferases (NATs). As a member of the NAT-family, NAA80 transfers an acetyl group from acetyl coenzyme (Ac-CoA) to the acidic N-termini of processed animal actins. These actins are integral parts of the cytoskeleton that is responsible for cell shape and motility, thus making NAA80 a viable target for the regulation of cytoskeletal functions. In this work, peptidic bisubstrate inhibitors based on Ac-CoA and the natural actin substrates of NAA80 were developed and for their synthesis we employed Fmoc-based solid phase peptide synthesis (SPPS) to prepare tetrapeptides, which were subsequently attached to coenzyme A through an acetamide linker. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy were used for structure confirmation of the inhibitors, whereas the potency and selectivity of the inhibitors was evaluated through acetylation assays. Furthermore, in complex with the most potent inhibitor, the crystal structure of NAA80 was determined. This work marks the basis for development of more potent and selective inhibitors of NAA80 thereby increasing the number of tools available for regulation of actin and the cytoskeleton.

P.18-066-Wed
Polyglutamine related aggregates serve as a potent antigen source for cross presentation by dendritic cells

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Protective MHC-I dependent immune responses require an overlap between the repertoires of proteins directly presented on target cells and cross-presented by professional antigen presenting cells (APC). How stable proteins that rely on defective ribosomal products (DRiPs) for direct presentation are captured for cell-to-cell transfer remains enigmatic. Here we address this issue using a combination of *in vitro* and in vivo approaches involving stable and unstable versions of ovalbumin model antigens, that display DRiP dependent and independent antigen presentation. Apoptosis induction in transfected donor cells resulted in robust global aggregate formation that captured stable proteins permissive for cross-presentation by APC. By using Imaging Flow Cytometry we were able to accurately quantify the amount of aggregation within the cells. Potency of aggregates to serve as cross-presentation antigen source was directly demonstrated using aggregates of poly-glutamine equipped model substrates. Collectively, our data implicate global protein aggregation in apoptotic cells as a mechanism that ensures the overlap between MHC-I epitopes presented directly or cross-presented by APC and demonstrate the unusual ability of dendritic cells to process stable protein aggregates.

P.18-067-Mon
Pyridylphenylene dendrimers influence the amyloid transformation of prion protein

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Dendrimers are actively used in biomedicine due to perfect characterization, nanometer size, internal cavities, high number and density of terminal groups, diverse chemical composition. The dendrimer application for treatment of neurodegenerative disorders is of particular interest. Among the different classes of dendrimers a special place belongs to cationic pyridyl containing aromatic dendrimers. The undeniable advantages of these dendrimers are the constancy of charge and shape as well as the independence of pH due to presence of quaternary nitrogen in the dendrimer pyridine moieties. These dendrimers were found to form extremely stable complexes with ovine prion protein (PrP) which causes several neurodegenerative disorders. The PrP molecule has two main sites possessing negative charge for the dendrimer binding and one more in the lateral side allowing the electrostatic interactions. Due to the presence of the phenylene groups in the dendrimer molecule, the hydrophobic interactions are also involved in the binding. It was observed that the contribution of the hydrophobic interactions increased from low to high generation dendrimers. The more pronounced hydrophobic interactions resulted in more efficient binding and higher dendrimer impact on the protein molecule. Complexation of PrP with the dendrimers also inhibited the fibril formation as well as formation of soluble protein aggregates known as oligomers which are currently believed to be the most toxic. CD spectra revealed that dendrimers prevented the $\alpha \rightarrow \beta$ transition of PrP molecule upon the oligomers formation. According to ThT fluorescence measurements and protease K digestion experiments, an addition of the dendrimers effectively inhibited the fibrillization process for all the generations studied. Importantly, the complexes formed do not cause the amyloidogenic transformation of the native PrP. TEM microphotographs displayed the inhibition of the process on the stage of structural intermediates.

P.18-068-Tue
Swi1 prion formation and deletional inactivation have different effects on the gene expression in *Saccharomyces cerevisiae*

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Prions represent infectious conformational states of some proteins and can exist in the same conditions as the normal conformational state. Most of known prions are amyloids i.e. highly-ordered protein fibrils. Prion formation by protein is considered to be equal to full or partial inactivation of the protein due to incorporation of protein monomers in the fibrils. Swi1 is one of the key chromatin remodeling factors in yeast Saccharomyces cerevisiae and have been found to form prion [SWI+]. The Swi1 deletional inactivation or prion formation have pleiotropic phenotypic effects and change expression of hundreds of different genes. Using NGS RNA sequencing we have shown that the effects of the deletion of SWI1 and prion formation of Swi1 protein on the expression of some yeast genes are different. In particularly, we have shown that nonsense suppression (growth on the media without adenine), which is induced in the [SWI+] and swi1Δ strains in the presence of both, the ade1-14UGA nonsense allele and mutant variants of SUP35 gene (encoding eRF3 release factor with decreased functional activity), are mediated by different mechanisms. Unlike the [SWI+] strain, where nonsense suppression occurs due to decrease in the amount of the SUP45 mRNA (encoding eRF1 release factor), nonsense suppression in the swi1Δ strains was caused by elevated expression of the ade1-14UGA. Another important finding was that about 20 genes that are downregulated in the swi1Δ strain are upregulated in the [SWI+] strain. Thus, prion formation and deletional inactivation of Swi1 show opposite effects on the yeast transcriptome and, in some cases, [SWI+] acts as the "gain-of-function" mutation. This study was supported by the Grant of the President of the Russian Federation (MK-3240.2017.4 to AAN) and the Russian Foundation for Basic Research (17-04-00816 to SGIV and 16-34-60153 to AAN). Sequencing of the transcriptomes was carried out in the Resource Center "Biobank" of SPbU.

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P.18-069-Wed
Identification of amyloid-forming proteins in higher plants

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Amyloids are protein fibrils with a highly-ordered spatial structure stabilized by intermolecular β-sheets. Due to such structure, amyloids acquire unique resistance to environmental factors and have been found to play an important role in functioning of many different organisms except plants. We analyzed 75 proteomes of plants involving 2.9 million of proteins using two different tools: Waltz, which seeks for short amyloidogenic regions, and SARP, which finds extended sequences enriched with amyloidogenic amino acids like glutamine Q and asparagine N. We found that the fraction of proteins harboring the amyloidogenic regions in the plant proteomes was not less than in organisms with known functional amyloids including fungi and animals, but varied significantly even within one plant genus, for example, *Oryza* sp. Proteins with Waltz predicted regions mostly belonged to a transporter group according to Gene Ontology enrichment test. QN-rich sequences were found in the proteins with different functions including nucleic acids binding, regulation, defense, etc. One ubiquitous group of plant proteins harboring QN-rich potentially amyloidogenic regions was seed storage proteins. Different seed storage domains were found to be QN-rich. Experimental analysis showed that several plant storage proteins form fibrils *in vitro* that demonstrate properties typical for amyloids, including unbranched morphology and apple-green birefringence upon binding of amyloid-specific dye Congo Red. Considering the importance of preservation of seed storage proteins, we hypothesize that formation of amyloids might be an important functional adaptation to stabilize these proteins. This work was supported by the Russian Science Foundation (Grant No 17-16-01100).

P.18-070-Mon
Discovery of NAA80 as actin's N-terminal acetyltransferase – a novel regulator of cytoskeleton dynamics

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More than 80% of all human proteins are cotranslationally N-terminal acetylated (Nt-acetylated) by to date six known N-terminal acetyltransferases (NATs). In contrast, actin, one of the most abundant proteins in the cytoplasm of animal cells, is Nt-acetylated posttranslationally, following a unique multi-step mechanism that has remained poorly characterized. Actin participates in countless cellular functions ranging from organelle trafficking to cell migration and regulation of gene transcription. Actin's cellular activities depend on the dynamic transition between its monomeric and filamentous forms, a process regulated in cells by several actin-binding proteins. Besides the association of regulatory proteins, several posttranslational modifications, including Nt-acetylation, control actin's functions. The physiological role and the biochemical mechanism of actin Nt-acetylation are poorly understood, since the NAT catalyzing this reaction was unknown. Here, we introduce NAA80, actin's N-terminal acetyltransferase. This modification is exclusively added to actin by NAA80, showed by mass spec-based COFRADIC analysis of control vs. *NAA80* knockout cells. Sequence analysis revealed that the expression of NAA80 is highly correlated with actin's unique N-terminal processing mechanism in the animal kingdom. Actin purified from NAA80 knockout cells, and thus 0% Nt-acetylated, shows significant differences in actin filament depolymerization and polymerization rates compared to 100% Nt-acetylated actin from control cells. Especially, actin filament elongation driven by formin-1 is affected, indicating a direct interaction between formin-1 and actin's N-terminus. In contrast, actin filament nucleation by Arp2/3 is not affected. Here, the role of the most conserved actin modification in animals has been revealed. In contrast to the established NATs often acting on ribosomes and target large groups of substrates, NAA80 is the first specific posttranslational eukaryotic NAT.

P.18-071-Tue
ESR spectroscopy as a probe of protein dynamics: an insight from long MD simulations

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Electron spin resonance (ESR) spectroscopy has established itself as a powerful tool to probe protein structural environment and dynamics. Typically, site-directed mutagenesis is used to introduce a unique cysteine residue at a site of interest; the cysteine side chain is then conjugated with the ESR-active methanethiosulfonate spin label (MTSL). It is known that the resulting ESR spectra are highly sensitive to dynamic status of the MTSL moiety, but the details of this relationship are largely lacking. In particular, it is often unclear what are the relevant contributions from protein backbone dynamics vs. rotameric jumps within the MTSL tag. To address this problem, we turned to MD simulations of MTSL-tagged B1 immunoglobulin-binding domain of protein G (GB1), for which the detailed experimental information is available. The MD trajectories with net length of 25 μs have been recorded for five GB1 variants with different MTSL attachment sites. The respective ESR spectra have been simulated on the basis of the MD data by means of the exact method involving direct propagation of the spin density matrix in the Liouville space. The significant length of the trajectories (20 times longer than anything previously used in this context) permitted the detection of rotameric jumps about the S-S bond which occur on slow (μs) time scale; we have shown that these jumps can influence the shape of the simulated spectra. As it turns out, in many cases the simulated ESR signals satisfy the validity conditions of the Redfield theory, so that the corresponding spectra can be successfully reproduced using Redfield formalism. In turn, this means that certain trademark features of the ESR spectra (e.g. multiplet asymmetry) can be nicely explained by invoking the concept of cross-correlations, which proved extremely useful in the context of Nuclear Magnetic Resonance (NMR) spectroscopy. This research was supported by RSF grant 15-14-20038 (modeling component) and NIH grant GM118664.

P.18-072-Wed
Saccharomyces cerevisiae growth assays and proteomics reveal functional conservation, redundancy and substrates of N-terminal acetyltransferases

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One of the most abundant protein modifications occurring in eukaryotic cells is N-terminal acetylation (Nt-acetylation) and the N-terminal acetyltransferases (NATs; NatA-NatH) catalyze this reaction. The evolutionarily conserved NATs cover more than 50% and 70% of the soluble yeast and human proteome, respectively. Recently, the human NatC substrate repertoire was uncovered by proteomics (COFRADIC), and to study the functional conservation of NatC we expressed human Naa30 in a *naa30*-Δ yeast strain (*Saccharomyces cerevisiae*). COFRADIC data showed restored Nt-acetylation of as many as 82% of the here-identified yeast NatC substrates by human Naa30, thus NatC is evolutionarily conserved between yeast and humans. Furthermore, these data added Met-Lys N-termini to the list of yeast NatC substrate specificity. We then investigated the functional conservation and redundancy of human NatC, NatE and NatF in yeast. The respective catalytic subunits (Naa30, Naa50 and Naa60) were individually expressed in NatC-inactive yeast, which was then challenged with glycerol, a non-fermentable carbon source, to induce the *nfs*$^-$ growth phenotype. Human Naa30 and Naa50 restored normal growth whereas Naa60 did not reverse the fermentation-dependent growth phenotype. In contrast, human Naa60, but not Naa50, Nt-acetylated and restored the subcellular Golgi localization of the NatC substrate Arl3. These findings clearly suggest that the *naa30*-Δ *nfs*$^-$ phenotype is dependent on Nt-acetylation of one or more NatC substrates independent of the impact on Arl3. Furthermore, our data suggest that NATs may have partially overlapping in addition to unique substrates *in vivo*. Finally, *S. cerevisiae* again stands its ground as a useful model system for functional protein studies.

P.18-073-Mon
Principles for creating biocatalysts with predefined functional activities

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Biocatalytic function is one of the most advanced functions inherent in living systems. This function is realized in a limited set of biological objects, the bulk of which are protein molecules – enzymes and antibodies. Biocatalytic activity is widely used in various fields of modern industrial and pharmaceutical biotechnology. Thus, it is obvious that for the successful implementation of research on the creation and directed change in the functional activity of biocatalysts, it is necessary to develop new methods for induction of catalytic activity and adapt it to a given biocatalytic process, and also to create new technologies for searching for biocatalytic activity and ways to ensure the prolongation of the therapeutic effect of drugs on the basis of biocatalysts. The presented thesis is devoted to new principles of creation of biocatalysts with new functional activities, and the results obtained in the course of its implementation solve the above-mentioned problems of a fundamental and applied nature. The aim of the work is to develop new principles for creating biocatalysts with predefined functional activities. To achieve the overwhelmed goal, we develop of new technologies for the production of biocatalysts based on antibodies (reactibody approach and QMMM prediction algorithm) and technology for high-performance screening of biocatalytic activity. This work was supported by RSF Grant #16-14-00191.

P.18-074-Tue
Oxidation-induced modifications of plasma fibrin-stabilizing factor at the different stages of its activation

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For the first time, by using HPLC-MS/MS, the peculiarities of oxidation-induced modifications of plasma fibrin-stabilizing factor (pFXIII) at different stages of its activation have been analyzed. pFXIII is a heterotetrameric proenzyme composed of two catalytic A subunits (FXIII-A_2) and two inhibitory/carrier B subunits (FXIII-B_2). The main function of the protein lies in cross-linking the polypeptide chains of the fibrin clot. The conversion of pFXIII into the enzymatic form FXIII-A_2* is a multistage process, accompanied by changes in the conformation of the molecule. The present study provides experimental data evidencing that (1) the set of amino acid residues (AARs) susceptible to oxidative attack and the oxidation degree of these AARs in FXIII-A_2 of non-activated pFXIII, activated by Ca^{2+}, and fully activated pFXIII with thrombin and Ca^{2+} differ; (2) in the process of conversion of the proenzyme into the enzyme, new earlier-unexposed AARs become available to reactive oxygen species (ROS) while some of the initially surface-exhibited residues are buried within the protein globule. The experimental data obtained in the present study combined with the data reported earlier regarding the functional activity of FXIII-A_2* strongly depending upon the stage of pFXIII conversion at which the oxidation was carried out enables one to suppose that some of the revealed easily oxidized AARs could serve as targets for ROS scavenging to protect the active site in the catalytic core domain. This mechanism may be among the leading factors that determine the capacity of proteins to maintain the native structure and function in the ROS-generating environment. That the degree of modification of the enzyme form is more pronounced than other samples can demonstrate the possible previously undescribed antioxidant function of the carrier subunit-B of pFXIII. The mass spectrometry measurements was supported by the Russian Science Foundation № 16-14-00181.

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P.18-075-Wed
Oxidative cellulose degradation orchestrated by cellulose dehydrogenase and lytic polysaccharide monooxygenase studied by structural mass spectrometry

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Cellobiose Dehydrogenase (CDH) and its redox partner Lytic Polysaccharide Monooxygenase (LPMO) are essential parts of cellulolytic system in wood degrading fungi. Their combined activity allows for oxidative decomposition of recalcitrant cellulose structures. The function of CDH depends on intramolecular electron transfer (IET) between CDH's disaccharide oxidizing flavin domain and electron transferring cytochrome domain, which then transfers electrons to copper-dependent cellulose oxidizing LPMO by yet not clearly described mechanism. We used various structural MS techniques namely H/D exchange and chemical cross-linking, to obtain more precise idea about the protein-protein and protein-substrate interaction, product formation and protein stability under the oxidative conditions. Using HDX-MS, changes were observed on CDH induced by lactose oxidation. Interestingly, no differences were observed on CDH when it interacted with LPMO either in its reduced or oxidized state. On the other hand, structural perturbance occurred on LPMO. In the fully functional system, when CDH or small molecular reductants are fueling LPMO with electrons, we saw increase in deuterium exchange around the active site and we attribute it to oxidative damage and protein unfolding. Several oxidative modifications of amino acids in peptides surrounding the active site were indeed identified. These adverse effects can be significantly diminished in the presence of substrate (PASC or cellulose) where we observed strong stabilizing effect on LPMO. So far, our findings indicate that the interaction between CDH and LPMO may be in fact just direct cofactor-cofactor interaction between the propionates of the heme group in cytochrome domain of CDH and a copper ion of LPMO. MALDI-MS product formation analysis also demonstrated, that reductant is necessary for reaction priming but the actual process requires nothing more than just hydrogen peroxide. Financial support: CSF (16-34818L)

P.18-076-Mon
Human nucleoporin Nupl1 forms amyloid-like aggregates

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Amyloids are a group of protein aggregates possessing a set of unusual features including high resistance to detergent or protease treatment and their ability to induce the transition of some proteins from soluble to aggregated form. Numerous investigations of amyloids are in the top of interest due to increasing incidence of amyloid-associated disorders, for instance Alzheimer's disease, Parkinson's disease, type II diabetes, etc. We analyzed a set of proteins, which physically interact with Htt (Huntington's disease) according to BioGrid database, for the ability to form amyloid aggregates. According to the prediction of ArchCandy program nucleoporin Nupl1 was one of the most probable candidates. This protein is a part of a midplane ring of nuclear pore complex, regulating nucleocytoplasmic traffic. Using advantages of yeast model system we demonstrated that the full length protein fused to EGFP forms fluorescent foci when overproduced. Nupl1 aggregates were resistant to cold SDS treatment according to the results of SDD-AGE analysis. Using C-DAG (curli-dependent amyloid generator) system we demonstrated that Nupl1 overproduction leads to staining of the bacterial cells with Congo Red. Taken together these results allow to suggest that Nupl1 is a candidate for new human amyloid. The research was supported by the Russian Science Foundation (17-74-10159).

P.18-077-Tue
Dynamic structural ensembles of selected folded and disordered postsynaptic proteins

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Protein internal dynamics is a key determinant of protein function and its regulation. Dynamic structural ensembles aim at providing an atomic-level description of protein motions by enumerating a number of possible conformers characteristic of the conformational space explored by the molecules at a given time scale. We have generated structural ensembles reflecting experimentally determined internal dynamics of the tandem PDZ1-2 domains of the postsynaptic density protein PSD95. In this domain pair ligand binding influences interdomain mobility. Dynamic structural ensembles of free and ligand-bound forms of the domain pair were generated with an in-house modified version of GROMACS and analyzed to understand the atomic basis of this behavior. Our models made it possible to point out the key determinants of interdomain mobility and the atomic-level basis of its changes upon ligand binding. In addition, we will present our efforts to generate structural ensembles of different intrinsically disordered segments of the postsynaptic scaffold protein GKAP, along with biochemical studies on its partner-binding properties. Our results highlight the usefulness of ensemble-based structural models in understanding interactions within the postsynaptic density.

P.18-078-Wed
Abscisic acid-induced re-localization of phytaspase

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Phytaspase belongs to a family of plant subtilisin-like proteases and possesses a rarely occurring aspartate specificity of hydrolysis. Phytaspase is a cell death-related protease. Upon autocatalytic proenzyme processing, active phytaspase is secreted out of the plant cell into the apoplast. However, death-inducing stresses cause retrograde transportation of phytaspase from the apoplast to inside the plant cell. Here we demonstrated that intracellular localization of phytaspase can be achieved in yet another, cell death-unrelated way. In *Nicotiana benthamiana* leaves transiently producing *Nicotiana tabacum* phytaspase, leaf treatment with phytohormone abscisic acid resulted in redistribution of the enzyme from the apoplast to the cell interior. Likewise, *Arabidopsis thaliana* phytaspase transiently produced in *A. thaliana* leaves became spread throughout the cell upon abscisic acid application. Consistent with these findings, co-production of the *A. thaliana* ABI3 transcription factor, a key regulator of abscisic acid

signaling pathway, together with the phytaspase, also resulted in re-localization of the protease. It seems likely therefore that some of the ABI3 target gene products may be responsible for phytaspase movement. Comparison of the intracellular phytaspase localization in response to cell death induction or to phytohormone treatment revealed marked difference between the two, suggesting that the underlying mechanisms of phytaspase re-import may be distinct. This work was supported by the Russian Science Foundation grant # 16-14-10043.

P.18-079-Mon
N-terminal acetylation stabilizes alpha-synuclein protein and modulates its toxicity

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Increased alpha-synuclein (aSyn) protein levels causes neurodegenerative diseases like Parkinson's disease or dementia with Lewy Bodies, grouped as synucleinopathies. Still, the mechanisms by which altered aSyn protein levels causes neuronal death are unknown. *In vivo*, all aSyn molecules are posttranslationally modified by an acetyl group attached to the alpha-amino group of the N-terminal amino acid by the enzyme NatB. Some *in vitro* evidences suggest that this modification stabilizes the helical structure of the aSyn N-terminal domain and affects the lipid binding and aggregation capacity of the protein. However, the biological significance of aSyn N-terminal acetylation in neurons has not been addressed. Recently, our laboratory has developed a neuronal model based on automated microscopy that enables longitudinal tracking of individual primary living neurons expressing aSyn and survival analysis. Using Cox proportional regression models the specific contribution of different factors (e.g. mutations and protein expression levels) can be quantified and dissected. To address the role of aSyn N-terminal acetylation in neurons we generated three N-terminal aSyn mutants; D2A and D2P that alter and block aSyn N-terminal acetylation respectively, and D2E, a conservative mutant. The toxicity of these mutants was evaluated by longitudinal survival analysis. Mutant D2P exhibited lower protein levels when expressed in primary cortical neurons and consequently it decreased the risk of neuronal death compared to normal aSyn forms. Using an optical pulse-chase methodology that allows measuring protein half-life *in situ* in primary neurons we found that D2P mutant decreased aSyn stability compared to wild-type aSyn. Our results strongly suggests that N-terminal acetylation stabilizes aSyn protein modulating its toxicity. We are currently evaluating N-terminal acetylation-dependent molecular mechanisms that lead to aSyn destabilization.

P.18-080-Tue
The effects of oxidation on the fibrinogen molecule and its functional properties

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Fibrinogen, which plays a key role in the physiological reaction providing plasma hemostasis, is the most vulnerable to reactive oxygen species among all the blood plasma proteins. Oxidation of fibrinogen damages its structure as well as affecting the protein function. The induced oxidative modifications of the all three polypeptide chains of the fibrinogen have been studied by the mass-spectrometry method. The alphaC-connector has convincingly been shown to be most vulnerable to oxidation as compared to other structural elements. The E region turned out to be the most protected area of the protein. Oxidative modifications of Tyr338, Met367, Met373 in the area 330–375 of the beta-nodules, oxidized Met476 and group modifications in the alphaC-region seem to contribute to the lateral aggregation damage. Detected modifications of Pro243 and Met264, Lys273, Tyr274 in gamma chain may influence the formation of interfacial D:D contacts. The mutation Ala68 in beta chain results in defective binding of thrombin to fibrin and consequent thrombosis, so the His67 modification possibly affects this binding too. Some structural elements of fibrinogen and a part of easily oxidizable amino acid residues are supposed to be endowed with antioxidant properties. The gained data on the identified oxidative modifications of fibrinogen appear to support the concept previously proposed by us that the fibrinogen structure is adapted to oxidation. The methods of confocal laser scanning microscopy and elastic light scattering revealed the disturbances in the structure of fibrin clots assembled from oxidized, thrombin-treated fibrinogen molecules. The study was supported by RFBR projects № 16-34-60244 mol_a_dk and № 18-04-01313a. The mass spectrometry measurements was supported by the Russian Science Foundation № 16-14-00181.

P.18-081-Wed
Overproduction of Sch9 leads to its aggregation and cell elongation in *Saccharomyces cerevisiae*

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The Sch9 kinase of Saccharomyces cerevisiae is one of the major TOR pathway effectors. It regulates such important processes in the yeast cell as progression through cell cycle, aging and regulation of response to nutrient. Sch9 belongs to the AGC kinase family. Even though amplification of AGC kinase genes has been connected with cancer in human, not much is known about the effects of Sch9 overproduction in yeast. We addressed this issue by developing a model system to monitor subcellular location and aggregation state of overproduced Sch9 or its regions fused to a fluorescent protein. We found an earlier unknown feature of Sch9 protein, formation of specific intracellular structures, at least some of which possess amyloid-like properties. In addition, we attempted to separately analyze functions of the different Sch9 domains and revealed specific effect of the C-terminal region overproduction on cell elongation and existing of several aggregation-prone regions in different parts of the protein. Even though this analysis may be further continued, we believe that

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our results add to the prior knowledge about Sch9 functions and create a basis for further investigation. The constructs we present can be used to create superior yeast-based model systems to study processes behind kinase overproduction in cancers. These potential model systems could also be useful for testing novel inhibitors of AGC kinases, for example p70S6K, inhibitors of which are already being developed. The research was supported by the Russian Science Foundation (17-74-10159).

P.18-082-Mon
Molecular insight in the interaction mechanism of farnesylated hGBP 1

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Human guanylate-binding proteins (hGBPs) belong to the dynamin superfamily of large GTPases and are upregulated in cells after stimulation with IFNγ-II. Three of the seven isoforms contain a C-terminal CaaX motif which can be isoprenylated. The isoprenyl anchor allows the hGBPs to attach to membranes within the cell and to artificial membranes in a nucleotide dependent manner *in vitro*. In absence of membranes farnesylated hGBP1 (hGBP-1_{fn}) reversibly polymerizes by addition of GTP to a tube-like structure. hGBP fulfils its main function in the innate immunity against bacteria and viruses by interacting with intracellular membranes. However, by now the molecular mechanism of interactions among the hGBPs when inserting into the membrane is poorly understood. For a better understanding a range of *in vitro* methods, including fluorescence microscopy and spectroscopy, turbidity assay and hydrolysis assays, have been performed to shed light on the missing knowledge. We showed that polymerization as well as membrane binding can be altered by introducing different point mutations to hGBP1_{fn}. These point mutations lead to changes in the crucial properties such as GTP hydrolysis, dimer formation or intramolecular rearrangements that are needed for the function of the farnesylated protein. With these findings, we gain a more detailed insight into the molecular interactions among the hGBPs in the process of polymerization and membrane binding.

P.18-083-Tue
Protein intrinsic disorder in postsynaptic density

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The synapse is a structure, which provides a way for neuronal communication. The postsynaptic density, responsible for modulating signal transduction and orchestrating a number of processes linked to learning and memory, is an elaborate protein network composed of a many different proteins with a high number of interaction sites. The molecular basis of its ability for dynamic rearrangement is still elusive. We investigated the possible role of intrinsically disordered segments of pre- and postsynaptic proteins and compared it to features of a number of other proteins associated with other well-characterized functions, such as the cytoskeleton, the immunome and signaling proteins. We took care to use consensus predictions as much as possible and also to exclude fibrillar segments like coiled coils from predicted disordered regions to get unbiased statistics. We have calculated different structural attributes based on intrinsic disorder and also on annotated protein-protein interactions from different sources. Our results, based on detailed analysis of the structural/functional features of the proteins, suggest intrinsic disorder is indeed an important feature of postsynaptic proteins and might comprise one of the main factors contributing to the versatility and dynamics of the postsynaptic network.

P.18-084-Wed
Local structural factors governing the fold-switching mechanism of RfaH

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Metamorphic proteins challenge the paradigms of protein folding by switching between distinctive functional and structural native states within biologically relevant timescales. The bacterial virulence factor RfaH constitutes a dramatic example of such behavior, with its C-terminal domain (CTD) refolding from a stable α-helical hairpin bound to and occluding the RNA polymerase binding site of the N-terminal domain (NTD) into a dissociated small β-barrel, enhancing transcription and enabling its coupling with translation. Here we explore in detail the metamorphic process of RfaH using a combination of multiscale molecular dynamics and biophysical experiments. Coarse-grained simulations using dual-basin structure-based models reveal that interdomain contacts control the fold-switching behavior of RfaH and highlight the role of specific NTD-CTD interactions in stabilizing the α-folded state, with the CTD residue F130 having a dual role in stabilizing both folds. Moreover, simulations using the AWSEM coarse-grained protein force-field correctly predict the NTD-CTD interface for the α-folded state of RfaH from sequence and local structure information alone. Finally, computationally predicted per-residue free energy differences using all-atom confine-convert-release simulations of RfaH unveil local structural preferences towards either native state, with the tip of the helical hairpin (CTD residues 129–146) having preferential thermodynamic stability towards the α-folded state whereas the rest of this domain is ~2 kcal/mol more stable if folded as β-barrel. Experimental evidence of these heterogenous local stabilities was obtained from deuterium incorporation kinetics using hydrogen-deuterium exchange mass spectrometry analysis of RfaH in both native states, showing remarkable spatial and magnitude consistency. Our results reveal how the stabilities of the native states of RfaH are locally encoded within its sequence. Funding: FONDECYT 11140601.

P.18-085-Mon
Interaction of human GOLPH3 with RAB1A and RAB1B: identification of a putative non-canonical effector

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Golgi phosphoprotein 3 (GOLPH3) is a trans-Golgi network protein implicated in several aspects of membrane trafficking. Intriguingly, GOLPH3 has been implicated also in tumorigenesis.

However, it is not well understood how GOLPH3 participates in these mechanisms. Interestingly, GTP promotes the association of GOLPH3 to Golgi membranes and vesicles. Nevertheless, it remains largely unknown whether or not this response is consequence of the function of GTP-dependent regulatory proteins, such as proteins of the RAB, ARF, or ARL families of small GTPases. Thus, we set to determine whether GOLPH3 is an effector of RAB proteins. We performed a mini yeast two-hybrid (Y2H) screen, and we found that GOLPH3 interacts with several RABs, including RAB1A and RAB1B. These interactions were further characterized by 1) mutational analysis using Y2H assays, 2) glutathione S-transferase (GST) and green fluorescent protein (GFP) pull-down assays, and 3) isothermal titration calorimetry. To investigate the functional role of the interactions, we performed RNAi-mediated knocking-down of RAB1A or RAB1B in cultured cells, and analyzed the effects on GOLPH3 by subcellular fractionation, immunoblot analysis and fluorescence microscopy, and of GFP-GOLPH3 by live cell imaging. The Y2H and GST and GFP pull-down assays showed that GOLPH3 binds to RAB1A and RAB1B in a non-canonical fashion. The knocking-down of RAB1A or RAB1B perturbed the subcellular distribution of GOLPH3, which relocated to distinct and unidentified, dynamic structures/organelles within the cell. We propose that GOLPH3 is a non-canonical effector of RAB1A and RAB1B. Supported by FONDECYT 1161252 and CONICYT 21151194.

P.18-086-Tue
Structure and function of human neuromodulator Lypd6

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Human Lypd6 is a membrane-tethered member of the uPAR protein family. Ly6/uPAR proteins share characteristic "three-finger" fold which is stabilized by four disulfide bonds. Lypd6 plays important role in embryogenesis by modulating the activity of Wnt/β-catenin pathway. It was shown that Lypd6 interacts with both Lrp6 and Frizzled8 proteins, – the members of the receptor complex on cellular membrane. It has been shown that Lypd6 increases the amplitude of the Ca^{2+} current in response to nicotine in the neurons of the mice trigeminal ganglion. In line with this, transgenic mice with Lypd6 overexpression demonstrate increased locomotor activity and visceral hyperalgesia, indicating an increase in cholinergic tone. Here we present structure and functional characterization of water-soluble variant of human Lypd6 (rLypd6). The structural properties of rLypd6 have been explored using high-resolution heteronuclear NMR spectroscopy. It was found that rLypd6 has structural elements typical for the Ly6/uPAR family, although with some unique features, particularly, an additional disulfide bond in the third loop and helical regions in the first and third loops. Electrophysiology experiments on *Xenopus* oocytes revealed that rLypd6 affects the sensitivity of α4β2 nicotinic acetylcholine receptors to acetylcholine. Computer modeling of the complex between rLypd6 and α4β2 receptor allowed to predict aminoacid residues of the neuromodulator important for interaction with the receptor. This work has been supported by the Russian foundation for Basic Researches (project #15-04-99517), and Russian Science Foundation (project #16-14-00102).

P.18-087-Wed
Alpha-synuclein oligomerization in the presence of the cholesterol and glycerophosphoglycerol

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Parkinson's disease is amyloid misfolding disease, where alpha-synuclein loses its function and starts to oligomerize. The protein changes from functional unstructured monomeric, dimeric and tetrameric state into soluble toxic oligomers and later into insoluble non-toxic fibrils. Soluble toxic oligomers of alpha-synuclein disrupt the calcium balance of mitochondria and leads to cell death. The reason why this oligomerization occurs is not yet fully understood but there is a clear indication that lipid environment is involved in the conversion process. The goal of our work is to use structural biology tools to identify the role of membrane components in the conversion of the native alpha-synuclein into toxic oligomers and later into inert, non-toxic fibrils. We used NMR in combination with novel lipid nanodiscs, SPR and fluorescence assays to monitor binding, oligomerization and fibrillation of alpha synuclein in the different lipid environments. We observed that two lipid components has significant effect on alpha-synuclein oligomerization process: glycerophosphoglycerol (PG) and cholesterol. PG increases affinity of alpha-synuclein monomers to the membrane and delays its oligomerization. On the other hand, cholesterol decreases affinity of alpha-synuclein and promotes oligomerization of the protein. We are now in process of resolving specific structural changes during binding of alpha-synuclein to the membrane models containing PG and/or cholesterol.

P.18-089-Tue
The features of water-soluble serum and ligand-switching proteins interaction with bilirubin- and bodipy-loaded Triton-X-based micellar coordination clusters

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Recently, it was shown that Triton-X-based micellar coordination clusters (MCCs) could be successfully used for many hydrophobic compounds solubilization and encapsulation. Based on these results, we've studied the interactions between hydrophilic proteins such as recently discovered apoUnaG and BSA with bilirubin (BR)- and several boron-dipyrrin (bodipy)-loaded MCCs, respectively. These proteins have a strong affinity to the studied molecules. Our results demonstrate that hydrophilic apoUnaG is unable to penetrate inside of the MCCs. However, due to the high binding constant of BR to UnaG, the protein partially extracts BR from MCCs leading to fluorescence increase during the interaction with BR-loaded MCCs. These interactions can be visually observed as a thin glowing layer on the MCCs interface indicating that the protein preserves its structure during this interaction due to the BR-UnaG (holoUnaG) complex formation. This part of research was supported by the Grant of the Russian Federation for young scientists No MK-2124.2017.3. Among all the natural hydrophilic proteins, only serum albumin (including BSA) may bind with Triton-X surfactants. Taking into account that this protein increases the fluorescence of several dyes upon interaction, we found that BSA may penetrate inside of the MCCs and interacts with bodipy dyes increasing its

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fluorescence signal, especially when being irradiated by UV-light. In contrast to UnaG, the addition of BSA leads to the fluorescence enhancement of the entire cluster indicating that its native structure remains unchanged upon penetration. In all cases, MCCs don't undergo significant changes of the shape or size during the interaction with proteins. Moreover, BSA and apoUnaG may selectively interact with MCCs contained both bodipy and BR simultaneously, leading to appropriate bright "turn-on" either of BR-UnaG or BSA-bodipy fluorescence responses. This part of research was supported by Russian Science Foundation grant No 17-73-10408.

P.18-090-Wed
Mms19 modulates mitotic spindle dynamics by recruiting Xpd to microtubules

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Mms19 is a component of the Cytoplasmic Iron-sulfur cluster assembly (CIA) machinery, which regulates the incorporation of iron-sulfur clusters in target proteins. Recent evidence, however points towards cell cycle regulatory activity of Mms19 as down-regulation of Mms19 in human cells leads to mitotic defects and mitotic tissues are absent or deformed in *Mms19* loss-of-function mutant *Drosophila* larvae. Apart from the CIA, Mms19 also forms a distinct complex, MMXD which consists of Mms19, Xpd, and Mip18. The mitotic defects in the *Drosophila* larvae can probably be attributed to the inhibition of Cdk activity by Xpd. But overexpression of the Cdk activating kinase complex in Mms19 mutant background did not fully rescue the mutant larval phenotype, indicating alternative pathways of mitotic regulation by Mms19. We performed Mass-spectrometry screen to identify novel Mms19-binding proteins, in which, we found tubulin and microtubule associated proteins. We confirmed these observations by additional microtubule (MT) binding assays. We were surprised to find that along with numerous mitotic spindle defects in Mms19 mutant larvae, the spindle microtubules polymerize at a reduced rate. In further MT-assays, we observed that the MMXD components Xpd and Mip18 also bind to MTs and if MMXD complex formation is inhibited, MT polymerization defects are seen *in vitro* and *in vivo*. Here, we establish that Mms19 recruits Xpd to spindle microtubules and the association of MMXD with MTs is crucial for regulating spindle structure and polymerization during mitosis.

P.18-091-Mon
The unique mechanism of promoter binding by phiKZ phage non-virion RNA-polymerase

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Non-canonical multisubunit non-virion DNA-dependent RNA-polymerase (nvRNAP) encoded by the bacteriophage phiKZ contains 5 subunits, 4 of which are homologs of β and β' bacterial RNAP subunits, a function of the fifth subunit (Gp68) is unknown. NvRNAP transcribes late phage promoters with ultimate short consensus TATG, where G is transcription start point. In experiments with double-strand DNA-templates was identified that for a specific transcription initiation every single pair of consensus nucleotides is very important. Using downstream fork junction templates (DFJ-templates) where DNA before the +3 nucleotide after transcription start site is presented as ssDNA for template or non-template strand, we revealed that phiKZ nvRNAP requires template strand for promoter interaction in single-strand form. It is the significant difference with bacterial RNAP, which specifically binds non-template strand in promoter consensus. Moreover, in binding with DFJ-templates, consensus nucleotides aren't important for effective nvRNAP transcription start. To study the possible role of the fifth subunit in promoter recognition and nvRNAP maturation two variants of co-expression systems were made: the first one encoded only 4 β/β'-like subunit and the second contains all 5 nvRNAP subunits. Both producing complexes from co-expression systems were tested by size-exclusion chromatography and native PAGE. 4-subunit system a small amount of 4-subunit (4-s) complex without any polymerase activity and DNA-binding capacity. 5-subunit system produced full recombinant nvRNAP, which transcribed late phage promoters with native nvRNAP specificity. Experiments with the addition of the fifth subunit to 4-s complex showed a significance of Gp68 to the formation of nvRNAP and its DNA-binding capacity. The work was supported by the Russian Science Foundation (grant № 17-74-10220) for M. Yakunina.

P.18-092-Tue
Stopped-flow kinetic study of DNA demethylation by Fe(II)/2-oxoglutarate-dependent dioxygenase AlkB

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Proteins of AlkB family are involved in the direct oxidative repair of alkylated DNA bases. Bacterial AlkB protein is a Fe (II)/2-oxoglutarate dependent dioxygenase that removes methyl groups from N^1-methyladenine, N^3-methylcytosine and exocyclic DNA adducts such as $1,N^6$-ethenoadenine (εA). The AlkB-mediated catalysis is accompanied by oxidation of 2-oxoglutarate to succinate resulting in formation of "oxyferryl" intermediate. The complexity of reaction mechanism requires multiple conformational changes within the enzyme-substrate complex including flipping of the alkylated base. In the present work to gain deeper insight into the damage recognition mechanism and catalytic step of reaction, we performed a pre-steady-state kinetic analysis of conformational transitions of AlkB and DNA in the course of their interaction. Using the stopped-flow fluorescence analysis together with a combination of biochemical assays, we have studied conformational transitions and transient kinetics of AlkB interactions with model ss and ds DNA containing damaged and undamaged nucleotides. An intrinsic fluorescence of the protein Trp residues, the fluorescent base 2-aminopurine and dye–quencher pair FAM/BHQ1 introduced into 15 bp oligonucleotides were applied for registration of kinetic traces. The data obtained under single-turnover conditions have been described quantitatively by equilibrium and rate constants. A comparison of kinetic data obtained by means of Trp and 2-aminopurine fluorescence and Förster resonance energy transfer (FRET) detection allowed us to elucidate the steps of specific and nonspecific DNA binding and catalysis. This work was supported by grant from the Russian Science Foundation (Grant № 16-14-10038).

P.18-093-Wed
Expression, purification and characterization of recombinant Zika viral proteins for antibody development and structural studies

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Zika virus, a member of the flavivirus, was declared a global public health emergency and causes microcephaly and other neurological disorders. Zika virus has a single-stranded positive-sense RNA genome and viral proteins consist of three structural proteins (capsid, pre-membrane and envelope) and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NA4B and NS5). The envelope protein is a vaccine target and the NS1 protein is a potential diagnostic marker of early infection. In this study, the NS1 and envelope genes of Zika virus are cloned into bacterial expression vectors and these proteins are expressed in *E. coli* in the form of insoluble inclusion bodies. The proteins are purified using affinity chromatography under denaturing conditions and refolded by dilution and dialysis. The purified recombinant viral proteins are characterized by size-exclusion chromatography, circular dichroism and dynamic light scattering. This study will help in future antibody development for rapid diagnostic tests and structural studies.

P.18-094-Mon
Dynamic light scattering study of base excision DNA repair proteins and their complexes

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Base excision DNA repair (BER) is very efficient process that involves the action of many enzymes and accessory proteins to correct the most common DNA lesions usually induced by ionizing irradiation and oxidative stress. The mechanisms defined the fidelity of the DNA repair synthesis strongly depend on assembling BER proteins into large dynamic "repairosome". In this study we have determined the hydrodynamic sizes and characterized the oligomeric state of several BER proteins (two key enzymes – DNA polymerase β (Polβ) and apurinic/apyrimidinic endonuclease 1 (APE1), one accessory enzyme – tyrosyl-DNA phosphodiesterase 1, regulatory/scaffold proteins – poly(ADP-ribose) polymerases 1 and 2 (PARP1, PARP2) and X-ray repair cross-complementing protein 1 (XRCC1)), their binary and multi-component complexes under true equilibrium conditions by using Dynamic light scattering (DLS) technique. Most of proteins and heterocomplexes examined have been revealed to exist predominantly in a dimerized form of the respective protomers (i.e. homodimers and dimers-of-heterodimers). PARP1 has been detected to be exceptionally unique in the possible formation of homotetramer upon the self-association. We have applied for the first time DLS technique to determine the hydrodynamic sizes of PARP1 and PARP2 molecules covalently bound with poly(ADP-ribose) (PAR) polymers synthesized upon the automodification reaction in the absence and presence of main BER enzymes – Polβ, APE1 and XRCC1. Both PARPs have been detected to form huge conglomerates stabilized via coordination of Mg^{2+} ions with PAR polymer; the conglomerates are destructed in the presence of EDTA due to disruption of the Mg^{2+} coordination bonds. We have revealed difference between PARP1 and PARP2 in the mechanism and efficiency of their regulation by XRCC1 protein. This work was supported partially under Russian State funded budget project (VI.57.1.2, 0309-2016-0001) and RFBR grant 17-04-00925.

P.18-095-Tue
Evaluation of the *B. subtilis* proteinase stability in the model conditions of the gastrointestinal tract of chickens

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The problem of improving of poultry industry with goal to supply of high-quality food protein for human consumption is still actual and important. Bacterial proteases effectively cleavage of protein complexes in components of chicken mixed fodder to increase the digestibility of nutrients. *B. subtilis* serine subtilisin-like proteinase has high proteolytic activity, stability and is not toxic to hens. The aim of the study was to establish the stability of subtilisin *B. subtilis* to the aggressive environment of the gastrointestinal tract of chickens in order to evaluate its potential as a probiotic supplement. The stability of subtilisin was studied in a model experiment that simulates the conditions (pH and time) of the gastrointestinal tract of chickens – a sequential transition of the enzyme to goitre conditions (pH 5.5 for 50 min), then the stomach (pH 3 times 90 min), small intestine (pH 6.5 for 30 min) and large intestine (pH 7.5 for 70 min) at 40°C. A Britten-Robinson universal buffer was used as the medium. At pH 5.5, the activity of the enzyme corresponded to the level of control. At pH 3, subtilisin activity decreased by 15–20%, but when the enzyme passed into alkaline medium (pH 6.5 and 7.5), it again rose to the level of control. It was also investigated the effect of bile on the activity and stability of subtilisin. In the spectrum of bile concentrations from 0.01% to 1% for an hour at 40°C, the enzyme activity remained at the level of control. At a bile concentration of 5%, the residual activity of the enzyme was 80%. The data obtained indicate that subtilisin is able to maintain high activity in the whole gastrointestinal tract of chickens. This work was performed in accordance with the Russian Government Program of Competitive Growth of Kazan Federal University. The experiments on the characterization of extracellular hydrolytic activity of bacilli were carried out on means of Russian Science Foundation (Project No. 16-16-04062).

P.18-096-Wed
Implication of the Hsp40 partner in the function of the Hsp70-based protein disaggregase system

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Diverse stresses disturb protein homeostasis within the cell. Among others, the molecular chaperones forming the Hsp70-based disaggregase system play a key role in maintaining a healthy proteome. This machinery has to be well qualified to confront the aforementioned events and solve them in a favourable way. The present work studies how different pairings of Hsc70 with its Hsp40 co-chaperones (DnaJB1 and DnaJA2) broaden the functionality and effectiveness of the former. Aggregation

prevention experiments with the amyloidogenic protein tau and refolding experiments with model substrates such as luciferase and glucose-6-phosphate-dehydrogenase prove that members of these J protein families differ in their holdase and foldase properties, as switching the co-chaperone substantially changes the output of the performed assays. These results may be underlined by the fact that DnaJB1 and DnaJA2 show different oligomeric states. As seen in crosslinking experiments, DnaJB1 appears to be a dimer whilst DnaJA2 also forms adducts of higher molecular mass. Furthermore, a deletion mutant of DnaJA2 lacking the C-terminal domain, which comprises an elongation compared to DnaJB1, shows a behaviour that appears to be in between those of the two full-length co-chaperones, both functionally and structurally. Taken together, these results highlight the importance of the pairings of Hsc70 with its co-chaperones for an optimal performance and provides some insights into their characterization.

P.18-097-Mon
Novel unspecific proteasome regulatory mechanism: evidences in favor

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Ubiquitin-proteasome system degrades most intracellular proteins. Its principal elements are the proteasomes. Free 20S proteasomes and proteasomes bound to regulators (19S, 11S, PA200) represent different forms of proteasomes. Besides regulator complexes, there are evidences of proteasome activity modulation by its potential substrates. Previously, we have shown that amyloid beta peptide and heat shock protein 70, both decreased the activity of purified 20S proteasomes, and both activated 26S proteasomes (20S complexes with 19S regulators). To study if the observed effects represent coincidence, or reflect putative additional mechanism of proteasome regulation, we investigated effects of a panel of peptides (four) and proteins (five) with different molecular weight on the activity of two main proteasome forms. We characterized 20S and 26S proteasomes activity in proteasome-enriched protein fractions from lysates of HepG2 cells using fluorogenic substrates. Activity in the "26S fraction" was increased by 50% following incubation with 10 μM of 36 a.a. peptide. Regardless of concentration (1 or 10 μM), short peptides (15 a.a.) did not affect the proteasome activity. Most of the used proteins in concentrations of 1 μM and 10 μM increased (from 30 to 150%) the proteasome activity in the "26S fraction". Interestingly, peptides and proteins exerted inverse effect on proteasomes in the "20S fraction". All peptides, except for one lacking predicted proteasome hydrolysis site, increased the proteasome activity in the "20S fraction" in the range 41–80%. Proteins showed either no effect or decreased the proteasome activity by 40–60%. Similar results were obtained using purified 20S and 26S proteasomes. Our data indicate putative presence of unspecific proteasome regulation mechanism. Study was supported by Russian President Foundation grant MK-3613.2017.4 and by the Program of fundamental research for state academies for 2013–2020 years (№ 01201363822).

P.18-098-Tue
[*PSI*+] prion variants belong to just two structural types

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The [*PSI*+] prion related to the Sup35 (eRF3) protein has phenotypic variants, or strains, which differ in the strength of nonsense suppression and mitotic stability. The number of possible variants is unclear, and may be very high. The structural basis of this variation is characterized poorly. To understand better the structure of [*PSI*+] variants, we mapped the proteinase K-resistant cores of Sup35 prion amyloids, prepared from many [*PSI*+] isolates. In all preparations, the major resistant region included fully protected first 31 residues and partly protected region up to residue 72. Two distinct digestion patterns were observed for this proximal 72-residue region, which correlated with the "strong" and "weak" [*PSI*+] phenotypes. Furthermore, all "strong" structures were tolerant to overproduced Hsp104 and caused cell inviability upon Sup35 overproduction, while "weak" structures showed opposite properties. The proteinase-resistant peptides were also found in four other regions (distal cores) within the Sup35 N (residues 1–123) and M (124–253) domains. Notably, only the N domain is usually considered amyloidogenic. Protection of these regions differed between [*PSI*+] isolates, but it appears that the distal cores did not significantly affect the [*PSI*+] phenotype. Protease resistant fragments of the [*RNQ1*] prion were found by serendipity, located at the extreme C terminus of Rnq1. Thus, Rnq1 prion structure mirrors the Sup35 prion structure. The work was supported by the Russian Scientific Foundation grant 17-14-01092.

P.18-099-Wed
Regulation of the Hsc70 chaperone system by the acidic insertion in the substrate binding domain of human Hsp110

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Hsp110 chaperones are relatively abundant proteins in mammalian and yeast cells, whose expression is regulated by different types of stress. These chaperones are closely related to the Hsp70 protein family, sharing both sequence and structural homology. Hsp110s are built by two domains: an N-terminal domain able to bind and hydrolyze ATP (NBD), and a C-terminal substrate binding domain (SBD) that contains a polypeptide binding site, which can be divided in α and β subdomains (SBD-α and SBD-β). The SBD of Hsp110s harbors the main structural divergences with Hsp70s, consisting in: i) the insertion of an acidic subdomain (AS) in the SBD-β and, ii) an intrinsically disordered C-terminal extension. The sequence of these protein regions also differentiates yeast and metazoan Hsp110, being both subdomains larger in metazoans. Together with Hsp70 and Hsp40 chaperones, metazoan Hsp110s unleash a potent protein aggregate reactivation activity both in vitro and in vivo. However the precise role of Hsp110 in protein disaggregation remains unanswered. Among other proteins in the eukaryotic cytosol, as Bag1 and HspBP1, Hsp110 possess a strong nucleotide exchange activity on their Hsp70 counterparts. Also, Hsp110 can act as chaperones protecting proteins from aggregation. Here we study how the protein aggregate reactivation activity of the Hsc70/Hsp40/

Hsp110 system is regulated by the AS of Apg2, one of the three representatives of the family in humans.

P.18-100-Mon
Intrinsic protein disorder in histone lysine methylation

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Histone lysine methyltransferases (HKMTs), catalyze mono-, di- and trimethylation of lysine residues, resulting in a regulatory pattern that controls gene expression. Their involvement in many different cellular processes and diseases makes HKMTs an intensively studied protein group, but scientific interest so far has been concentrated mostly on their catalytic domains. In this work we set out to analyze the structural heterogeneity of human HKMTs and found that many contain long intrinsically disordered regions (IDRs) that are conserved through vertebrate species. Our predictions show that these IDRs contain several linear motifs and conserved putative binding sites that harbor cancer-related SNPs. Although there are only limited data available in the literature, some of the predicted binding regions overlap with interacting segments identified experimentally. The importance of a disordered binding site is illustrated through the example of the ternary complex between MLL1, menin and LEDGF/p75. Our suggestion is that intrinsic protein disorder plays an as yet unrecognized role in epigenetic regulation, which needs to be further elucidated through structural and functional studies aimed specifically at the disordered regions of HKMTs.

P.18-101-Tue
Phage display antibodies (selected to Ectromelia virus) capable of Variola virus in vitro neutralization: implementation for p35 neutralize epitope mapping

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Five phage display antibodies (pdAbs) against ectromelia virus (ECTV) were selected from vaccinia virus (VACV)-immune phage-display library of human scFv antibodies. ELISA demonstrated that selected pdAbs could recognize ECTV, VACV, and cowpox virus (CPXV). Atomic force microscopy visualized binding of the pdAbs to VACV. Three of the selected pdAbs neutralized variola virus (VARV) in the plaque reduction neutralization test. Western blot analysis of ECTV, VARV, VACV, and CPXV proteins indicated that neutralizing pdAbs bound orthopoxvirus 35 kDa proteins, which are encoded by the open reading frames orthologous to the ORF H3L in VACV. The fully human antibody fh1A was constructed on the base of the VH and VL domains of pdAb, which demonstrated a dose-dependent inhibition of plaque formation after infection with VARV, VACV, and CPXV. To determine the p35 region responsible for binding to neutralizing pdAbs, a panel of truncated p35 proteins was designed and expressed in Escherichia coli cells, and a minimal p35 fragment recognized by selected neutralizing pdAbs was identified. In addition, peptide phage-display combinatorial libraries were applied to localize the epitope. The obtained data indicated that the epitope responsible for recognition by the neutralizing pdAbs is discontinuous and amino acid residues located within two p35 regions, 15–19 aa and 232–237 aa, are involved in binding with neutralizing anti-p35 antibodies. This study was supported by the Russian Scientific Foundation (project #16-14-00083).

P.18-102-Wed
The C-type lectin-like receptor Nkrp1b: structural features affecting protein conformation and interactions

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NK cells represent substantial part of the innate immunity as they promptly recognize virally infected and tumor cells or regulate adaptive immune responses. These functions are controlled by the NK receptors. This project elucidates structure of the inhibitory C-type lectin-like receptor Nkrp1b and highlights its several structural features (the stalk, loop and oligomerization state), which might affect whole protein conformation or interactions. To study these structural aspects, two Nkrp1b protein forms were recombinantly prepared: entire ectodomain and ligand-binding domain lacking the stalk. Both protein forms fold as monomers and homodimers, therefore, their functionality was tested. Bone-marrow derived cells expressing Nkrp1b ligand were incubated with fluorescently labeled recombinant proteins and their interaction was analyzed by scanning fluorescence microscopy. Thereafter, homology models of the protein forms were proposed using distance restrains obtained by chemical cross-linking and disulfide bonds characterization. Distance constrains related to dimerization interface were taken in account as well. Finally, homology models were validated by ion mobility-mass spectrometry, when their collision cross sections were compared to the experimentally obtained values of native proteins. Taking our results together, we suggest the stalk region does not affect protein fold, nor Nkrp1b dimerization as it was thought for decades, or even its ability to interact with the ligand. Moreover, we show that only monomeric Nkrp1b forms are able to interact, nevertheless, homodimers occur. This might point out the regulatory function of the stalk region. This work was supported by the projects no. 200816, LH15010, LD15089, "NPU II" project LQ1604, LM2015043 CIISB for CMS BIOCEV; LTC17065, CZ.1.05/1.1.00/02.0109 BIOCEV, CZ.1.05/4.1.00/16.0347 and CZ.2.16/3.1.00/21515.

P.18-103-Mon
Isolation of mycobacterial monooxygenase EthA

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Protein EthA is NADPH dependent, FAD-containing mycobacterial monooxygenase that catalyses activation of thioamide and thiourea drugs, like ethionamide, isoxyl or thiacetazone. These antitubercular drugs inhibit the biosynthesis of mycolic acids, which are the hallmark of the cell envelope of mycobacteria, including the important human pathogens *Mycobacterium tuberculosis* and *Mycobacterium leprae*. EthA encoded by the *ethA*

gene, which is conserved across the *Mycobacterium* genus, belongs to the Baeyer-Villiger family of monooxygenases. The key role of monooxygenase EthA in the activation of ethionamide was revealed in 2000, however important knowledge regarding the nature of this protein and its physiological function in mycobacteria is still missing. Moreover, the efforts to crystallize this protein and to solve its structure were not successful yet. The results of our work indicated that the recombinant EthA isolated from non-pathogenic strain of mycobacteria, *M. smegmatis* overproducing this protein by an inducible expression system, is more stable and is more active in modification of isoxyl and thiacetazone, than the EthA protein isolated from *E. coli* producer. We tried to produce and to isolate the orthologs of EthA from *M. tuberculosis* ($EthA_{tb}$), *M. smegmatis* ($EthA_{smeg}$) and *M. thermoresistibile* ($EthA_{thermo}$) strains. All of the proteins were isolated by affinity chromatography and their activity was determined by monitoring their ability to modify thiourea drug isoxyl. While the proteins $EthA_{tb}$ and $EthA_{smeg}$ precipitated during desalination by gel filtration and lost their activity, the protein $EthA_{thermo}$ was isolated in the active form. This protein will be used for crystallography purposes and further biochemical characterization of this clinically important monooxygenase.

P.18-104-Tue
Regulation of chaperone – co-chaperone interaction by 14-3-3 proteins

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Maintenance of stable protein homeostasis is ensured by complex chaperone network cooperation within which the chaperone protein specificity is determined by interaction with its co-chaperones. One of such modulator of chaperone action is Tomm34. This protein contains a conserved solvent exposed linker sequence between its TPR domains which is engaged in the regulation of Tomm34 ATP-dependent binding to Hsp70. Simultaneously the linker comprises overlapped binding motifs for Protein Kinase A (PKA) and 14-3-3 protein family. We have verified that Tomm34 can be phosphorylated *in vitro* and showed that the phosphorylation is crucial for binding to 14-3-3 proteins. We then employed combination of size exclusion chromatography and structural mass spectrometric techniques to study the effect of Tomm34 phosphorylation in its subsequent interaction with 14-3-3γ in more detail. We prepared selected Tomm34 serine mutants and showed that Ser160 located in the interdomain linker is the key residue for the interaction. Interestingly, Ser93 from the first TPR bundle had also some role in the binding. We then further studied how 14-3-3 influences the binding of Tomm34 to chaperone Hsp70. This project is supported by the Czech Science Foundation (16-20860S). Access to the MS facility was enabled through MEYS/EU financial support (CZ.1.05/1.1.00/02.0109, LQ1604 and LM2015043 CIISB).

P.18-105-Wed
Characterization of protein interactions between nonstructural protein 1 and human guanylate binding protein 1

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Nonstructural protein 1 (NS1) is one of the 11 known proteins expressed in *influenza A virus (IAV*) which causes influenza in many animals as well as humans. It interacts with cellular proteins including IFN- induced proteins to antagonize the host immune response against the viral infection. Human guanylate binding proteins (hGBPs) which combat intracellular bacteria and parasites are one group of the superfamily of IFN-inducible effector molecules. Particularly hGBP-1, the best characterized member of the hGBP family, was described to possess antiviral activity in living cells against *IAV* replication. However, the antiviral activity of hGBP-1 was antagonized by the virus protein NS1 and co-localization of both proteins was detected in living cells. We cloned the cDNA of NS1 (*IAV*, strain H1N1; PR8) into bacterial expression vector, and expressed and purified both recombinant proteins NS1 and hGBP1. We quantified a direct interaction of NS1 and hGBP1 in micro molar range using microscale thermophoresis (MST) and observed that the interaction is independent of nucleotide binding of hGBP1. The protein hGBP1 is composed of an N-terminal large GTPase (LG) domain, a middle domain and a C-terminal GTPase effector domain (GED). We generated different truncated mutants of hGBP1 and identified both the GED and LG domain as two distinct interaction sites for NS1 with different binding affinities. Moreover, we found no interference of NS1 in dimerization of full-length hGBP1. We also examined the influence of NS1 on enzymatic activity of full-length hGBP1 which showed no substantial impairment in contrast to the published data. This may suggest that additional proteins or cellular factors may be required for NS1 to cause the inhibitory effect on the GTPase activity of hGBP1 found in cellular studies.

P.18-106-Mon
Scaffold attachment factor A binds in the oct4 gene promoter preferentially to guanine quadruplex over the double helical DNA

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Scaffold attachment factor A (SAF-A) belongs to the family of human heteronuclear ribonucleoproteins. SAF-A contains two nucleic acid binding regions: the N-terminal domain, known as SAP-domain, which is highly conserved in DNA-binding proteins and the C-terminal part containing RGG boxes that are generally responsible for RNA binding. Recent works suggest that RGG boxes have structurally specific affinity to guanine quadruplexes (G4); G4 are non-canonical secondary structures that might be adopted by certain G-rich sequences of nucleic acids under specific conditions. In genomes, they are often found at telomeres or promoter regions of various genes, where their regulatory function is supposed. The parallel form of G4 was observed at in the promoter region of the *oct4* gene. The Oct4 is well known as key regulator of pluripotency of mammalian stem cells. It was demonstrated that SAF-A positively regulates expression of *oct4* via binding to the G-rich region of its promoter. To elucidate the SAF-A binding mode, we prepared GST-tagged full SAF-A

protein and its two potential DNA binding domains, using pGEX/*E. coli* expression system and performed interaction studies with respect to the conformation of target DNA: electromobility shift assay, as well as, filter binding assay (DOT-blot) showed, that the N-terminal domain does not bind to the region, irrespectively of its conformation. N-domain may loss its binding activity due to bulkiness of N-terminal tag. In contrast, the C-terminal domain does bind, but without any DNA-structural selectivity. The full protein SAF-A preferably binds to G4 form of *oct4* promoter region. To characterize this interaction in more detail we performed the ITC calorimetry experiments, followed by CD spectroscopy measurements, which indicate changes of the secondary structure of interacting partners. This work was supported by the CSF (17-19170Y)and by the project SYMBIT reg. nr. CZ.02.1.01/0.0/0.0/15 003/0000477 financed by the ERDF.

P.18-107-Tue
Metabolism of different types of polyubiquitin chains

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Ubiquitination is one of the major post-translational modifications of proteins in eukaryotic cells. In addition to the well-known functions such as the targeting of substrates for proteasomal degradation and DNA repair, polyubiquitin chains are also involved in other major cellular processes, for example, they participate in the regulation of the cell cycle and functioning of the immune system. The incredible diversity of polyubiquitin signals derives from the ability of ubiquitin to form chains of various branched types via isopeptide bonds between the ε-NH_2 group of any of the seven lysines (K6, K11, K27, K29, K33, K48, K63) of one ubiquitin with the C-terminus of the other ubiquitin. Abundance of these chains in the cell is different. It has been supposed that each of these different linkages imparts the unique structural and dynamic properties of the polyubiquitin chain. The function of the polyubiquitinated chains in proteasome degradation and DNA repair consisting of K48 and K63 bonds, respectively, are well studied. The cellular functions of other, so-called non-canonical chains of ubiquitin, are substantially less defined. This study examined the intracellular metabolism of polyubiquitinated conjugates and the substrate specificity of the proteasome for different ubiquitin linkage types, using a combination of flow cytometry and high throughput sequencing. The reported study was supported by Russian Scientific Foundation project #14-14-00585.

P.18-108-Wed
Interactions of a bacterial Argonaute protein with DNA targets *in vitro*

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Argonaute proteins are central components of RNA interference in eukaryotes but the functions of homologous proteins in prokaryotes remain largely unknown. *Rhodobacter sphaeroides* Argonaute protein (RsAgo) was shown to preferentially recognize foreign genetic elements *in vivo* suggesting its role in RNA interference in bacterial cells. RsAgo was proposed to use guide RNAs to recognize complementary target DNA, leading to inhibition of transcription and also its nucleolytic cleavage by accessory nucleases. However, the mechanisms of specific DNA targeting by RsAgo and, in particular, the details of its interactions with double-stranded DNA molecules are unknown. In the present study, we analyzed the interactions of guide-loaded RsAgo with dsDNA targets *in vitro*. Using the gel shift assay, we showed that successful loading of RsAgo onto dsDNA requires prior DNA melting. The boundaries of the assembled ternary complex of RsAgo with guide RNA and dsDNA were revealed by footprinting methods. Possible interactions of RsAgo with RNA polymerases of *Escherichia coli* and *R. sphaeroides* were tested using the bacterial two-hybrid system, and the domains of the β- and β′-subunits of RNA polymerase that are likely involved in interactions with RsAgo were identified. The results suggest that recognition of dsDNA targets by RsAgo *in vivo* may be facilitated by DNA replication and/or transcription, a hypothesis that is now under investigation. This work was supported in part by the Grant of the Ministry of Education and Science of Russian Federation 14.W03.31.0007.

P.18-109-Mon
Intrinsically disordered proteins – how the solvent influences the interaction between the intrinsically disordered transcription factor c-Myb and its interaction partner CBP

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Intrinsically disordered proteins (IDPs) are characterized by a partial or global lack of secondary structure elements under physiological conditions, which allows them to bind to their interaction partner with low affinity and high specificity. A common feature among IDPs is a folding upon binding process, during which the IDP samples through its funneled energy landscape in search of favorable intra- and/or intermolecular interactions, stabilizing the final complex. This structural plasticity enables IDPs to play key roles in cellular signaling and regulatory pathways. However, thermodynamics, kinetics and binding mechanisms of IDPs are poorly understood. In this work we present the impact of solvent and co-solutes on the interaction of the intrinsically disordered transcription factor c-Myb and its interaction partner CREB-binding protein (CBP). We use different techniques, including temperature jump relaxation and microscale thermophoresis to study both kinetics and thermodynamics of the interaction. By applying a variety of co-solutes, such as osmolytes, electrolytes, artificial polymer crowders, as well as protein crowders like BSA and Lysozyme we are able to quantify the effect of each co-solute on the interaction of c-Myb and CBP. Further work will include a mutagenic study, revealing the impact of the solvent at nearly residue level.

P.18-110-Tue
In vivo study of copper transporter 1 ectodomain (NdCTR1) chelating properties

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Copper (Cu) is an essential trace element because it is a cofactor for variety of vital enzymes and it also acts as a modulator of transcription factors, cell cycle, apoptosis and signalling. In opposite

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to plenty Cu pathways inside the cell, there are only two Cu importers into the cell: highly specific CTR1 and nonspecific DMT1. NdCTR1 contains three metal-binding motives and plays crucial role in import by catching Cu(II) from extracellular donors and guiding them as Cu(I) to the membrane pore. Besides Cu NdCTR1 can also bind silver (Ag) ions, which are isoelectronic to Cu(I), and cisplatin. Mechanism of NdCTR1 action during Cu import by CTR1 is not fully understood yet, also remains unclear whether it participates in Cu(II) reduction prior to transport. In present work hNdCTR1 fused with GST was cloned in *E. coli*. As was shown by AAS bacteria synthetizing GST-NdCTR1 accumulated significantly more Ag and Cu compared to GST-producing strain. NdCTR1 increased resistance of bacteria to Ag and Cu ions as well as to silver nanoparticles. Gel filtration and immunoprecipitation experiments with subsequent measurement of metal concentration in fractions revealed that major amount of it is bind to NdCTR1. As GST-NdCTR1 formed inclusion bodies, however GST alone did not, we performed bioinformatical analysis of its structure and showed that last 13 a.r. of NdCTR1 demonstrate high hydrophobicity. Due to presence of 8 (13%) histidine residues in NdCTR1, GST-NdCTR1 was isolated on Ni-charged IDA-Sepharose column in a buffer with 6M guanidine chloride. Now we are obtaining NdCTR1 without mentioned hydrophobic cluster to purify NdCTR1 for further experiments. In present work we've shown that NdCTR1 chelates Ag and Cu ions *in vivo* and increases *E. coli* resistance to heavy metals. It's important that as NdCTR1 binds Cu and cisplatin, potentially, it can be used in treatment of Cu-related disorders, characterised by presence of unbound copper, and therapy of malignancies.

P.18-111-Wed
Conformationally predetermined segments of polypeptide chain in proteins: their characteristics and role of context

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A beta-hairpin included beta-bend can be treated as an example of composite local structure which components (beta-bend and fragment of beta-structure) play different structural, energetic and geometric role. In accord to our conception, the fragment of antiparallel beta-structure characterized by increased stability imposes to the linker the definite set of conformations. Wherein, the number of independence conformational variables reduces due to pseudo-cycle formation across hydrogen bonds. As a result the overall number of conformations in this system does not exceed two for main types of beta-bends. Thus the conformation of beta-bend is determined by context, namely assigning beta-structural part of the whole structure. It must be underline that definite conformation is enforced to beta-bend independently to the primary structure of beta-bend itself even if this conformation encounters sterically hinders (so called 'disallowed' conformations). In current study the primary and three-dimensional structures of both parts of the composite structure have been studied. On the basis of representative sample it can be found that the sequence and the structure provide enlarge stability of the context part. In spite to predetermined character of the beta-bend part of the whole structure some peculiarities in amino acid composition rather than in sequence take place. Contacts within beta-hairpin and its part as well as long-distance contacts were estimated by Voronoi-Delaunay tessellation. Degree of conservation of residues in beta-structural part and in beta-bend separately was determined by multiple-alignments of homologous structures and sequences of corresponded protein domains using various matrices of amino acid similarities. Possible biological implementations are discussed. This work was supported by RFBR (project No 17-04-02105) and the Program of fundamental research for state academies for 2013–2020 years (No 01201363825 and No AAAA-A16-11611610173-5).

P.18-112-Mon
Design of lipoate ligase library with following selection of variants for intracellular site-specific protein labeling by derivates of lipoic acids

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Nowadays there is a wide diversity of methods for protein visualization in a living cell. However, existent approaches have major deficiencies and limitations such as cell toxicity or substrate impermeability, large size and low substrate specificity, therefore alternative methods are intensively developed. Currently, there are several variants of lipoate ligase (LplA), an enzyme of 338 amino acids and 38 kDa known to be involved in bacterial oxidative metabolism, which are capable for attaching fluorophores with red and blue fluorescence. Here we report design of the lipoate ligase library with a subsequent selection of variants for intracellular site-specific protein labeling by derivates of the lipoic acid. We selected seven amino acid residues in the wild-type lipoate-ligase sequence that form the substrate-binding pocket. These amino acid residues are replaced by each of 20 amino acid residues, thus creating all sorts of combinations, each of which will form a unique configuration of the active center. The library is constructed from a set of overlapping primers containing random nucleotides at positions encoding the corresponding amino acid residues. The reported study was supported by Russian Scientific Foundation project #14-14-00585.

P.18-113-Tue
Mechanistic insight into HsNMT1-mediated acylation

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N-myristoylation is an essential and irreversible lipid modification in eukaryotes, corresponding to the addition of a myristate group (C14:0) on the N-terminal of a glycine. This modification is carried out co-translationally after excision of the initiating methionine directly on the nascent protein chain by the N-myristoyltransferase enzyme (NMT) or by post-translational manner after proteolytic cleavage of certain proteins. This modification has the role of targeting a protein to specific membranes where it can interact with other partners triggering signaling pathways essential for many cellular processes. The NMTs are monomeric enzymes composed of two GCN5-related N-acetyltransferase (GNAT) fusing domains, a large superfamily of enzymes that use as substrates acyl-CoA derivatives to acylate their substrates. Nevertheless, previous studies suggested that the catalytic mechanism of NMTs is different from that of other GNAT proteins. We present several 3D structures of hsNMT1 in complex with its

two substrates and its two products (Myr-CoA/Octapeptide and Coa/Myristoyl-peptide), recapitulating snapshots of the different steps of catalysis and providing unprecedented details at the active site pocket. Here, we propose a brand-new portrait to both protein catalysis and substrate specificity.

P.18-114-Wed
Knockdown and inhibition of the N-terminal acetyltransferase Naa10/NatA in zebrafish

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N-terminal acetyltransferases (NATs) catalyze the addition of an acetyl group to the N-terminal amine moiety of a protein. Although this modification is extremely common, occurring on 80–90% of human proteins, little is currently known about the physiological role of N-terminal acetylation. The major eukaryotic NAT, NatA, of which Naa10 is the catalytic subunit, acetylates distinct subpopulations of N-termini on the ribosome. Both NatA and Naa10 are important for cancer cell survival and proliferation and may be therapeutic targets. Recently, mutations in the *NAA10* gene were implicated in several developmental disorders, suggesting that this protein may have an important role in development. We employ knockdown techniques, in vitro enzyme assays, in vivo NAT inhibition and label-free quantitative shotgun proteomics to investigate the functional role of the NatA complex in developing zebrafish. We identified the zebrafish orthologue of the human Naa10, determined its expression pattern, and assessed its knockdown phenotype. Furthermore, we developed selective NatA-inhibitors and found overlapping developmental phenotypes when targeting NatA and Naa10. Label-free quantitative shotgun proteomics on inhibitor-treated embryos identifies potential downstream targets of NatA which may contribute to the observed phenotypes.

P.18-115-Mon
Alfa-synuclein amyloid fibrils structure and formation probed by the fluorescent dye thioflavin T

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Alpha-synuclein amyloid fibrils formation accompanies the development of the Parkinson's disease, one of the most common neurodegenerative diseases. Although, alpha-synuclein amyloid fibrils attracted the researchers attention for a long time, not all the features of their structure and formation are revealed. In this work alfa-synuclein amyloid fibrils structure and fibrillogenesis were investigated using the fluorescent dye thioflavin T (ThT). Alfa-synuclein amyloid fibrils formation was visualized by electron microscopy and the kinetics of this process was detected by absorption, fluorescence and CD spectroscopy (absorption, fluorescence, fluorescence excitation spectra and fluorescence lifetime of ThT and CD spectra of fibrils were determined). Formation of amyloid fibrils was also proved by confocal microscopy in the presence of ThT. For correct determination of photophysical characteristics of ThT incorporated into mature alpha-synuclein fibrils and ThT-amyloid fibrils binding parameters a special approach based on spectroscopic investigation of solutions prepared by equilibrium microdialysis was used. One mode (type) of ThT binding to alpha-synuclein fibrils with binding constant about 10^4 M^{-1} and binding stoichiometry of ThT per protein molecule about 1:4 was observed. The comparative analysis of the results obtained by a wide range of physical-chemical approaches for amyloid fibrils formed from different amyloidogenic proteins, allowed to assume the possibility of the existence of one more ThT binding mode to alpha-synuclein fibrils with higher affinity and fluorescence quantum yield of the bound dye, which is difficult to detect because of the very small amount of these binding sites. This work was supported by grants of the RFBR (16-04-01614, 18-54-00022_Bel, 18-34-00366_mol_a and 18-34-00965_mol_a) and Fellowship of President of the Russian Federation (SP – 841.2018.4).

P.18-116-Tue
Protein-nucleic acid complexes: insight into their gas-phase stability

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Interactions between proteins and nucleic acids play a crucial role in all kinds of cell regulations. Understanding of this part of regulation pathways depends on the knowledge of the protein-nucleic acid complex structure and its dynamics. Field of structural mass spectrometry has undergone a remarkable development in recent years. This include a huge increase of commercial availability of instruments coupling ion-mobility to mass-spectrometry. Native mass spectrometry is capable of gently transferring macromolecules into the gas phase and so preserving their tertiary and quaternary structure. Ion mobility can then separate these macromolecules or macromolecular assemblies according to their size and shape. Stability of macromolecules can thus be studied under different conditions by observing the shifts of a drift-time caused by changes of the shape during the unfolding event. We demonstrate here the power of this technique to observe the increase in stability of the protein when interacting with its specifically recognized ligand counterpart – nucleic acid. Further we support these results by high-resolution mass spectrometry measurements, that reveals an involvement of low molecular weight adducts in protein-nucleic acid interaction stability in gas-phase. Well characterized complex of the DNA-binding domain of Forkhead-box transcription factor (FOXO4), with its target DNA sequence – DAF-16 DNA native response element was used as an object of this study. Acknowledgement: This work was supported by the Czech Science Foundation (grant numbers 16-24309S), the Ministry of Education of the Czech Republic (project LH15010; programs "NPU II" – LQ1604 and LM2015043 CIISB for CMS BIOCEV – LTC17065), and the European Regional Development Funds (BIOCEV – CZ.1.05/1.1.00/02.0109).

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P.18-117-Wed
A Mason-Pfizer monkey virus Env-mCherry fusion vector allows visualization of viral precursors transport in cells using fluorescent microscopy

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Mason-Pfizer's monkey virus (M-PMV), as a D- type retrovirus preassembles its immature particles at the pericentriolar region. Upon their assembly, the particles are subsequently transported to the cytoplasmic membrane and released from the infected cell. Trafficking of the immature particles to the plasma membrane (PM) is facilitated by the envelope glycoprotein (Env) that is brought to the pericentriolar assembly site via recycling vesicles. It has been also shown that Env- containing vesicles mediate efficient anterograde transport of preassembled capsids. By using markers of a panel of intracellular vesicles and fluorescently labeled Gag and Env proteins, we have identified those vesicles, where the immature particles meet with the Env. Based on this, we propose a putative mechanism of intracellular trafficking of Env and assembled Gag, where a portion of Env is recycled from the PM through Rab5 sorting endosomes and then it is transported to the C7 cisternae and Rab9 vesicles, where it co-localizes with Gag particles. Then both proteins are co-transported on the vesicles to the plasma membrane.

P.18-118-Mon
Effect of glycation by methylglyoxal on prion protein structure and properties

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We focused on the study of prion protein modification by methylglyoxal, identification of its possible sites, influence on the physico-chemical properties and ability to form different types of amyloid aggregates. Protein glycation occurring under hyperglycemia in diabetes mellitus is involved into the development of various pathologies and might play an important role in the development of neurodegenerative disorders, such as prion diseases, Parkinson and Alzheimer's diseases, etc. It is known that non-enzymatic protein glycation by reducing sugars follows by a cascade of reactions finally forming the so-called "advanced glycation end products" (AGEs). Highly reactive 3-deoxyglucosone, methylglyoxal and glyoxal can modify with amino, sulfhydryl, and guanidine groups of proteins. To study the effect of modification by methylglyoxal on the structure and properties of prion protein glycation was carried out in 0–2.5 mM methylglyoxal concentration range. The formation of AGEs exhibiting intrinsic fluorescence at 335 nm excitation wavelength, is observed after the 20-h modification. It has been shown that the modification of prion protein by methylglyoxal, one of the main in vivo glycation adducts, leads to the formation of the modified arginine residue 27, with arginine residue 51 also a possible target, as well as the generation of AGEs. The glycated prion protein does not aggregate under the conditions of a 75-fold molar excess of methylglyoxal. A slight change in the secondary structure is apparently the cause of impairing in the formation of intermediate oligomers and amyloid fibrils in "seeding" experiments. However, the prevention of the formation of oligomeric forms of prion protein after glycation under certain conditions demonstrated in this work, indicates an ambiguous role of this type of posttranslational modification in the development of neurodegenerative pathologies. The work was supported by Russian Scientific Foundation, project No 16-14-10027.

P.18-119-Tue
Molecular basis of 14-3-3 protein-dependent regulation of calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2)

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Calcium/calmodulin-dependent protein kinase (CaMK) signaling cascades serve as molecular detectors of intracellular calcium ion concentration elevation and transduce this signal by activation of corresponding transcription factors. Protein kinase CaMKK2 is a member of the CaMK family which is involved in energy balance, glucose homeostasis, neuronal development and plasticity, inflammation and cancer. The activity of CaMKK2 is regulated through interactions with its multiple binding partners. Four PKA phosphorylation sites have been identified in CaMKK2 and its catalytic activity is partly inhibited through phosphorylation by PKA. Besides the direct PKA-mediated inhibition, CaMKK2 activity is also regulated through an additional PKA-dependent mechanism based on interaction with 14-3-3 proteins. The molecular mechanism of this interaction is, however, still unclear due to the lack of structural data. In this study, we performed biophysical and structural analysis of CaMKK2 bound to 14-3-3 using analytical ultracentrifugation and small angle X-ray scattering. Obtained data suggest that a monomer of phosphorylated CaMKK2 binds to a 14-3-3 dimer with the kinase domain of CaMKK2 directly participating in this interaction. Previous studies have shown that the 14-3-3 binding inhibits the kinase activity of CaMKK1. To check whether this is also the case for CaMKK2, the effect of 14-3-3 binding on CaMKK2 kinase activity was examined using two physiological downstream substrates of CaMKK2: CaMKI and AMPK as well as a synthetic peptide containing the sequence of the AMPK activation loop. Interestingly, our data suggest that the complex formation does not inhibit but rather increases the catalytic activity of phosphorylated CaMKK2. This suggests that the 14-3-3 protein may play different roles in the regulation of CaMKK1 and CaMKK2. This study was supported by the Czech Science Foundation (Projects 16-02739S).

P.18-120-Wed
Protein quality control enzyme activities and carbonylated protein content in the skeletal muscles and cardiomyocytes of reared rainbow trout, Oncorhynchus mykiss

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Oxidative stress in farming fish resulted from numerous stress-inducing factors including hypoxia and overcrowding causes fish growth delay, high susceptibility to infectious agents, and high percent of lethality. To overcome this limitation for fish farming we explored the idea to enrich commercial diet by the additives with antioxidant capacity such as dihydroquercetin. Two groups

of rainbow trout, Oncorhynchus mykiss, fed by standard or dihydroquercetin-containing (experimental) diet were compared. Besides the level of protein oxidation estimated by carbonylated protein content, the activities of proteases of the main proteolytic pathways, such as proteasome, calpains, and cathepsins, were measured in trout muscles. Oxidized proteins are normally targeted and eliminated by protein quality control machinery with intracellular proteases as the key executors. In fish muscles, including cardiomyocytes, calpains, or calcium-dependent proteases, disassemble myofibrillar protein complexes before free polypeptides could be hydrolyzed by ubiquitin-proteasome system. Dietary antioxidant resulted in partial decrease both in protein carbonyl concentration and calpain but not proteasome activity in trout muscles. Significant effect of dihydroquercetin on protein oxidation level has been observed since a month on experimental diet consumption while decreased total proteolytic activity has been detected in an antioxidant-fed group throughout the experiment. Experimental diet was also associated with substantial increase in individual growth rate and lower lethality. Thus, an antioxidative capacity of dihydroquercetin and their efficiency as a food supplement for reared fish was experimentally confirmed. The KarRC RAS Equipment Sharing Facilities were used in the study. The work was financially supported by the Russian Science Foundation, project no. 17-74-20098.

P.18-121-Mon
Processing of hepatitis B virus e antigen precursor is regulated by conserved cysteine residues within the signal peptide sequence

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Hepatitis B is a liver infection caused by hepatitis B virus (HBV) which can induce both acute and chronic disease. According to WHO an estimated 257 million people are infected worldwide and hence HBV represents a major global health problem. Hepatitis B virus, a member of the *Hepadnaviridae* family, is a small enveloped DNA virus with a genome containing only four open reading frames (C, P, S, and X) that largely overlap and encode multiple proteins using different in-frame start codons. For example, the HBV preC-C gene gives rise to two different products translated from distinct mRNAs – core protein (HBc) and precore protein (HBe). Despite their high sequence similarity, these proteins exhibit different functions and subcellular localizations. HBc is a cytosolic protein with a molecular weight of 21 kDa responsible for the assembly of icosahedral viral particles and pre-genomic RNA encapsidation. On the other hand, the precore precursor (p25) which represents a longer non-particulate version of the core protein (HBc) undergoes a two-step maturation process resulting in production of the extracellular immunomodulatory HBe antigen. A signal sequence directs this protein to the endoplasmic reticulum (ER), where cleavage of the signal peptide (sp) gives rise to the first processing product, p22. P22 can be retro-translocated from the ER back into the cytosol and also can be found in the nucleus of transfected cells, or it can enter the secretory pathway and undergo a second cleavage event at the C-terminus, resulting in secreted p17 (HBe). Here, we report that p25 is not completely translocated into the ER and further processed. A fraction of p25 is phosphorylated and remains present in the cytoplasm and the nucleus. We found that simultaneous mutations of all of the three cysteine residues in the sp sequence do not interfere with the mature p17 secretion, upregulate the efficiency of sp cleavage and causes mislocalization of the intracellular precore pool.

P.18-122-Tue
The interaction of the C-terminal domain of ecdysteroid receptor from *Aedes aegypti* with Zn^{2+} and Cu^{2+}

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Involved in vitellogenesis process, ecdysteroid receptor (EcR) exhibits typical, for nuclear receptors, canonical structure. There is an N-terminal domain, responsible for transactivation, DNA and ligand binding domains and an elastic hinge between them. However, at the C-terminus occurs an additional region F (AaFEcR), which structure and function has not been described yet. In the context of the number of annual infections with dengue and Zika fever viruses, no efficient cure or vaccine against diseases they cause, deepening the knowledge about ecdysteroid receptor functioning may help with planning successful strategies concerning mosquitoes reproduction. The data we obtained, not published yet, about the isolated C-terminal domain of EcR indicate that AaFEcR belongs to intrinsically disordered proteins (IDPs) family and putatively conforms to pre-molten globule-like (PMG-like) shape. Amino acid composition *in silico* analyses revealed the presence of motif (IonBM), that might be responsible for zinc and cooper ions binding. In order to investigate whether AaFEcR is capable of binding Zn^{2+} and Cu^{2+} we conducted ESI-TOF mass spectrometry. For samples of AaFEcR with Zn^{2+} (molar ratio 1:1) we observed forming of the following complexes: ZnAaFEcR, Zn_2AaFEcR. When the molar ratio of AaFEcR with respect to Cu^{2+} concentration equaled 1:1, CuAaFEcR, Cu_2AaFEcR and Cu_3AaFEcR complexes occurred. These results correspond with the hydrodynamic radius decrease in the presence of increasing metal ion concentration, tested with size-exclusion chromatography (SEC). However, examination of the interaction between AaFEcR and Zn^{2+} or Cu^{2+} with circular-dichroism technique did not affect the content of the secondary structures in protein. We suggest that occurring interaction with IonBM does not result in AaFEcR structuring but in its structure contraction.

Structural biology

P.19-001-Mon
Characterization of a second N-terminal domain in Pcf11 from the 3′ processing complex CFIA

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Pcf11 protein is an essential component of the yeast pre-mRNA 3′ processing machinery, conserved in *metazoa*. One subunit of this assembly is Cleavage/polyadenylation Factor IA (CF IA), consisting of Pcf11 as well as a dimer of the scaffold protein Rna14, two copies of the RRM-domain protein Rna15, a ATP-binding protein Clp1. CF IA is required for both the cleavage and polyadenylation of newly transcribed pre-mRNA. The N-terminus of Pcf11 harbours the Carboxyl-terminal domain Interaction Domain (CID) that has been structurally characterized both in isolation as well as bound to ligands based on the carboxy terminal domain (CTD) of RNA polymerase II. A small region near

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the C-terminus has also been studied in complex with Clp1. Other known domains, although less characterized, include a region involved in association with Rna14/Rna15, a region rich in glutamine and two zinc-finger domains. We have identified a novel domain adjacent to CID and have completely determined the backbone and side chain 1H, 13C and 15N chemical shift assignments for the bacterially produced construct. Relaxation analysis indicates that the domain is independently folded and composed of three well-defined alpha-helix. The final high resolution structure confirms the helical fold. The proximity of this previously uncharacterized domain close to the CID prompts speculation for a putative role in modulating CTD and RNA binding, or intermolecular contacts within CF IA. We have used NMR spectroscopy to characterize the RNA binding by this domain and to reveal its role in enhancing RNA affinity by cooperating with the adjacent CID. Despite this cooperativity, we have found motional independence between CID and this domain by using paramagnetic relaxation enhancement (PRE) and 15N relaxation studies. Deletion and site-directed mutagenesis indicate preliminary effects of cell growth when the domain is absent or disrupted. Further tests will aim to pinpoint the processes affected by these mutants.

P.19-002-Tue
Pathogenesis mechanism of familial "Australian" L723P mutation of Amyloid-β precursor protein revealed by structural and dynamical NMR data

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Despite some progress in study of the molecular mechanisms of initial steps of Alzheimer's disease pathogenesis are still unknown. Amyloidogenic Aβ-peptides forming plaques in the brain are products of intramembrane cleavage of Amyloid-β Precursor Protein (APP). At the same time cholesterol interactions with APP have been rather extensively explored, and Alzheimer's disease pathogenesis is known to be closely correlated with the membrane concentrations of various lipid species, the dependencies on cholesterol content being especially frequently discussed. "Australian" (L723P-APP) mutation is identified to be associated with developing early onset Alzheimer's disease. Using of cell-free expression we produced the 13C/15N-isotope labeled, wild-type and mutant APP686-726 fragments. We performed high-resolution NMR in membrane mimetics followed by Molecular Dynamics simulations. We found L723P-APP mutation to cause local unfolding of the last C-terminal turn of the APP transmembrane domain helix and increased accessibility to water. Moreover, we found that unlike the wild-type fragment, the mutant fragment APP686-726 gradually converts from the α-helical to the β-sheet conformation. With the aid of a spin-labeled cholesterol analogue we also characterized the sterol interactions with the APP transmembrane domain in two different membrane-mimicking systems we described novel site near central hinge region of the APP transmembrane helix. The observed diversity of sterol interactions with APP implies an important role of overall state of lipid environment in the Alzheimer's disease development. These findings suggest a straightforward mechanism of the pathogenesis associated with "Australian" mutation, which is able to shift the distribution between the alternative APP transmembrane domain cleavage cascades, resulting in the accumulation of the pathogenic forms of the amyloid-β peptide. This work was supported by RFBR 17-04-02045-a.

P.19-003-Wed
Bacterial synthesis, purification and structural study of the Toll-like receptor 1 cytoplasmic domain

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Today special attention is paid to transmembrane proteins, widely represented by ion channels and membrane receptors. The family of Toll-Like Receptor (TLR) refers to the first type of MPs. TLRs play a critical role in the innate immunity as the first line of host defense. Despite the facts that general scheme of TLRs functioning is known and that the structures of some individual domains of several TLRs are solved, the detailed mechanism of signal transduction into the cell remains unclear. Obtaining the full-length receptor structures, or at least junctions of individual domains, would provide an answer to many questions of this problem. To date, structural problems are solved mainly employing methods of cryoelectron microscopy, X-ray diffraction analysis and NMR spectroscopy. However, main success in the field of first type MPs investigation was achieved by NMR spectroscopy. NMR study of the MPs in membrane-mimicking environment allows not only to obtain an information about the structure of the protein but also about its mobility, and to follow the conformational changes directly in the process of interaction with other proteins or ligands. Such data makes it possible to make an accurate model of MP functioning and shed light on the process of signal transduction into the cell. The results of bacterial expression, purification and NMR spectra assignment of the intracellular domain of TLR1 are shown. The interactions of this domain with the model membrane were studied. The obtained data allow us to clarify previous results about the mechanism of the Toll-Like Receptors functioning, serving the starting point to study the mechanism of signal transduction into the cell when TLR is activated. This work was supported by Russian Science Foundation (project No 14-14-00573).

P.19-004-Mon
Construction of recombinant fragments of tick-borne encephalitis virus E glycoprotein in order to determine an epitope recognized by protective chimeric antibody Encemab

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An antibody-based therapeutic drug for tick-borne encephalitis post-exposure prophylaxis and treatment is under development in Novosibirsk, Russia. This drug is based on high affinity protective chimeric antibody Encemab derived from murine 14D5 antibody against tick-borne encephalitis virus (TBEV) E glycoprotein. When developing a therapeutic drug based on antibody Encemab, it is necessary to determine its antiviral activity mechanism as well as localize an epitope recognized by this antibody on the surface of E glycoprotein. The major goal of this study was to find the epitope by constructing various fragments of E glycoprotein and examining the binding of the antibody to these fragments. At the first step, location of the epitope was estimated by creating three recombinant fragments of E

glycoprotein: rED3_294 and rED3_301 proteins, being two variants of domain D3 and differed by N-terminus, as well as rED12 protein representing domain D1 together with domain D2 of E glycoprotein. Western blot analysis showed that the antibody Encemab binds to both variants of the D3 domain and does not bind to the rED12 protein. Surface plasmon resonance-based affinity measurements revealed that both D3 variants have a comparable affinity for the antibody. This suggests that amino acid residues 294–300, which are only present in rED3_294 protein, do not appear to be part of the epitope region. Next, several recombinant fragments of domain D3 were generated. Western blot analysis revealed that the antibody Encemab can bind only to the N-terminal fragment of the D3 domain of the glycoprotein E, which includes amino acid residues 301 to 339. These results were also complemented by *in silico* docking of predicted 3D structure of the antibody to the D3 domain of E glycoprotein. The research was supported by the Russian Science Foundation (project # 17-74-10146).

P.19-005-Tue
Exploring a proteinaceous inhibitor of the human dUTPase: pioneering structural model of the inhibitory complex

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As a part of preventive repair, dUTPase enzyme prohibits uracil misincorporation into DNA by hydrolysis of dUTP. This important role of dUTPase entitles it as an essential enzyme in most organisms. It has been shown that Stl, a *Staphylococcus aureus* pathogenicity island repressor protein interacts with several trimeric dUTPases. Upon dUTPase-Stl complex formation dUTPase enzymatic activity is inhibited while the repressor function of Stl protein is also perturbed. Besides the possible perspective of designing new antibacterial drugs after the detailed mechanism is revealed, our group has shown that this system has also a potential to design human dUTPase inhibitors for chemotherapy, which are recently in the scope of medicinal interest. We investigated the structure of human dUTPase-Stl protein-protein complex by applying an integrated structural biology approach, while combining the results of *in vitro* laboratory techniques (native gel electrophoresis, steady-state and transient kinetics, isothermal titration calorimetry, native and hydrogen-deuterium exchange mass spectrometry (HDX-MS)) with synchrotron radiation structure determination techniques as circular dichroism spectroscopy, small-angle X-ray scattering (SAXS) and X-ray crystallography. The obtained hitherto unprecedented structural models revealed that upon dUTPase-Stl complex formation the functional homodimer of Stl repressor dissociates, which abolishes the DNA binding ability of the protein. Active site forming dUTPase segments were directly identified to be involved in the dUTPase-Stl interaction by HDX-MS, explaining the loss of dUTPase activity upon complexation. Strikingly a relatively short, 10–15 residues-long peptide segment of Stl showed the most significant decrease in H/D exchange rate upon complexation with both human and phage dUTPases. This peptide therefore will be subjected to further studies as a potential candidate for development of a new, peptide-type inhibitor against this enzyme.

P.19-006-Wed
Lectin RS20L from plant pathogen *Ralstonia solanacearum* – stability studies and crystallization

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Ralstonia solanacearum is a Gram-negative β-proteobacterium, inhabiting water and soil and causing wilt in more than 200 plants. Plant pathogens use protein-carbohydrate interactions for host invasion. Lectins are non-enzymatic carbohydrate binding proteins of non-immunoglobulin nature. Lectin RS20L (the *Ralstonia solanacearum* 20 kDa lectin) has no sequence similarity to any known lectin amino acid sequence. Multiple sugars were tested for RS20L binding by isothermal titration calorimetry. Despite negative testing of monosaccharides, addition of mannosylated oligodendrimers or mannosylated BSA provided positive results. RS20L without a sugar ligand showed low solubility. Additives can suppress precipitation. A selected panel of additives was tested using the nano differential scanning fluorimetry (nanoDSF). This method is based on intrinsic fluorescence of aromatic amino acids in proteins. Fluorescence intensity and the fluorescence maximum heavily depend on the surroundings of the tryptophan and can indicate protein unfolding. $MnCl_2$ with basic pH 8.1 dramatically increased the stability of RS20L. The resulting protein solution was used for crystallization screening. Crystals emerged in 0.1 M Hepes pH 7.5 with 10% (v/v) PEG3350. Selenomethionine substitution is currently chosen to solve the phase problem by multi-wavelength anomalous dispersion (MAD). Beside the expected 20 kDa monomer, a higher molecular form of this protein appears on the SDS-PAGE. Analytical ultracentrifugation confirmed the trimeric form of RS20L in solution at room temperature. Similar properties have been described for the model porin protein OmpF from *Escherichia coli*. Despite the different architecture of porins from Gram-positive and Gram-negative bacteria, antiparallel ß-sheets in secondary structure represent a common denominator. This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601).

P.19-007-Mon
Structure-based design of human carbonic anhydrase XII inhibitors

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Carbonic anhydrase XII (CA XII), one of the isoform of carbonic anhydrase overexpressed in solid hypoxic tumours, was recently identified target for cancer therapy and diagnostics.

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Specific inhibition of CA XII was shown to affect P-glycoprotein (Pgp) mediated tumour cell chemoresistance. Canonical CA inhibitors are mostly aromatic compounds substituted by a sulfonamide, sulfamide or sulfamate group i.e. functions known to bind tightly to the zinc atom in the active site of CAs. Most of the currently used CA inhibitors lack selectivity therefore a current challenge is the design of compounds that can inhibit specific isoforms. Carboranes and metallacarboranes substituted by a zinc-coordinating group were also reported to act as specific and potent CA inhibitors. Here we report on recent advances in the structure-assisted design of carborane and metallacarborane inhibitors specifically targeting CA XII. Recombinant catalytic domain of CA XII was prepared by heterologous expression in *E. coli*. CA XII was successfully crystallized and the crystal structure was determined and refined. The comparison of structures of different CA isoforms further helped to identify inhibitors for the selective inhibition of CA XII. We tested inhibitory property of selected inhibitors containing carborane clusters and we proved their inhibitory potency and selectivity. Furthermore, inhibitors were successfully co-crystallized with CA XII. The knowledge of binding of these inhibitors in the active site of CA XII can further help with the designing of CA XII specific inhibitors.

P.19-008-Tue
Towards the structure of the DeoR metabolic repressor of *Bacillus subtilis*

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DeoR is involved in carbon catabolite repression in *B. subtilis* as the local repressor of the catabolism of deoxyribose and deoxyribonucleosides. The structure of the C-terminal effector-binding domain of DeoR has already been reported. However, to fully understand the allosteric effects of the repressor during the metabolic regulatory process, it is necessary to resolve the 3D structure of the full-length protein in complex with its operator DNA. To achieve this, we initiated structural studies by X-ray diffraction. Recombinant DeoR was prepared by heterologous expression in *E. coli* and purified with yield of 3.4 mg per 1 l of bacterial culture. Biochemical and biophysical analysis revealed that protein forms dimer and binds DNA duplex derived from the operator sequence. Crystallization of DeoR in complex with DNA was carried out using the vapour diffusion sitting- and hanging-drop techniques. Needle-shaped crystals were obtained and presence of DNA was confirmed by fluorescence microscopy in the presence of SYBR-Gold®. The best X-ray diffraction results were obtained to a resolution of 3.9 A. Crystallization conditions are now being optimized to obtain better-diffracting crystals. This project was supported by the Ministry of Education of the Czech Republic (programme "NPU I") project LO1304.

P.19-009-Wed
Structural and molecular characterization of the 14-3-3 protein-dependent activation of yeast neutral trehalase (Nth1)

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14-3-3 proteins form family of highly conserved molecules which were found in all eukaryotes. They interact with and regulate the function of hundreds of different proteins by recognizing specific phosphorylation motifs. Thus 14-3-3 proteins are involved in many important physiological processes such as: regulation of cell cycle, signal transduction, gene transcription or apoptosis. We focused on understanding of the 14-3-3 protein function in the regulation of the neutral trehalase (Nth1, EC 3.2.1.28) from *Saccharomyces cerevisiae*. This enzyme hydrolyses disaccharide trehalose and helps yeast to survive different stress conditions. Nth1 activity is enhanced by the yeast 14-3-3 protein (Bmh1) binding and/or by Ca^{2+} binding within the EF-hand-like motif. For our studies we used site-directed mutagenesis, enzyme activity measurements, MST, H/D exchange coupled to MS, SAXS and protein crystallography. We found that Bmh1 binding induces a rearrangement of the whole Nth1 molecule and enables the proper three-dimensional configuration of the Nth1 catalytic and calcium-binding domains relative to each other. The complex formation stabilizes an intrinsically disordered N-terminal part of Nth1 and moreover Bmh1 directly interacts also with the separate Ca-binding domain of Nth1. Thus the EF-hand-like motif can function as an intermediary through which Bmh1 modulates the structure and function of the catalytic domain of Nth1. This process stabilizes the flexible part of Nth1 active site, so called "lid" loop, which completes the active site of Nth1 by providing side-chains important for catalysis and Nth1 activation. Our crystal structure of fully active Nth1 bound to yeast 14-3-3 protein provides the first high-resolution view of the neutral trehalase from eukaryotic organism as well as highlights the ability of 14-3-3 proteins to modulate the tertiary structure of a multi-domain binding partner.

P.19-010-Mon
Initiation translation complex of *Staphylococcus aureus* ribosome

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Staphylococcus aureus (S. aureus) is a pathogenic bacteria that causes a range of illnesses such as pneumonia and has a high resistance to most commonly used antibiotics. Several antibiotics inhibit protein synthesis, specifically affecting initiation stage, a dynamic and rate-limiting step that should be strictly regulated. There is a certain number of peculiarities in the structure of ribosomes and translation factors as well as in translation regulation in different species. There are specific mRNAs such as *spa* mRNA that due to its structure can easily bind to the *S. aureus* ribosome without the help of proteins S1. Recently cryo-EM structure of empty *S. aureus* ribosome have been determined.

Here, we show the cryo-EM structures of initiation complexes with a constituent small ribosomal subunit (30S), three specialized translation initiation factors: IF1, IF2 and IF3, *spa* mRNA and fMet-tRNA. Our structures could be used for new therapeutic developments in the design of new antibiotics against this bacteria.

P.19-011-Tue
The function of N-terminus in human 4-hydroxyphenylpyruvate dioxygenase

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4-Hydroxyphenylpyruvate dioxygenase (HPPD) is an important enzyme in tyrosine catabolism which catalyses the conversion of 4-hydroxyphenylpyruvate (HPP) to homogentisate (HG). It belongs to the member of non-heme iron(II) alpha-keto acid-dependent dioxygenase. This enzyme is a target for herbicides and pharmaceutical treatment of tyrosinemia. Human HPPD is composes of two domains for each monomer. The active site is located in the C-terminal domain which contains a 2-His-1-carboxylate facial triad to bind the ferrous ion for the activity. This study explored the role of N-terminal segment in the function of HPPD. Truncation of 4 (△S5 mutant) and 8 (△A9 mutant) residues from the terminus showed *ca*. 20% reduction in the activity. Significant decrease in the activity (over 80%) was occurred after deletion of 12 (△R13 mutant) residues. Compared to wild-type enzyme, the k_{cat} and k_{cat}/K_m values were decreased by *ca*. 6- and 9-fold for △R13 mutant enzyme, respectively. Interestingly, the activity was recovered by *ca*. 60 after truncation of 17 (△H18 mutant) residues. Compared to ΔR13 mutant enzyme, the k_{cat} and k_{cat}/K_m values were increased by 4- and 15-fold, respectively. However, the stability of ΔH18 mutant was significantly reduced. The result suggested that residues of Lys10 and Glu12 might play the critical role in catalytic function. It is possible due to their locations in the structure which form H-bonds with E233, Y105 and K109, respectively, to stabilize the domain interfaces.

P.19-012-Wed
Structural characterization of the N-terminal domain of the MexXY-OprM efflux pump response regulator ParR

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Pseudomonas aeruginosa (PA) is one of the most important infectious agents in cystic fibrosis. Introduction of last resort antibiotics such as colistin and polymyxin B against multi-resistant strains of PA shows activation of the two-component system ParRS leading to MexXY efflux pump overexpression. The presence in the environment of PA of such drugs and their interaction with lipopolysaccharide triggered uncharacterized signal that is detected by the membrane-sensor Histidine Kinase ParS. In response, ParS auto-phosphorylates its cytosolic domain on a conserved histidine residue. A phospho-transfer allows the activation of the response regulator ParR on a conserved aspartate residue. Once ParR is activated, it will regulate the expression of several genes including *mexXY* operon. In this study, we purpose the structural characterization of the wild-type response regulator ParR together with a gain of function mutant M59I from the strain PAOW2. Our first results show that unphosphorylated ParR (u-ParR) is a monomeric protein in solution with two domains as suggested by homology modeling and confirmed by SAXS experiment. Interestingly, we show that u-ParR is not able to bind its DNA sequence target. SAXS result also shows folded protein containing flexible region. This was confirmed by limited proteolysis, which leads to the C-terminal domain (CTD) isolation. In addition, the N-terminal domains carrying (NTDw2) or not (NTDwt) the mutation M59I was cloned and purified. Structural investigation in solution showed that NTDwt is a well-folded and globular domain. Crystallization screens on NTDwt gave diffracting crystals that were optimized and X-ray data were then collected at ESRF and SOLEIL synchrotrons. Molecular replacement solution and structure refinement of this domain is now in progress. These structures will shed light on the role of the particular mutation M59I of ParR and will offer a new target for *in silico* drug-design in order to restore AG activity.

P.19-013-Mon
Porphyromonas gingivalis HmuY and *Tannerella forsythia* Tfo – two homologous proteins with different heme-binding properties

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Periodontal diseases belong to a group of infectious diseases, initiated by both an ecological shift in the microbial composition of the subgingival biofilm, and an exaggerated host immune response, leading to inflammation and destruction of tooth-supporting tissues. Most abundant bacterial species isolated from subgingival samples associated with the clinical features of chronic periodontitis are characterized by the presence of microorganisms belonging to so called 'red complex'. Our hypothesis was that members of this complex *Tannerella forsythia* and *Treponema denticola* might utilize similar mechanisms to the *Porphyromonas gingivalis hmu* heme uptake system, with leading role played by HmuY hemophore-like protein, to acquire heme and increase their virulence. Although comparative and phylogenetic analyses suggested differentiation of HmuY homologs in a variety of bacteria and low conservation across phyla of heme-coordinating histidine residues of HmuY, no homologous protein to *P. gingivalis* HmuY was identified in *T. denticola*. We showed that *T. forsythia* produces a HmuY homolog, termed Tfo, which bound heme, but with lower affinity, preferentially in the ferrous form, and in a manner different to that of HmuY. Analysis of the three-dimensional structures confirmed differences between Tfo and HmuY, mainly in the fold forming the heme-binding pocket and the lack of two histidine residues coordinating heme in HmuY, being replaced by two methionine residues in Tfo. Heme binding to apo-HmuY was accompanied by a movement of the loop carrying the His166 residue, thus closing the heme-binding pocket. Molecular dynamics simulations demonstrated that such a conformational change also occurs in the case of Tfo. We conclude that specific accommodation of heme-binding pocket that could be generated exclusively in *P. gingivalis* and other bacteria maintain *hmu* genes allowing transport of heme or other nutrients, depending upon redox conditions.

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P.19-014-Tue
Prevotella intermedia produces two homologous proteins to *Porphyromonas gingivalis* HmuY with different heme-binding properties

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Periodontitis is a group of multifactorial, infectious diseases, initiated by an ecological shift in the composition of the subgingival biofilm, resulting in inflammation and destruction of the tooth-supporting tissues, eventually leading to tooth loss. Among the putative periodontopathogens, *Porphyromonas gingivalis* is considered as the main etiologic agent and key pathogen responsible for initiation and progression of chronic periodontitis. *P. gingivalis* is a heme auxotroph and uptake of this compound is central for survival and efficient infection establishment by this bacterium. Our hypothesis was that *Prevotella intermedia* might utilize similar mechanisms to the *P. gingivalis hmu* heme uptake system, with leading role played by hemophore-like HmuY protein, to acquire heme and increase its virulence. Comparative and phylogenetic analyses suggested differentiation of HmuY homologs in a variety of bacteria and low conservation across phyla of heme-coordinating histidine residues of HmuY. The analyses also showed that the homologs were subjected to duplications before divergence of Bacteroidetes lineages, which could facilitate evolution of the residues binding heme, and functional diversification. In agreement with these findings, we found that *P. intermedia* produces two HmuY homologs, termed PinO and PinA. Both proteins bound heme, but with lower affinity, preferentially under reducing conditions, and in a manner different to that of HmuY. Analysis of the three-dimensional structures confirmed differences between PinO and HmuY, mainly in the fold forming the heme-binding pocket and the lack of two histidine residues which coordinate heme in HmuY, being replaced by two methionine residues in PinO. We conclude that specific accommodation of heme-binding pocket could be generated exclusively in *P. gingivalis* and other bacteria maintain *hmu* genes allowing transport of heme or other nutrients, depending upon redox conditions.

P.19-015-Wed
Purification and structural analyses of the C-terminal part of germ-cell expressed protein from *D. melanogaster*

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Drosophila melanogaster is a model organism in the study of molecular basis of organism development. The growth and maturation of insects are controlled by the coordinated action of two hormones: 20-hydroxyecdysone (20E) and juvenile hormone (JH). Although the 20E receptor has been studied extensively, the identity and function of the JH receptor has been long unknown. Recently, Wilson and Fabian identified the Methoprene tolerant protein (Met), acting as JH receptor. Met binds JH and mediates JH action preventing the precocious differentiation. The deletion of Met is lethal for insects. However in *D. melanogaster* exists Met paralog: Germ cell-expressed protein (Gce), ensuring survival of Met null mutants. The functions of Met and Gce are not fully redundant and proteins exhibit tissue specific distribution. Bioinformatic analyses assign Met and Gce to the bHLH-PAS transcription factors family. Their sequence homology is limited to defined domains while C-termini (MetC and GceC) exhibit significant differences. These fragments are predicted as intrinsically disordered regions (IDRs). The differences between C-termini of Met and Gce can be crucial for proteins distinction. In this study, we present purification protocol and initial structural characterization of GceC. The size exclusion chromatography (SEC) was used to estimate apparent molecular weight (MW) and Stokes radius (Rs). Other analyses were performed using analytical ultracentrifugation (AUC). The Circular Dichroism (CD) was used for the secondary structures content determination. All results demonstrate the disordered character of the GceC with residual secondary structure and propensity for folding. We hypothesize that high flexibility and a range of conformations adopted by the GceC are essential for its activity. Some subtle structural differences between GceC and MetC, in example localization of short structured fragments, could be crucial for their distinction during *D. melanogaster* development.

P.19-016-Mon
Deep-structure of the genetic code and the origin of replication: path invariants as pre-LUCA attachment sites

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The genetic code conserves an imprint of its evolution before the Last Universal Common Ancestor (LUCA), over $3.5x10^9$ years ago, as a model with the time-order of codon assignments based on amino acid synthesis path-length unifies its diverse structural features. Consistent with evidence for pre-LUCA attachment to a cofactor/adaptor tRNA, intermediates of all amino acids (except histidine) retain a free α-carboxyl. Reductive pentose-phosphate cycle (RPC) intermediates, likewise, retain an invariant, a free PO_4^{2-}, and attributing it to a pre-LUCA polyphosphate scaffold led to evidence of the origin of replication. Method: Model-building software, optimized by restricted Hartree-Fock fragment molecular orbital calculations, was used to construct the poly(-sugar-phosphate) replicators responsible for the RPC invariant. Result: D-ribulose-1,5-bisphosphate (RuBP) molecules, linked by terminal PO_4^{2-}, form a ladder-like polymer with parallel polyphosphate scaffolds, crosslinked by the RPC pentose. Hydrolytic cleavage at the C2-C3 bond within carboxylated-poly (RuBP), in an RPC-like manner, yields two 3-phospho-glycerate (PG) bearing strands. The modified glyceraldehyde monomer obtained on strand separation links the origin of replication to the spontaneous, autocatalytic formose cycle. Anti-parallel strands of the 2-ketopentol additionally form a H-bonded duplex, equipped to bind by charge-attraction to a cationic mineral surface. Incorporation of a second monomer – a H-bonding 3-keto-RuBP (anoxic conditions), or non-bonding ribose (spacer) – yields a hetero-poly(pentotide) duplex with anti-parallel strands as an antecedent of an RNA double helix. Conclusion: Invariant groups in the ancient pathways of central metabolism and amino acid synthesis were attributed to pre-LUCA attachment to a scaffold, or cofactor. Evidence resulted that ladder-like polypentotides bridged the interval between pre-sugar autocatalysis and RNA replication in the origin of life.

P.19-017-Tue
Crystallographic studies of the Fab based on high potency VHH specific for IL-17A

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Cytokine interleukin-17A (IL-17A) is a target for therapy of immuno-inflammatory rheumatic diseases and psoriasis. Recently the company BIOCAD (Russia) has developed a novel human IgG1/κ based on VHH to IL-17A as a promising potential drug candidate for autoimmune disease therapy. First, antibodies from *Lama glama* specific for IL-17A were obtained by immunization and phage display methods. Then, based on the obtained monodomain antibody, the classical antibody (BCD-085) was constructed. BCD-085 has very high (KD = 2 pM) affinity to human IL-17A and neutralizes the interaction of IL-17A with the receptor. Currently, BCD-085 is put on the stage of clinical trials. At present time was known two crystal structures of IL-17A in complex with Fab fragments of different specific antibodies (PDB ID: 2VXS, 4QHU). Each of the complexes contained one functional homodimer of IL-17A sandwiched between two Fab fragments. We have shown by using the gel filtration method that the complex of the homodimer of IL-17A with Fab BCD-085 is formed in the ratio 1:1. We speculate that in this case could be another type of interaction cytokine with antibody like interaction the IL-17A with the receptor IL-17RA (PDB ID: 4HSA). We present preliminary structural studies of the Fab BCD-085. The crystals belonged to the space group $P2_12_12_1$, with unit-cell parameters a = 90.56, b = 111.92, c = 165.43, $\alpha = \beta = \gamma = 90$. Diffraction data were collected to 1.9 A resolution using a synchrotron-radiation source ESRF (Grenoble, France). The crystal structure of Fab BCD-085 playing an important role in the initial phase structural investigations of Fab BCD-085/IL-17A and can help to provide new insights into the molecular details antibody-antigen interactions in this complex. This work was supported by the Russian Science Foundation (grant № 17-74-10156). We acknowledge the Structural Biology Group of the European Synchrotron Radiation Facility for granting access to the synchrotron beamlines.

P.19-018-Wed
Hibernating ribosomes of *Staphylococcus aureus* as a potential target for new therapeutics

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Bacteria and especially pathogenic species often cope with stress and nutrients limitations, and their survival strongly depends on the ability to preserve energy. One of the most beneficial mechanism to save energy for bacterium is deactivation of its own translation machinery, namely hibernation of ribosomes. In *S. aureus* this process is prompted by binding of stress protein SaHPF (*S. aureus* hibernation promoting factor) to 70S ribosomes, which triggers their association into inactive 100S ribosome dimers (disomes). In such stand-by mode ribosomes are preserved from degradation and from binding of several antibiotics, at the same time, they can rapidly (within 1–2 min) be transformed back into active ribosomes upon energy recovery. Unlike other bacteria, in *S. aureus* SaHPF is constitutively expressed in the cell, and part of ribosomes are converted into hibernating 100S even in rich media. Finding the way to prevent dimerization may significantly decrease survival ability of *S. aureus*. Our recent cryo-EM studies demonstrated that integrity of giant 100S disomes (5 MDa) is maintained primarily by interactions between C-terminal domains (CTD) of SaHPF with each other. Based on NMR and crystal structures of SaHFP-CTD (see Fatkhullin et al., this issue) we predicted the key amino acids and point mutations which may distort SaHPF dimers. Predicted mutants were purified and analyzed using several biophysical methods (i.e. AUC, DLS, gel filtration coupled MALS, native PAGE). According to our data, hydrophobic residues F160, Ile173, Tyr175 are the key players in SaHPF dimerization. Selected mutations will be next introduced into *S. aureus* cells in order to analyze their survival rate as well as their infectivity in mice. Concluding, our work paves the path for exploring of complementary approaches to combat this severe pathogen. This work was supported by the Russian Science Foundation (grant 16-14-10014), Agence Nationale de la Recherche ANR-10-LABX-0036_NETRNA.

P.19-019-Mon
Studies of inhibition mechanisms, dimerization and DNA binding of the transcription regulator Fur

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Facing the public health threat of bacterial resistance to antibiotics, the development of new strategies to fight pathogens should be a priority. The new ideal therapeutic targets should exert weak evolutionary pressure, be able to disarm or weaken the pathogen and be unique to the microorganism. An interesting strategy is to interfere with the "Battle for Iron" that takes place between hosts and pathogens as iron is vital for all forms of life. To do so, we must target iron dependent sensors like the Ferric Uptake Regulator (Fur) protein, a transcription regulator with a large regulatory network implicated in iron homeostasis and bacterial virulence. Fur proteins from different bacterial species have a relatively similar tertiary structure but their quaternary structures vary: some are homo-dimers and others are homo-tetramers. Our main objective is to study the inhibition mechanisms of Fur proteins and design new inhibitors. To reach this goal, structure-function studies of the relationship between Fur proteins from different species and their inhibitors have been performed and *in silico* models of the interactions (Docking, Free Energy Profiles) have been proposed providing us with mechanistic insight into Fur complexes. These models will guide the experiments answering the same problematic using techniques like X-ray absorption spectroscopy and crystallography. The structural information gathered from this work will be used to better understand the inhibition mechanism of Fur proteins and the development of new anti-virulence molecules as an alternative to antibiotics to which pathogens are becoming resistant.

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P.19-020-Tue
CW-domain of ASHH2 methyltransferase: structural basis of ligand binding and specificity

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The CW-domain is a histone-tail modification reader which is found in many different protein families involved in epigenetic regulation and chromatin remodeling, and functioning by recruiting and directing the proteins to the chromatin. Studies have shown that CW is specific towards methylated H3K4 modification, however specificity is different for different protein families, thus, CW from ASHH2 was shown to be more specific to H3K4me1, while MORC3 and ZCWPW1 are more specific to H3K4me3. It is not well understood what determines the specificity of the domains. The aim of the study is to understand the structural requirements underlying the specificity of ASHH2 CW-domain. The objectives were approached by structural and calorimetric analysis of interaction of wild type, followed up with mutation studies. Results show that specificity of ASHH2 CW-domain towards H3K4me1 is conditioned by energetically favorable enthalpy driven interaction. Study of mutants showed that C-terminal α-helix is an important determinant in stabilization of the structure and binding, functioning via intradomain interaction of L915 and I915 residues with the tryptophan side chains in the binding pocket. Changes in chemical environment around tryptophan side chains can affect the structure of the binding pocket and structure of the whole domain.

P.19-021-Wed
Cryo-high voltage electron microscopic tomography (Cryo-HVEMT) of whole cells of the immortalized murine microglial cell line, BV-2

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Cryo-ET allows three-dimensional (3D) visualization of cellular structural details at nanometer resolution in a near-native state. Acquiring high resolution 2D projection images is a key point of high quality 3D reconstruction. Thick sample suggests abundant structural information with augmented 3D sample volume. However, low penetration power of conventional cryo-EM only can resolve samples embedded in thin (200–500 nm) layer of ice. Sectioning or thinning thick vitrified samples has been suggested as a solution, but it mitigates given specimen volume and comes with difficulties in handling delicate vitrified sample. Here, we use high voltage electron microscopy (HVEM) at accelerating voltage of 1250 kV to reconstruct 3D structure of large mammalian cell, BV-2 without sectioning. Despite the fact that our cryo-HVEMT has been performed manually between −20° and 60° as asymmetric tilting angles and the lack of high tilt angle projections in one side acts as the missing wedge, this study shows the first visualization of thick part (~1000 nm) of the whole cells in 3D using an advanced technique combining HVEM and cryo-ET. We anticipate that the methodology established here will not only constitute a useful tool for studying virus-host cell interactions at various stages during infection, but will also open new ways to investigate cell signaling events and many other cellular processes in general.

P.19-022-Mon
X-ray structure of *Arthrobacter globiformis* M30 ketose 3-epimerase which is useful for D-allulose production

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D-Allulose (or D-psicose) is one of rare sugars of which physiological functions are focused for human healthcare, such as moderating a blood glucose level and fat accumulation. In recent years, the effects of a non-caloric sweetener, a growth regulator to plants and an immune enhancer against pathogenic bacteria have been also reported as biological functions. Several enzymes for the production of D-allulose have been reported so far, however the structural information of those enzymes is limited. The available structures of the potential enzymes for D-allulose production from abundant sugar D-fructose, are *Pseudomonas cichorii* D-tagatose 3-epimerase (homo dimer, PDBID, 2QUL), *Clostridium cellulolyticum* D-psicose 3-epimerase (homo tetramer, 3VNK), and *Agrobacterium tumefaciens* D-psicose 3-epimerase (homo tetramer, 2HK1). Here we determined the X-ray structure of *Arthrobacter globiformis* M30 ketose 3-epimerase which was previously found as D-allulose 3-epimerase (AgD-AE) due to its similar substrate specificity to the known D-psicose 3-epimerases. There are two molecules of AgD-AE in asymmetric unit, and AgD-AE forms homo tetramer by two-fold symmetry operation. The overall structure is similar to that of the known structures of *C. cellulolyticum* D-psicose 3-epimerase and *A. tumefaciens* D-psicose 3-epimerase, but rather similar to the structure of *Mesorhizobium loti* L-ribulose 3-epimerase (homo tetramer, 3VYL). In addition, we found that AgD-AE shows the highest enzymatic activity for L-ribulose. Therefore, AgD-AE could be categorized to be L-ribulose 3-epimerase. Nevertheless, AgD-AE shows the activity for D-allulose with 65% of L-ribulose that is different from the substrate specificity of *M. loti* L-ribulose 3-epimerase which has almost no activity for D-allulose.

P.19-023-Tue
Structural basis of interactions between 14-3-3 proteins and the 14-3-3 binding motifs of calcium/calmodulin-dependent protein kinase kinase

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Calcium/calmodulin-dependent protein kinase kinases (CaMKK1 and CaMKK2) are members of the Ca^{2+}/calmodulin-dependent kinase (CaMK) family involved in adiposity regulation, glucose homeostasis and apoptosis. These upstream activators of CaMKI, CaMKIV and AMP-activated protein kinase are negatively regulated by phosphorylation, which also triggers an association with the scaffolding protein 14-3-3. Studies have shown that CaMKKs bind to various 14-3-3 isoforms and that the conserved N-terminal motif containing phosphorylated Ser74 in

CaMKK1 (Ser100 in CaMKK2) functions as the primary 14-3-3 binding site. Furthermore, a second 14-3-3 binding motif located at the C-terminus containing phosphorylated Ser475 in CaMKK1 (Ser511 in CaMKK2) has also been suggested. Although the C-terminal 14-3-3 binding motif (sequence RSLpSAP) is a canonical "mode I" 14-3-3 binding site, the N-terminal motif (sequence RKLpSLQE) contains a Gln residue at the position +2 relative to the phosphorylated residue pSer. Bioinformatics survey of 14-3-3 binding sites revealed that Gln is seldom found at +2 because the Pro residue and, to a lesser extent, Ser, Gly and Asp also, are frequently found at this position. To elucidate the structural basis of interactions between 14-3-3 proteins and the 14-3-3 binding motifs of CaMKK, we solved the crystal structures of phosphopeptides containing both 14-3-3 binding motifs of CaMKK bound to 14-3-3. The structures showed that both phosphopeptides interact with the amphipathic groove of 14-3-3 similarly to other 14-3-3 complexes. Nevertheless, in the case of the N-terminal motif, the interaction between the side-chain of Gln at the position +2 relative to pSer and the phosphate group appears to abruptly change the direction of the polypeptide chain. This study was supported by the Czech Science Foundation (Projects 16-02739S) and the Initial Training Network, funded by the H2020 Marie Curie Actions of the European Commission under Grant Agreement 675179.

P.19-024-Wed
Structural bases of inactivation of methionine γ-lyase by suicide substrates

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Pyridoxal 5′-phosphate-dependent methionine γ-lyase (MGL; EC 4.4.1.11) catalyzes the γ-elimination reaction of L-methionine and the β-elimination of S-substituted L-cysteine analogues to produce alkyl mercaptans, ammonia and α-keto acids. The enzyme was identified in a number of pathogenic bacteria and primitive eukaryotes. It is absent in mammalians. Thus MGL might be considered as a target in pathogens. We demonstrated that the enzyme catalyzes the γ-elimination reaction of L-methionine and L-homocysteine sulfoxides and the β-elimination reaction of S-alk(en)yl-L-cysteine sulfoxides which are accompanied by an inactivation of the enzyme in both γ- and β-elimination reactions. Three SH-groups of MGL were found to be modified by thiosulfinates, the products of the γ- and β-elimination reactions of sulfoxides. Spatial structures of MGL inactivated in the reactions with L-methionine sulfoxide and S-ethyl-L-cysteine sulfoxide were solved at 1.46 A and 1.59 A. Three SH-groups of the enzyme, Cys4, Cys115 and Cys245 were found to be oxidized to S-alkyl-cysteines. Inactivation of MGL in the β-elimination reaction depends on a length of the thioalkyl groups at the active site residue Cys115. Thioethyl group "prevents" effective substrate binding, while thiomethyl does not. Inactivation of the enzyme in the γ-elimination reaction is mainly due to the oxidation of Cys115. Analysis of the structures revealed that the presence of either thiomethyl fragment or thioethyl fragment at Cys115 results to an inability of Tyr113 to be a general acid catalyst at the stage of methyl mercaptan elimination. Sulfoxides of S-alkyl (allyl)-L-cysteines, suicide substrates of the enzyme, may be potential antimicrobial agents in MGL-containing pathogens. The work was supported by the Russian Science Foundation (project No. 15-14-00009).

P.19-025-Mon
Effect of various additives on the processes of aggregation and dissociation of coat proteins of several helical plant viruses

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Factors determining the direction of aggregation and dissociation processes of the coat protein (CP) of several helical plant viruses have been studied. The ability of various additives (polyelectrolyte's, surfactants and same peptides) to prevent protein aggregation was analyzed. We have shown that the CP of *potyvirus* – Potato Virus A (PVA) – forms associates – clusters consisted of 30 subunits of CP's, that retain in water-salt solution with increased ionic strength and pH (pH 10.5, 0.5 M NaCl). To analyze a low-resolution structure of the protein in solution, we used a method of Small-Angle X-ray Scattering. It was shown that particles of CP PVA do not dissociate under the influence of 10 mM dextran sulfates (15, 100 kDa) but dissociate under the action of 5 mM sodium dodecyl sulfate (SDS). We observed formation complexes of "CP-SDS", consisting of 10–12 small particles joined together as "necklace and bead" complex. The similar effect of SDS is shown for serum albumins (BSA, HSA). Spectral methods show the preservation of secondary structure and loss of tertiary one of the complexes of CP PVA -SDS. The effect of additives on the process of aggregation was studied in the model of amorphous aggregation CP's of *potyvirus* PVA and *tobamovirus* TMV, induced by heating to 42C. We tested specially selected neuropeptides (5–7 aa) and immunomodulatory peptide (2–3 aa). The kinetics of aggregation was monitored by UV absorption at 320 nm by UV-2600 Spectrophotometer and Dynamic Light Scattering by Malvern ZetaSizer instrument. Among some tested peptides, several peptides were effective in suppressing aggregation; others accelerated this process. The possible mechanism and role of disordered segments of CP's plant viruses in the processes their aggregation and dissociation are discussed. The work was supported by the Russian Foundation for Basic Research (projects 18-04-00525) and by the Federal Agency of Scientific Organizations (Agreement N 007-Г3/Ч3363/26) in part of SAXS.

P.19-026-Tue
Structural studies of human superoxide dismutase (SOD1) with its metallochaperone

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The final stages of Cu-chaperone (hCCS) dependent maturation of human Superoxide dismutase (hSOD1), which involves the transfer of copper and disulfide oxidation, have not been fully elucidated. The difficulty is in obtaining human hSOD1-hCCS complexes that are stable over time for crystallisation and

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subsequent crystallographic studies. The complex and the full length hCCS have been regarded as recalcitrant to crystallization due to the highly flexible structure of hCCS. Previous crystallographic work on this interaction focused on the yeast CCS orthologue in combination with copper site SOD1 mutants. However, comparison between hCCS and yCCS reveals significant differences such as absence of Zinc loop and binding sites in yeast. The objective of this work was to understand the structural basis on hSOD1 recognition and maturation catalysed by hCCS. Complex stability experiments on combinations of hCCS and SOD1 mutants confirmed that disulphide formation seems to be both the last step in the SOD1 maturation and the regulator of complex formation with hCCS. Based on these observation, we chose a set of complexes suitable for crystallographic assays. Heterodimer crystals were obtained using a variety of crystallisation screens and optimized using different techniques e.g. microbatch, protein/precipitant proportion, micro, macro and streak seeding. Data collections were performed at Soleil and Diamond synchrotrons and structure was solved using Molecular replacement. The results showed a novel conformation of the SOD1 disulphide sub-loop communicating the presence of hCCS to the SOD1 active site, and orchestrate the timing of copper and disulphide transfer, complexation and complex dissociation.

P.19-027-Wed
Glutamate transporters in archaea and eukaryotes

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Glutamate transporters catalyse the thermodynamically unfavourable transport of anionic amino acids across the cell membrane by coupling it to the downhill transport of cations. This coupling mechanism is still poorly understood, in part because the available crystal structures of these transporters are of relatively low resolution. In this contribution I will present the crystal structures of the archaeal transporter GltTk in the presence and absence of aspartate resolved to the highest up to date resolution, and will report results of molecular dynamics simulations and binding assays to show how strict coupling between the binding of three sodium ions and aspartate takes place. Furthermore I will present the latest results, including Cryo-EM structure of a mammalian homologue, which has revealed a few very interesting peculiarities when compared to archaeal homologues.

P.19-028-Mon
Characterizing substrate specificity of guanosine deaminase from *Arabidopsis thaliana*

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Nitrogen is an essential element for growth and reproduction of plant. In the past decade, it has been characterized in plants and many microbes that purine, a nitrogen-rich compound, is subject to enzyme-dependent degradation and the resulting products are ammonia and/or urea. In brief, the ureide pathway in plants is capable of utilizing urate, an early product from purine degradation, as substrate and undergoes sequential enzyme reactions to produce ammonia, glyoxylate, and/or urea. This ureide pathway has been suggested as one of the possible metabolic pathway for recycling nitrogen in plants. Unlike the downstream of purine catabolism by ureide pathway, the early biological events for purine degradation have been studied recently. In *Arabidopsis thaliana,* among those early events in purine degradation, guanosine deaminase (GSDA), a plant-specific enzyme, was characterized to be responsible for converting guanosine into xanthosine. Product xanthosine is then catalyzed into xanthine for further conversion into urate, a substrate for the ureide pathway. Presence of plant specific GSDA is very unique, because in most organisms guanine serves as a substrate of guanine deaminase (GDA) to produce xanthine. In order to understand substrate specificity of GSDA from *Arabidopsis thaliana* (AtGSDA) and its mechanistic features, we are undergoing structural studies of AtGSDA. In particular, AtGSDA is related in sequences to GDA but differs in its function. Specifically, they contain catalytic residues common for the cytidine/deoxycytidylate deaminase superfamily but their substrate specificities are unclear. Our analyses will provide structural basis for substrate specificity of AtGSDA and characterize enzyme mechanism in the early events of purine degradation in plants. This work was supported by Next Generation BioGreen 21 program of Rural Development Administration (Plant Molecular Breeding Center) of Republic of KOREA.

P.19-029-Tue
Staphylococcus aureus hibernation promoting factor interaction with 70S ribosome determined by high resolution NMR spectroscopy

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Recent structural cryoelectron microscopy studies have shown that in human pathogen *Staphylococcus aureus*, 100S ribosome formation occurs in the presence of long two domain protein SaHPF and its N-terminal domain (NTD) binds to the 30S subunit at the P-site and A-site similarly to its homologs from *E. coli,* EcHPF, EcYfiA and a plastid specific YfiA and may inhibit protein synthesis by preventing mRNA and tRNAs from accessing the 70S ribosome. Additionally, it occludes the binding of several antibiotics such as hygromycin B, tetracycline, edeine, pactamycin, kasugamycin. C-terminal domain (CTD), connected by a linker of 35 amino acids, protrudes out of each ribosome through the E-site and forms a main contact in 100S dimer through homodimerization with SaHPF CTD from the second ribosome. In order to improve interpretation of recently obtained cryoEM data and provide more detailed characterization of the interaction between SaHPF NTD and the functional sites of *S. aureus* ribosome we performed HETSOFAST NMR experiments and observed an intensity changes of SaHPF NTD signals in the presence of 70S ribosomes in solution during imino-protons saturation. For free SaHPF NTD sample we did not observe any influence on the amide signals intensity, while after the addition *S. aureus* 70S ribosome a strong effect (up to ~25%) was observed, which means that we observe a chemical exchange between protein and ribosome protons. Obtained intensity changes allow us to determine that several amino acid residues of SaHPF, located in close proximity to the 16S rRNA, may be involved in contacts formation. Information on SaHPF protein-ribosome contacts will provide crucial knowledge for further antibiotic modification strategy. This work was supported by the Russian Science Foundation (grant 161410014).

P.19-030-Wed
Structural studies of the UxuR and ExuR proteins, regulators of hexuronate metabolism in gammaproteobacteria

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An alternative branch of sugar metabolism that allows bacteria to survive under stress is the Ashwell pathway involving catabolism of D-hexuronic acids. This metabolic pathway is essential for the formation of colonies and motility of *E. coli*. The final product of hexuronic acid metabolism of hexuronic acids is pyruvate that eventually results from the transfer of the Ashwell cycle passing into the Entner-Doudoroff pathway. Processes of hexuronate utilization are controlled by the two paralogous – transcription factors, UxuR and ExuR. ExuR has likely emerged by duplication of an ancestral *uxuR* gene. Both proteins belong to the GntR family transcription factors, whose members share high structural similarity. To date, 3D structures of only few GntR-family proteins have been solved. Based on amino acid sequences, closest relatives of UxuR and ExuR with known structure are FadR from *Escherichia coli* and GntR from *Bacillus cereus*. However, the amino acid identity of these proteins does not exceed 25%. To understand how UxuR and ExuR interact with their DNA targets for cooperative regulation of hexuronate metabolism, one needs a reliable structure of at least one of these regulators. In order to study their structure, ExuR and UxuR have been isolated and purified, and first crystals have been just obtained. This work was supported by Russian Scientific Foundation.

P.19-031-Mon
Cooperation between the C-terminal domains of nucleoplasmin is key to assemble the histone octamer prior DNA deposition

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Histone chaperones are key components of the machinery that regulates chromatin dynamics. We have previously shown that the distal face of nucleoplasmin (NP), the first histone chaperone described, is the protein region involved in substrate binding, and that the complexes formed between NP and the distinct core histones differ in stoichiometry and overall architecture. One NP pentamer binds five H2A-H2B dimers, with each C-terminal domain interacting with one dimer. In contrast, a more complex structure is obtained in the case of H3-H4 and the histone octamer, in which two NP pentamers face each other through their distal face, encaging the basic ligand. In this work, we characterize the NP/octamer complex and present its cryoEM structure. We also demonstrate that post-translational NP modifications contribute to stabilize the NP/histone octamer complexes and that several C-terminal domains must cooperate to form stable complexes with H3-H4 and histone octamers, in contrast with what is observed with H2A-H2B dimers. Finally we show that, *in vitro*, NP is capable of transferring the histone octamer to DNA, assembling nucleosomes. This activity could have biological relevance in processes in which the histone octamer must be rapidly removed from or deposited into the DNA, such as DNA replication in the first steps of embryo development and transcription in highly active genes.

P.19-032-Tue
Development of a model system for studying stiffness on the leading edge of fibroblasts by the method of force spectroscopy

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The mechanical properties of cells are responsible for maintaining the shape of the cells, their mobility and response on various biophysical and biochemical impacts. Cell stiffness often is used as a biomarker for cancer cells identification, as they are softer than healthy ones. The higher elasticity and less adhesiveness of cancer cells increases their mobility and the deformation ability, as well it helps them to migrate during metastases. Thus, there is a certain correlation between rigidity and mobility: the softer the cell, the more mobile it is. The stiffness of cells depends on the reorganization of the cytoskeleton, including intermediate filaments, such as vimentin, which maintains cellular integrity and ensures cell resistance to stress. Measurement of the stiffness (Young's modulus) of cells is carried out using an atomic force microscope. Usually, the cells are located either as monolayer or as single rounded cells on the flat substrate and are pushed by modified probes, which have microsphere shapes. In order to measure the stiffness at the leading edge of the cell, special lavsan substrates, coated with thermoplastic and with grooves at a frequency of 1.6 μm and a depth of 350 nm, were used. On these substrates the cells grow predominantly elongated along the grooves with a well-identifiable leading edge. Unlike measurement on a flat surface, it was obvious that in the case of substrates with grooves, their relief can affect the results of measuring the stiffness of cells. In this connection, additional experiments were carried out, which showed that the variations of the relief do not affect the measured Young's modulus. Thus, the developed approach makes it possible to measure the rigidity of cells precisely at the leading edge. In the course of further experiments, the stiffness of rat and mouse fibroblasts with mutant vimentin, unmutant vimentin and its absence was investigated. The work was supported by a grant from the RSF (14-14-00234).

P.19-033-Wed
Structural insight into glucose repression of the mannitol operon

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Carbon catabolite repression is a regulatory mechanism to ensure sequential utilization of sugars and is usually accomplished by repression of genes for the transport and metabolism of less preferred sugars by a more preferred one. Although glucose and mannitol share the general components, enzyme I and HPr, of the phosphoenolpyruvate-dependent phosphotransferase system (PTS) for their transport, glucose represses the transport and metabolism of mannitol in a manner dependent on the mannitol operon repressor MtlR in *Escherichia coli*. In a recent study, we

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identified the dephosphorylated form of HPr as a regulator determining the glucose preference over mannitol by interacting with and augmenting the repressor activity of MtlR in *E. coli*. Here, we solved the structure of the MtlR-HPr complex to understand how phosphorylation of HPr impedes its interaction with MtlR. The phosphorylation site His15 of HPr is recognized by Glu108 and Glu140 of MtlR and phosphorylation at His15 induces electrostatic as well as steric repulsion between the two proteins. Based on this structural insight and comparative sequence analyses, we show that determination of the glucose preference over mannitol solely by the MtlR-HPr interaction is unique among members of the *Enterobacteriaceae* family.

P.19-034-Mon
Towards characterizing substrate specificity of DAO1 from *Arabidopsis thaliana*

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Auxin is a central plant hormone, regulating many aspects of plant growth and development. Therefore, maintaining its concentration at a critical level is a key biological event for plant. Local concentrations of auxin have been known to be diverse among various types of plant cells, suggesting that its regulation is crucial for plant development. Auxin homeostasis requires a metabolic balance of its biosynthesis and degradation. In the past years, there have been intensive studies for IAA (indole-3-acetic acid) biosynthesis, a major form of auxin, whereas its modification or degradation has not been investigated. It is only recently identified that *DIOXYGENASE FOR AUXIN OXIDATION 1 from Arabidopsis thaliana* (*AtDAO1*) catalyzes a conversion of IAA into oxIAA (2-oxoindole-3-acetic acid), an oxidized form of IAA, which is a major IAA catabolite and subsequently subjected to further degradation. Sequence analysis indicates that AtDAO1 is a member of the Fe(II)/α-ketoglutarate-dependent dioxygenase, and further suggests that AtDAO1 also follows an enzyme mechanism for IAA oxidation essentially identical for other dioxygenases. In plant kingdom, AtDAO1-like gene is well conserved including corn, rice, potato etc. From sequence perspective, AtDAO1 is one of the α-ketoglutarate-dependent dioxygenase family, but its substrate specificity for IAA remains unclear. In order to decipher a substrate specificity of AtDAO1, we initiated structural study of AtDAO1 using X-ray crystallography. Our structural analysis will provide a platform for understanding substrate specificity and mechanistic features of AtDAO1. This work was supported by Next Generation BioGreen 21 program of Rural Development Administration (Plant Molecular Breeding Center) of Republic of KOREA.

P.19-035-Tue
Tandem domains go a long way: the repetitive region in a biofilm-forming protein forms an elongated stalk

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Staphylococcus aureus is an opportunistic Gram-positive bacterium. It can colonise the surface of in-dwelling medical devices, forming a biofilm. Cell wall-anchored (CWA) proteins such as *S. aureus* surface protein C (SasC) mediate biofilm formation. These multi-domain proteins feature a similar organisation: the N-terminal domain mediates adhesion and the C-terminus is attached to the cell wall, while the function of the central repetitive region is unknown. We hypothesise that the repetitive region forms an elongated structure with the necessary rigidity to project the functional N-terminus away from the cell wall surface. We redefined the domain boundaries of the structural units in the repetitive region, Domains of Unknown Function (DUF1542), using bioinformatics approaches. The structure was determined by X-ray crystallography and showed elongated helical bundles, arranged in tandem. Importantly, the new domain boundaries for DUF1542 were verified. Biophysical characterisation confirmed that the interface between tandem domains was essential for both intra- and inter-domain structural stability. We assessed by Quasi-Elastic Light Scattering (QELS) that the shape of DUF1542 domains in solution was elongated rather than globular. The approximate size in solution measured by Small Angle X-ray Scattering (SAXS) for 4 DUF1542 domains showed elongation to the maximum length predicted from the crystal structure, indicating rigidity. Taken together, the structural insight gained for the repetitive region of SasC suggests that the biofilm-mediating domain is displayed at the tip of an elongated and putatively rigid stalk, projecting it away from the cell wall surface. Knowledge of the structure and function of domains in CWA proteins contributes to a better understanding of the mechanism underlying protein-mediated biofilm formation.

P.19-036-Wed
Hydrogen bond network near OH group of 6-(p-hydroxyphenyl) substituent of coelenterazine determines the bioluminescence spectra differences among hydromedusan calcium-regulated photoproteins

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Ca^{2+}-regulated photoproteins are responsible for the light emission of many marine coelenterates. All hydromedusan photoproteins are single-chain polypeptides to which the oxygen-activated substrate, 2-hydroperoxycoelenterazine, is tightly but non-covalently bound. Bioluminescence results from oxidative decarboxylation of 2-hydroperoxycoelenterazine, generating a protein-bound product, coelenteramide, in an S_1 excited state. The bioluminescence spectral maxima of photoproteins from different organisms vary in the range 462–495 nm, despite a high degree of identity of amino acid sequences and spatial structures of these photoproteins. Based on the studies of obelin and aequorin mutants with substitution of the residues located near oxygen of 6-(*p*-hydroxyphenyl) substituent of 2-hydroperoxycoelenterazine by amino acids with different donor-acceptor properties of side chains we found that the hydrogen bond network surrounding the oxygen atom influences a light emission spectrum of the photoprotein. For instance, the replacement of Phe to Tyr and Tyr to Phe in obelin and aequorin, respectively, makes bioluminescence spectrum of obelin similar to that of aequorin and the bioluminescence spectrum of aequorin – to that of obelin. Determination of the crystal structures of some photoprotein mutants before and after bioluminescent reaction clearly supports the suggestion that different hydrogen bond patterns near the hydroxyl group of 6-(*p*-hydroxyphenyl) substituent of the substrate molecule account for spectral differences among

hydromedusan photoproteins. This work was supported by RFBR grant 17-04-00764 and a China-Russia international collaboration grant from the Chinese Academy of Sciences and the Natural Science Foundation of China.

P.19-037-Mon
X-ray structural study of a novel S-type cardiotoxin (CTX) from *N. naja*

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Cobra cardiotoxins (CTXs) are amphiphilic three-finger (loops L1-L3) basic cytolytic polypeptides (59–61 residue-long) that bind to cell membranes, causing a wide range of effects. These include depolarization and necrosis of heart and skeletal muscles, lysis of blood and epithelial cells, induction of toxicity in cortical neurons and various types of cancer cells. Based on binding activities of CTXs to zwitterionic membranes, two distinct types of CTXs, P- (Pro-30-containing) and S- (Ser-28-containing), have been identified, of which the P-type CTX interacts more strongly than the S-type with the membranes. We have isolated several CTXs from *Naja naja* cobra venom using gel-filtration, ion exchange and reversed-phase HPLC and analyzed the amino acid sequences of isolated toxins by mass-spectrometry. The most abundant was an S-type CTX 16-1, a L48V49 analogue of CX3 (P24780) *N. naja*. In the present work, its 3D structure was established by X-ray crystallography. Crystals of CTX 16-1 belonging to $C222_1$ and $P6_422$ space groups were grown by the hanging drop or counter diffusion method. Structures of hexagonal (with three molecules in the asymmetric unit) and orthorhombic (with six molecules in asymmetric unit) crystal form were solved at 2.3 and 2.5 A respectively. X-ray analysis reveals unusual conformation of L2 functional loop in all subunits of hexagonal structure and most subunits of orthorhombic form, while the remaining models reveal a structure reported earlier. Distinctly different is the tip of the L2 in the structures of both crystal forms. This exhibits striking similarity to that found by NMR in dodecylphosphocholine-bound cytotoxin I from *N. oxiana*. Thus, for the first time, via crystallization we obtained a membrane-bound form of an S-type cardiotoxin. This work was partly supported by the Federal Agency of Scientific Organizations and in the frame of Federal Space programm 2016–2025 (ISS *"Science")*.

P.19-038-Tue
Structural study of interactions between the F-BAR domain protein Hof1 and the formin Bnr1

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The formin Bnr1 is recruited to the bud neck in *S. cerevisiae* to assemble actin cables and enable cargo transport between the mother cell and the growing daughter cell. This process is regulated by Hof1, which directly binds to Bnr1 and inhibits its actin nucleation activity. In the absence of Hof1 the actin network structure becomes disorganized, and vesicle traffic is impaired; however, the details of Hof1-Bnr1 interactions have remained unclear. Here, using single particle electron microscopy, we reveal the structures of the FH2 domains of Bnr1 in complex with full-length Hof1 or with its F-BAR domain. The Hof1-Bnr1 complex is a dumbbell-shaped structure, in which the tips of an elongated F-BAR dimer hold two FH2 dimers apart, and these interactions appear to obstruct the actin-binding surfaces on the FH2. In support of this view, bulk actin assembly assays and TIRF microscopy assays show that the F-BAR domain of Hof1 directly inhibits Bnr1-mediated actin nucleation. Taken together, our results allow us to describe a mechanism for how Hof1 controls Bnr1 activity. The authors acknowledge funding from the Russian Science Foundation (grant #14-14-00234 to O.S.), the grant of the President of the Russian Federation (grant MK-2614.2018.4 to T.S.-K.), and the National Institutes of Health (RO1 GM083137 to B.G.).

P.19-039-Wed
Novel coiled coils observed in the C-terminal domains of human septins may aid in filament bundling

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Classical dimeric coiled coils are composed of two α-helices which may be orientated either parallel or anti-parallel with respect to one another. They are characterized by heptad repeats (*abcdefg*) in which hydrophobic residues occupying the *a* and *d* positions stabilize the helical interface. Characteristic residue patterns can often be used to predict their relative orientation, however, as far as we are aware, the predominance of hydrophilic interactions at the interface has yet to be described. Here we observe this phenomenon for the first time in the crystal structures of the short coiled-coils present in the C-terminal domains of human group III septins. Septins are filament forming GTP-binding proteins involved in a wide variety of intracellular events most of which involve membrane remodelling or barrier formation. Crystal structures of peptides derived from human septins 1, 4 and 5 at <2 A resolution show examples of both the parallel and anti-parallel arrangement. In the latter the *a* position is exclusively occupied by hydrophilic residues (including Asp and Glu) which form a continuous chain of hydrogen bonds down one strip of the interface. However, the amino-acid sequence and the anti-parallelism guarantee that the charged residues are never consecutive along this chain. On changing to the parallel orientation those residues which previously occupied the *a* position move out of the interface. Simultaneously those occupying the *d* position move into *a* and a new group of hydrophobic resides assume the *d* position. The sequences appear therefore to be chameleon in nature, possessing two partially-overlapping alternative interfaces. Neither of the two orientations appear to provide great stability and we speculate that dynamic interchange may be physiologically relevant to the formation of individual filaments when parallel and cross-bridges between them when anti-parallel. This work was supported by FAPESP.

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P.19-040-Mon
Biophysical characterization of Rad50 zinc hook domain from thermophilic archeon *Thermoplasma acidophilum*

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Rad50 protein is an essential part of Mre11 complex that plays central role in processes of DNA repair. It is highly conserved among all domains of life which is the consequence of its fundamental biological function. It functions as a homodimer consisted of two long antiparallel coiled-coil arms ended with globular ATP-binding domains, that protrude from the Zn(II)-binding interface called zinc hook. The Zn(II)-mediated assembly is essential for the complex formation. To date, only two significantly different X-ray structures of zinc hook domain were resolved which come from hyperthermophilic archeon *P. furiosus* and *H. sapiens*. It remains unclear how zinc hook assemble to form interprotein zinc binding site with such sufficient stability to work at low intracellular free Zn(II) concentrations. The aim of the study was to biophysically characterize zinc hook domain, which will shed more light on structure stability and thermodynamics of the complex. For this purpose, a central 130 amino acid long fragment of Rad50 from thermophilic archeon *T. acidophilum* was chosen as a subject of study. Experiments were performed using several different techniques: UV-VIS spectroscopy, circular dichroism, fluorimetry and isothermal titration calorimetry. Application of these methods allowed to obtain details regarding Zn(II) affinity towards hook motif, stoichiometry of formed complex and its thermostability. The study provided substantial information about the role of Zn(II) in Rad50 zinc hook structure formation and stability, which is crucial for the functioning of the Mre11 complex. The work was supported by the National Science Centre (2016/21/B/NZ1/02847). Participation supported by National Scientific Leadership Centre Wrocław 2014–2018.

P.19-041-Tue
Oligomerization of ovine prion protein in solution characterized by SAXS

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Recent findings have demonstrated that many neurodegenerative diseases, such as Creutzfeldt-Jakob, Alzheimer's, and Parkinson's diseases, as well as the frontotemporal dementias, are all caused by proteins which are transformed into prions, accumulate, and cause disease. Despite numerous studies, the physiological functions of the cellular prion proteins as well as the pathogenesis and molecular basis of neuronal cell death in prion diseases remain still enigmatic. To date, no treatment is available to revert, stop, or even delay the clinical course of any of these disorders. Mammalian PrP was the first protein to be discovered that can convert into a prion conformation. Its alternatively folded isoform, designated PrPSc, causes Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy in cattle, and scrapie in sheep. The detailed mechanisms underlying this conversion and subsequent aggregation into higher-order aggregates are still unknown. To get better insight into the molecular mechanisms of PrP oligomerization, we analyzed structural behavior of the ovine prion protein in solution at neutral pH using SAXS. Our investigation demonstrates that PrP in solution aggregates and forms oligomeric species of elongated shape of determined size. The pathway leading to formation of these oligomers might be a clue to unravel their biological roles and have fundamental importance to elucidate the pathogenesis of prion diseases. This work was supported by the Russian Foundation for Basic Research (project no 17-00-00487) in part of conducting the SAXS experiments and the Federal Agency of Scientific Organizations (Agreement No 007-Г3/Ч3363/26) in part of data interpretation and modeling.

P.19-042-Wed
The role and potential benefits of structural disorder in viral replication machinery: structural study of Influenza A virus M1 protein

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Matrix proteins are essential components of most enveloped negative-sense RNA viruses. They are the most abundant components of any viruses; being generally globular they are usually crystallized as infinite 2D lattices. However, some parts of these proteins, presumably flexible or unstructured, are not amenable to crystallization, and thus there are no full-length high resolution structures for a number of them. Well known example is the influenza A matrix protein M1, in which only two thirds of the macromolecule are crystallized. Proteins of this type, called intrinsically disordered proteins (IDPs), exist as dynamic ensembles of conformations that do not have a stable folded structure, but nevertheless they carry out their biological functions, often very varied. The concept of IDPs can be applied to explain multifunctional character of viral matrix proteins' activity. Here we employed small-angle X-ray scattering, atomic force microscopy and zeta-potential measurements to characterize the overall structure and association behavior of the full-length M1 at different pH conditions. We demonstrate that the protein consists of a globular N-terminal domain and a flexible C-terminal extension. The globular N-terminal domain of M1 monomers appears preserved in the range of pH from 4.0 to 6.8, while the C-terminal domain remains flexible, and the tendency to form multimers changes dramatically. A predicted electrostatic model of M1 self-assembly at different pH revealed a good agreement with zeta-potential measurements, allowing one to assess the role of M1 domains in M1-M1 and M1-lipid interactions. Together with the protein sequence analysis, these results provide insights into the mechanism of M1 scaffold formation and the major role of the flexible and disordered C-terminal domain in this process. This work was supported by the Russian Federal Agency of Scientific Organizations (Agreement № 007-GZ/C3363/26).

P.19-043-Mon
Crystallization in starved *E. coli* cells studied by synchrotron small-angle scattering

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The study addresses fundamental problems of structural biology regarding the growth of highly structured intracellular ensembles, *in cellulo* crystallization. The importance of the study of biocrystallization is determined by a number of important reasons. First of all, *in vivo* crystallization of proteins and their complexes with nucleotides, beyond crystallization *in vitro*, provides a unique opportunity to investigate their structures directly in the cell. It is known that crystals formed in artificial conditions may be substantially different from those grown natively. Knowledge of the structure of *in cellulo* crystallites is important to understand the basic functional properties of living matter, and for the development of new antibacterial and antiviral drugs. The aim of this study is a characterization of biocrystallization by high brilliance synchrotron X-ray scattering as a major tool for a rapid detection of the crystallization inside the cells and for subsequent determination of the overall structural parameters of the crystallites. We conducted small-angle X-ray scattering (SAXS) studies of activated and inactivated (as a control) *E. coli* cells at the EMBL P12 beamline of Petra-3 (project EMBL SAXS-691). Solutions of DNA-binding protein of starvation (DPS) with and without DNA were also measured. The SAXS signals from the activated cells and from the solutions of DPS with DNA demonstrate well-defined peaks suggesting the presence of ordered crystallites in both cases. However, the positions and characteristics of the peaks are significantly different *in cellulo* and *in vitro*. The cellular peaks point to the formation of lamellar structures whereas in solution, three-dimensional nanocrystals seem to be formed. This work was supported by the Russian Federal Agency of Scientific Organizations (Agreement № 007-GZ/C3363/26).

P.19-044-Tue
Structures of subgroup I septins and the plasticity of the NC interface

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Septins are guanine-nucleotide-binding proteins capable of polymerizing into filaments. Important biological roles have been associated with these filamentous structures which generally involve membrane remodelling. Subgroup I of human septins, composed of SEPT3, SEPT9 and SEPT12, form important homotypic interactions via an NC interface which is essential for filament stability. The current work aims to consolidate the observations made for subgroup I and to study how a specific mutation resulting in male infertility affects the GTP-binding domain of SEPT12 (SEPT12G). Expression of SEPT12G and the SEPT12G^{T89M} mutant was performed in *E. coli* cells, Rosetta(DE3) strain, using the pET28a(+) expression vector. The recombinant proteins were purified by Ni^{2+}-affinity chromatography. Crystals of SEPT12G-GDP, SEPT12G-GMPPNP and SEPT12G^{T89M}-GDP were obtained and diffraction data collected on beamline I24 at Diamond Light Source (Didcot, England) resulting in high resolution datasets. The studies showed that for SEPT12G the switch I region is ordered and the threonine coordinates Mg^{2+}, whereas for SEPT12G^{T89M} the methionine is unable to coordinate Mg^{2+}, resulting in the interruption of the universal switch mechanism and the ability to hydrolyze GTP. Common structural aspects for this subgroup were observed, such as the differential orientation of the α5′ helix and the presence of two NC interface types (open and closed). SEPT3-GDP, SEPT3-GMPPNP and SEPT9-GTPγS structures present the closed conformation, while SEPT9-GDP and SEPT12G^{T89M}-GDP were in the open conformation. Surprisingly, SEPT12-GDP and SEPT12-GMPPNP structures presented both conformation within the same filament. The plasticity of the NC interface occurs due to flexibility in the interactions established by conserved residues capable of forming alternative sets of salt bridges, leading to alterations in the relative positioning of helices α2 and α6. Financial support: FAPESP.

P.19-045-Wed
Carborane and metallacarborane inhibitors of carbonic anhydrase IX, promising compounds for cancer therapy

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We have previously identified metallacarboranes and carboranes as a promising class of specific inhibitors of HIV protease (HIV-PR) and Carbonic Anhydrase IX (CA IX) enzymes. Here we report on recent advances in the molecular design of carborane and metallacarborane inhibitors targeting CA IX isoenzyme. This enzyme, which is associated with solid hypoxic tumors, belongs to newly identified targets for cancer therapy and diagnostics. The scope of currently available site-directed modifications on various boron cages is overviewed, with an emphasis on the progress in the synthesis of carboranes and metallacarboranes substituted by sulfamide, sulfonamide and other similar groups, *i.e.* functions known to bind tightly to the zinc atom in the active site of CA-IX. The new generations of polyhedral inhibitors of CA-IX, based on the careful selection of boron cages and optimized substitutions, exhibit significantly enhanced *in vitro* activities with corresponding Ki values in the range of tenths of pM to several nM. The structure-activity relationship (SAR) observed within a small library of ca. 70 substituted carboranes and metallacarboranes is discussed. These results are complemented by synchrotron structures of enzyme-inhibitor complexes and by a short overview of pharmacologically relevant factors such as plasma protein binding, cell membrane penetration, and basic results from toxicology and pharmacokinetic studies (mouse model) performed on a panel of the selected inhibitors of CA IX enzymes. Due to promising inhibitory properties, these compounds are thus primarily considered as candidates for drugs applicable in cancer treatment.

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P.19-046-Mon
Structural studies of plant omega amidase (*M. truncatula*)

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Omega amidase (systematic name: omega-amidodicarboxylateamidohydrolase; synonym: Nit 2, Nitrilase-like protein 2) is an enzyme metabolically linked to asparagine and glutamine transamination. The role of omega amidase is to remove potentially toxic intermediates by converting alpha-ketoglutaramate and alpha-ketosuccinamate to biologically useful alpha-ketoglutarate and oxaloacetate, respectively. Subsequently, alpha-ketoglutarate and oxaloacetate can enter the TCA cycle as an energy source. We overexpressed, purified and crystallized recombinant omega amidase from *Medicago truncatula* (MtOA). Synchrotron X-ray diffraction data were collected and two MtOA structures were determined, with the catalytic loop in an open and closed conformation. The protein exists as a dimer with alpha-beta-beta-alpha fold in both structures. The quaternary arrangement of the subunits is also observed in solution using small-angle X-ray scattering (SAXS).

P.19-047-Tue
Structural and functional study of translation initiation factor e/aIF2

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In Eukarya and Archaea, translation initiation factor 2 (e/aIF2α,β, γ) plays a key role in delivering initiator Met-tRNA to the small ribosomal subunit. Structures of the eukaryotic 43S and 48S complexes bound to the translation initiation factors have been determined (Hashem et al., 2013; Husain et al., 2014). However, there is no interpretable density for eIF2β and γ. Therefore, no model building or refinement was done for eIF2β and γ, and its placement was based on the structure of the archaeal aIF2 (Schmitt et al., 2012; Stolboushkina et al., 2013). aIF2 and eIF2 have a similar structural organization, but the eukaryotic polypeptides have additional terminal extensions, most strongly pronounced in the case of the β subunit. Structure of the eukaryotic eIF2 is a great interesting. Before all attempts to produce and isolate recombinant eukaryotic eIF2γ were unsuccessful. Here, we report about the soluble isolated recombinant human and yeast eIF2γ. In this study, we have used a strong ionic detergent SDS for solubilization of inclusion body proteins and refolded proteins by removing SDS at lower temperature with KCl. At present, we are testing this eIF2γ for binding with other subunits eIF2 and tRNA. After that we plan to use the reconstructed heterotrimeric eIF2 for crystallization. Moreover, we have reconstructed chimeric e/aIF2 as an attractive tool for studying the role of the each subunit of archaeal and eukaryotic e/aIF2 in translation. Using the toeprinting and yeast cell-free translation system, we have received first results. Chimeric e/aIF2 can support ribosomal scanning during eukaryotic translation initiation but archaeal γ in complex with eukaryotic α and β inhibits translation. This research requires continuation and was supported by the Program for Basic Researches on Molecular and Cellular Biology and Post-Genomic technologies of the Presidium of RAS to E.A.S.

P.19-048-Wed
Unravelling dimerization of the epigenetic reader LEDGF/p75

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The Lens Epithelium Derived Growth Factor/p75 (LEDGF/p75) is a chromatin reader recognizing H3K36me2/me3 marks through the N-terminal PWWP domain. Versatile integrase binding domain (IBD) of LEDGF/p75 serves for interaction with several other partners, e.g. Mixed-lineage leukemia (MLL) protein, the Myc interacting factor JPO2, Zinc-finger protein PogZ, transcription elongation factor IWS1, HIV-1 integrase, and tethers them to actively transcribed genes. Our major aim is detailed structural characterization of LEDGF/p75 and a thorough understanding of its physiological functions in order to advance in drug discovery efforts. LEDGF/p75 was found to dimerize and using NMR, we identified the minimal dimerization domain to comprise amino acid residues 345–467. Surprisingly, the structured part of the dimerization interface partially overlaps with the binding site recognized by all known LEDGF/p75 cellular binding partners. We will therefore follow the effects of LEDGF/p75 dimerization on binding to various cellular partners and try to identify dimerization defective LEDGF/p75 variants to decipher physiological relevance of LEDGF/p75 dimerization. Besides NMR spectroscopy, size exclusion chromatography, small angle X-ray crystallography (SAXS), analytical ultracentrifugation, cross-links and native electrophoresis are employed to investigate LEDGF/p75 dimerization.

P.19-049-Mon
3-D structure of L254N carboxypeptidase T mutant and its complexes with transition state analogues

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Metallocarboxypeptidase T is a zinc-dependent exopeptidase. Like other proteins of this family, carboxypeptidase T cleaves C-terminal amino acid residues from proteins and peptides, but, unlike other enzymes of this class, it has broad substrate specificity, cleaving, albeit at different rates, peptide bonds formed by C-terminal hydrophobic, as well as positively and negatively charged amino acid residues. Carboxypeptidase T from Thermoactinomyces vulgaris, for which a spatial structure is known, is a convenient object for constructing mutant forms with altered substrate specificity. One such form is L254N carboxypeptidase T mutant. It was shown that L254N carboxypeptidase T mutant has a specificity in comparison with the wild type with respect to the charged substrate. In present study 3-D structures L254N carboxypeptidase T mutant and its complexes with transition state analogues (N-sulfamoyl-L-glutamic acid, N-Sulfamoyl L-Arginine, N-Sulfamoyl L-Leucine) have been determined by X-ray analysis method. The analysis of the obtained spatial

structures allowed to determine how the replacement of leucine with asparagine affects the binding of substrates. The work was supported by the Russian Science Foundation (Project 17-14-01256) in part of macromolecule production and X-ray analysis and by the Central Research Institute for Mechanical Engineering, ROSKOSMOS in part of microgravity crystallization.

P.19-050-Tue
Structure-function studies of formate dehydrohenase from pathogenic bacterium *Staphylococcus aureus*

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Bacterium *Staphylococcus aureus* is one of the most dangerous hospital infection due to high multiple antibiotics resistance. The resistance increases more when *S. aureus* exists as biofilms. Genome analysis of *S. aureus* showed presence in chromosomal DNA of gene of formate dehydrogenase (FDH) which showed very low homology with FDH from other pathogenic and methylotrophic bacteria as well as with ones from yeasts, fungi and plants. It was shown that at biofilm conditions level of FDH mRNA is in the third place among all mRNAs. We have cloned and expressed in *E. coli* gene of *S. aureus* FDH (SayFDH). Recombinant enzyme was purified and characterized. SauFDH showed one of the highest chemical and thermal stability among all described formate dehydrogenase. The enzyme has high specific activity and very high Km values with formate and NAD^+. Multiple alignment of FDH amino acid sequences showed that in some conservative regions of active site the SauFDH has different amino acids residues. We carried out site-directed mutagenesis experiments to check the role of differences in stability and catalytic activity. Some amino acid changes resulted in improvement of Km values. To determine structure of SauFDH the crystallization experiments (totally 1728 variants) were performed. Crystals of apo- and holo- forms were obtained but its quality was not enough for data collection. Optimization of crystallization conditions resulted in crystals which were used for data collection. Preliminary structures of apo- and holo- forms of SauFDH were solved with resolution 2.3 and 2.7 angstrom, respectively. Parts of this work were supported by Russian Science Foundation (grant 16-14-00043) and Russian Foundation for Basic Research (grant 17-04-001662a).

P.19-051-Wed
Insights into the structural mechanisms controlling the dynamic nanodomain organization of REMORINs

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Membrane proteins and lipids are dynamically organized in domains or compartments, orchestrating important cellular event such as trafficking, signalling and pathogen response. Structural and mechanistic principles governing membrane organization are often uncharacterized because of the challenging nature of the dynamic membrane-protein system. The plant-specific REMORINs (REMs) are proteins regulating hormonal crosstalk and host invasion. Located in the plasma membrane, they spatially segregate into nanodomains and represent the best-characterized nanodomain markers in plants. In order to establish a mechanistic description of the interplay between REMs and membrane nanodomains we use magic-angle-spinning solid-state NMR (ssNMR), a powerful emerging technique to reveal protein structure and flexibility in the native membrane environment, in conjunction with static ssNMR to assess membrane order, thickness, phase transition and curvature. Our studies of a short N-terminal peptide, conferring membrane anchoring and nanodomain organization of REMs, reveal an original mechanism regulating the dynamic nanodomain targeting of REMs, mediated by the interaction of the anchor peptide with phosphoinositolphosphate PI4P. MD simulations and solid-state NMR propose unconventional binding motif of the anchoring peptide. While the membrane anchor revealed essential for nanodomain organization, the role of REM trimerization via a coiled-coil domain remained obscure; we therefore invested in a detailed dissection of the structural mechanisms driving trimerization, and their implications for native REM organization. We show that the coiled-coil domain controls targeting of REMs into the PM and, combining solid-state NMR with molecular modeling, we propose a model of the REMs when associated to the PM.

P.19-053-Tue
Characterization of L-serine O-succinyltransferases involved in L-cysteine biosynthesis in prokaryotes

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Until very recently, acetylation was the only known way for activating L-serine in the penultimate step of L-cysteine biosynthesis, in bacteria and fungi. This reaction is catalyzed by L-serine *O*-acetyl transferases (SAT), encoded by *cysE* and *srpH*. These enzymes belong to the transferase hexapeptide repeat family. Nevertheless, we demonstrated that L-serine is succinylated in *Schizosaccharomyces pombe* by an enzyme unrelated to the classical SAT (Bastard and Perret et al., Nature Chemical Biology, 2017). This unique class of L-serine succinyl-CoA transferases (SST), called Cys2, is encoded by *metX* homologs whereas these latter are known to only be involved in L-methionine biosynthesis. The conservation of Cys2 in fungi suggests that the biosynthesis of L-cysteine *via* the novel metabolite *O*-succinyl L-serine is a common treat in this kingdom. In contrast, few Cys2 homologs have been identified in bacteria, particularly in Xanthomonadales species. The phylogenetic proximity of these enzymes, along with the structural similarity of their active site suggest they share the same function. To support the *in vivo* role for *O*-succinyl-L-serine in Xanthomonadales, we kinetically characterized Cys2 from both *Xanthomonas campestris* and *Frateuria aurantia*. Their catalytic efficiency (*i.e.* k_{cat}/K_m) is in favor of the formation of *O*-succinyl-L-serine. We also report that their L-cysteine synthase (the next enzyme in L-cysteine biosynthesis pathway) are actually *O*-succinyl-L-serine sulfhydrylases. This new metabolite was detected in the metabolome of *X. campestris*. Together, these results demonstrate the role for this novel metabolite in L-cysteine biosynthesis in bacteria, too.

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P.19-054-Wed
Revealing *Ciona intestinalis* septins heterocomplex

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Septins are GTP-binding proteins that interact with each other to form heterocomplexes as filaments and higher order structures, usually membrane associated. These filaments are important for cytokinesis and have been reported to be involved in a series of other cellular processes, but only a few of the mechanistic details are known. *Ciona intestinalis* is a simple model for studying septin complex organization in animals. As demonstrated by phylogenetic analysis, this non-vertebrate chordate presents only a single representative homologue of each of the four mammalian septin subgroups. Due to their great similarity with human septins, we have named these proteins *Ci*SEPT2, *Ci*SEPT6, *Ci*SEPT7 and *Ci*SEPT9. In this study, we present the characterization of the *C. intestinalis* septins individually as well as an analysis of their self-assembly into a heterocomplex. For this purpose, these septins were expressed and characterized biophysically and biochemically revealing that *Ci*SEPT2 and *Ci*SEPT7 are capable of hydrolyzing GTP. We have also evaluated the interaction strength among monomers and oligomers using microscale thermophoresis (MST), which revealed a higher binding affinity for the latter. Co-purification and analysis by Transmission Electron Microscopy (TEM) confirmed the formation of a heterocomplex. Additionally, class averaging of the particles has revealed the dimensions of the hetero-oligomeric core particle, which will enable comparative studies with the canonical human SEPT2/6/7 heterocomplex. This work was supported by FAPESP.

P.19-055-Mon
Crystal structure of the membrane attack complex assembly inhibitor BGA71 from Lyme disease agent *Borrelia bavariensis*

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Borrelia bavariensis along with *B. burgdorferi*, *B. afzelii*, *B. garinii*, and *B. spielmanii* is the causative agent of Lyme disease. Lyme disease spirochetes reside in infected *Ixodes* ticks and are transferred to the mammalian hosts during tick feeding. To successfully colonize the new host organism spirochetes must fight against the innate immune system. At least five different outer surface proteins including CspA, CspZ, ErpA, ErpC, and ErpP have been described and characterized in *B. burgdorferi* known to inhibit the complement system either by binding the complement regulators or by terminating the formation of the membrane attack complex. *B. bavariensis* can inhibit the complement system by disrupting the formation of the membrane attack complex by the outer surface protein BGA71. We have determined the crystal structure of the essential *B. bavariensis* OSP BGA71 at 2.9 Á resolution. The structure revealed a cysteine cross-linked homodimer. Based on the crystal structure of BGA71 and structure-based sequence alignment with the homologous protein CspA from *B. burgdorferi* we have proposed the potential binding site for the complement pathway components C7 and C9 involved in the formation of the membrane attack complex. The results shed light to the molecular details used by the pathogenic *Borrelia* species to overcome the mammalian innate immune response and to cause the Lyme disease. The results aid in the understanding of Lyme disease pathogenesis and in the development of new strategies to hold the spread of Lyme disease. This work was supported by the ERDF grant Nr. 1.1.1.2/VIAA/1/16/144 "Structural and functional studies of Lyme disease agent Borrelia burgdorferi outer surface proteins to reveal the mechanisms of pathogenesis with the intention to create a new vaccine"

P.19-056-Tue
Structural analysis of the highly flexible PI4KB complexes using SAXS

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Small angle X-ray scattering is increasingly used to elucidate the structures of highly flexible protein complexes or of proteins containing intrinsically disordered regions [1]. Here, we have combined SAXS, molecular dynamics simulations and other biophysical techniques to characterize the protein complexes formed by the lipid kinase phosphatidylinositol 4-kinase IIIβ (PI4KB) that is responsible for the synthesis of the Golgi and trans-Golgi network (TGN) pool of PI4P. PI4P is the defining lipid hallmark of Golgi and TGN and, therefore, its synthesis must be tightly regulated [2]. PI4KB is recruited to the Golgi by ACBD3 protein and is further regulated by PKD and 14-3-3 proteins [3]. We have structurally characterized PI4KB:ACBD3 and PI4KB:14-3-3 protein complexes [4] to revealed how 14-3-3 protects PI4KB. The project was supported by Czech Science Foundation grant 17-05200S and RVO: 61388963.

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P.19-057-Wed
Crystal structure of β-carbonic anhydrase from *Candida albicans*

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Pathogenic yeasts of the genus *Candida* represent the most prevalent cause of mycotic diseases worldwide. They behave as opportunistic pathogens, which means that they can live in human

hosts as harmless commensals, being kept under the control by the host immune system. One of the key survival strategies of fungal pathogens is the ability to proliferate in different carbon dioxide concentrations. CO_2 is among the most important gases for living organisms. In nature, the concentration of CO_2 is balanced by interconversion to hydrogen carbonate however, its average amount required by organism is much greater than the amount produced spontaneously from CO_2. Therefore, bicarbonate production requires a fine tuned regulation. For this reason, a highly diverse family of enzymes has evolved that are able to accelerate the interconversion reaction up to 10 000-fold. The members of this family, carbonic anhydrases (CAs) are able to catalyze reversible hydration of CO_2 to bicarbonate. CAs evolved in all three domains of life, and are divided into five, evolutionarily unrelated classes (α, β, γ, δ, and ζ) that independently arose from different precursors during convergent evolution. ß-CAs are present in many pathogenic microorganisms but not in the mammalian hosts and therefore represent possible target for drug development. We determined the crystal structure of CA form *Candida albicans* (CaNce103) at 2.2 A resolution. CaNce103 assembles as a tetramer, with the active site localized at the interface between two monomers. At the bottom of substrate pocket, the zinc ion is coordinated by three highly conserved residues Cys78, His133 and Cys136 in addition to water molecule. Activity assays of full length and truncated versions of CaNce103 indicated that the N-terminal region is indispensable for enzymatic activity. This work was supported by grant GA17-08343S from the Czech Science Foundation, by project NPU LO1302.

P.19-058-Mon
Flavivirus methyltransferases as a target in antiviral treatment

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Viruses of the *Flaviviridae* family are widespread vector-borne pathogens causing large epidemics. Members of the *Flaviviridae* family consist of a large group of enveloped viruses with a +RNA genome. Dengue virus (DENV), yellow fever virus (YFV), tick-borne encephalitis virus (TBEV) and Zika virus (ZIKV) are all emerging or reemerging pathogens. Their RNA-dependent RNA polymerase (RdRp) consists of RdRp subunit and methyltransferase (MTase) subunit that is responsible for N-7 and 2′-O methylation of the viral RNA cap which protects the RNA from being recognized by host sensors. We have already shown that the RdRp can be targeted by nucleotide analogs [1] and we performed structural analysis of the Zika MTase [2]. Now, we explore the possibility to target different (ZIKV, TBEV, DENV and YFV) MTase domains by the same compound. We produced recombinant MTase domains in E. coli and assayed them enzymatically. A key step was the removal of S-adenosyl-L-methionine (SAM) – the methyl donor that co-purifies with the MTases. We have already designed several analogs of SAM. Our next aim is the crystallization of MTases with our compound and also with small molecule fragments to obtain starting hits for inhibitor design. We believe that our approach can help the development of efficient antivirals against Flaviviruses.

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P.19-059-Tue
Structural basis of STING activation by its fluorinated agonists

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STING (stimulator of interferon genes) is a protein with four transmembrane domains localized on the endoplasmatic reticulum membrane that is involved in innate immune signaling. After triggering by cyclo dinucleotides (CDNs) of bacterial or host origin, it is capable to activate both NF-κB and IRF3 transcription pathways to induce type I interferons (INF-α and INF-β), which leads to potent anti-bacterial and anti-viral state of organism. Therefore, STING is being investigated as a potential pharmacophore for the treatment of viral diseases. A natural endogenous agonist of STING is 2′3′-cyclo-GMP-AMP (2′3′-cGAMP). This CDN is produced by cytosolic microbial DNA and RNA sensor cyclic-GMP-AMP synthase (cGAS). On the basis of 2′3′-cGAMP and also cyclo-dinucleotides of bacterial origin (c-di-AMP, c-di-GMP or c-di-IMP), novel STING agonists were designed. Among the very interesting ones are fluorinated analogs of CDNs, which exhibit higher potency in induction of type I interferons than their non-fluorinated analogs such as fluorinated 3′3′-cGAMP. Mechanism how the fluorine atoms influence CDNs ability to activate STING is unclear. Aim of our work is to compare structures of human STING in complex with fluorinated and non-fluorinated agonist using protein crystallography. To this date we have successfully co-crystallized human wild type STING with fluorinated 3′3′-cGAMP and 3′3′-c-di-AMP. The protein:ligand structures were solved at 2.8 A and 3 A resolution in space group P 41212. The Project was supported by Project InterBioMed LO1302 from Ministry of Education of the Czech Republic, the support of the Academy of Sciences of the Czech Republic (RVO: 61388963) is also acknowledged.

Systems biology

P.20-001-Mon
Regulatory network of *Bacillus subtilis* alternative σ factors during spore germination and outgrowth: kinetic modelling, meta-analysis and experimental verification

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σ factor is an essential transcription initiation factor in bacteria. A fundamental task is identification of target genes of various σ factors. Each bacterial species has one primary and several alternative σ factors. Primary σ factor drives gene expression in a bacterial cell under favorable conditions, while alternative σ factors take control when diverse stresses come. In past years, there was a burst of sequencing data accumulation based on ChIP-seq and RNA-seq data. These approaches provide information on singular interactions between σ factor and its potential targets.

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Nevertheless, this information is fragmentary and does not provide comprehensive view of the gene regulatory network in the studied organism. A highly useful tool for discovering gene regulatory networks that complements sequencing approaches is kinetic modelling of gene expression. *Bacillus subtilis* is a widely used Gram-positive model organism containing 18 σ factors. In our previous study we used data of gene expression profiles from 14 time points to model gene regulatory network of the primary σ factor – σ^A – in *B. subtilis* during spore germination and outgrowth, subsequently verifying selected predictions experimentally. In this study we focused on the regulatory network of alternative *B. subtilis* σ factors during spore germination and outgrowth. We used kinetic modelling and meta-analysis to create regulatory network between alternative σ factors and their targets. For selected σ factors σ^B, σ^D, σ^H, σ^1, σ^M we set up *in vitro* transcription systems to verify predicted interactions experimentally. The outcome is a comprehensive overview of alternative σ factors regulatory network in *B. subtilis*.

P.20-002-Tue
Measuring interdependence of bacterial optogenetic circuit and microbial growth

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Light can replace the chemical effector for programming on-demand gene expression in bacteria and optogenetic bacterial circuits with wavelengths ranging from near infrared to UV have been developed over the past decade. While system biology has always aimed to decipher complex interactions of biological systems and identify mathematical models that best depict their behavior. Recent study reveals that these synthetic genetic circuits can be strongly affected by the physiological states of the organism, and often the growth rate of cell and the level of gene expression are often intimately intertwined. Characterization of this dependence is also of practical importance because these synthetic circuits must be placed under different growth conditions. In this work, we report the measurement of the coupling of optogenetic circuit and microbial growth using our recently developed bioreactor array that enables automated, *in vivo* parallelizable simultaneous monitoring of gene expression and growth. The CcaS-CcaR light sensing system in *Escherichia coli* is chosen as a model system for such study. The CcaS-CcaR light sensing system consists of membrane bound histidine kinase CcaS and a response regulator CcaR. The interdependence between microbial growth and optogenetic gene expression was confirmed in a growth experiment, varying light intensity and three effectors of microbial growth (carbon source, oxygenation, and antibiotic drug concentration). Specifically, the demand of optogenetic circuit for resource usage from the host is measured using light intensity as a tuning parameter. Growth under different carbon sources and oxygenation levels shows the gene expression decreases as the growth rate increases. These studies again confirm resource allocation trade off picture, recently proposed by Terence Hwa' group.

P.20-003-Wed
The language of the genome – semantics of the minimal cell organization

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Mycoplasmas as the most reduced group of Mollicutes are believed to have extremely reduced gene expression control systems along with the general genome reduction. They demonstrate broad transcriptional response and absolute transcriptional changes are relatively small and protein changes at the same time are negligible. We used TSS's map and cross-species conservation analysis to reconstruct transcription control network based on known regulators. We concluded that conserved transcriptional regulators are mostly involved in control of metabolic pathways, which are reduced in mycoplasmas compared to other Mollicutes. To reveal both adaptation strategy and underlying regulatory network in mycoplasmas we performed a combination of –omics studies with computational methods. We constructed a quantitative model of gene expression in *M. gallisepticum*. We identified that *M. gallisepticum* features unexpectedly high amount of genes that undergo regulation, a substantial amount of housekeeping genes including components of translation machinery and glycolytic enzymes require regulation as well. We used sub-diffraction microscopy to directly quantitate translation efficiency and protein distribution in population under different combinations of promoters and translation determinants. We identified that *M. gallisepticum* may feature high heterogeneity and the protein distribution pattern close to all-or-nothing. *M. gallisepticum* demonstrated the ability to retain the altered proteome state during long-term cultivation on artificial medium after the infection. Using genetic tools we identified several key players (SpxA, YebC) that are involved in support of this transition of phenotype. We conclude that under physiological conditions mycoplasma demonstrate strong ability of regulation and adaptation and is capable of transition to stable phenotypic states (attractors), while the exact mechanism is yet to be discovered. This work was funded by RSF grant 14-24-00159.

P.20-004-Mon
Effect of repeated stress on gene expression in the basal conditions in human skeletal muscle

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Repeated cellular stress leads to expression of specific proteins which improve physiological function of cell and tissue. This adaptation is related to transitory changes in gene expression after each acute stress. However, repeated stress may affect the expression of many genes during the basal conditions (several days after the last stress stimulus). Importantly, acute and repeated stress-related changes in gene expression usually are evaluated in cell culture and in animal models. The goal of our study was to investigate the effect of repeated stress on changes in the transcriptome in the basal conditions in human skeletal muscle. We used regular physical exercise (exercise training) as a model of repeated stress. The transcriptome in the basal conditions was evaluated prior to and after exercise training (8 wk cycling training, 5/wk, 1 h/day) in biopsy samples from m. vastus lateralis of 7 males. RNA-seq was performed by NextSeq 500. As expected 8 wk training increased endurance performance, ADP-stimulated mitochondrial respiration in muscle fibers, and content of proteins related to mitochondrial complexes I-V. Previous studies showed that acute exercise affects the expression of several hundred genes. Surprisingly, here, repeated stress changed the expression of 2000 genes in the basal conditions (48 h after the last exercise) and a half of these genes were down-regulated. Many genes, encoding mitochondrial proteins, protein kinases, and transcriptional regulators, were both up- and down-regulated, while genes, encoding cytokines and growth factors, were mainly up-regulated. These up- and down-regulated genes were associated with different biological process. In conclusion, the adaptation of human muscles to repeated stress associates not only with the transitory changes in gene expression after each acute stress, but also with the marked changes in gene

expression in the basal conditions. This work was supported by the Russian Science Foundation (14-15-00768).

P.20-005-Tue
Identification of novel mechanisms of transcriptional regulation in reduced bacteria using machine learning and high-throughput identification of promoters

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Bacteria of class Mollicutes and in particular mycoplasmas represent good approximation of minimal cell due to the severe reduction of genome. Previous studies revealed that mycoplasmas feature very limited repertoire of transcriptional regulators and poorly react to different stimuli in perturbation models. These findings led to conclusion that the systems of gene expression regulation degraded in mycoplasmas along with the general reduction of genome. We applied a high-throughput method of the identification of transcription start sites and the quantitation of their activity with machine learning to construct the quantitative model of bacterial promoter on the model of *Mycoplasma gallisepticum*. The model was able to predict promoter strength from its sequence. Further we identified promoters, which predicted strength deviated from their measured activity. Thus we identified candidate promoters, which activity was putatively regulated. This group included the targets of few known regulators as well. Surprisingly we identified numerous regulated promoters. Further using genetic engineering we identified novel DNA sites and RNA structures, responsible for the activator or repressor effect on the promoters. We conclude that the gene expression regulation in mycoplasmas is complex. The regulated genes code for both stress-response proteins and housekeeping proteins. The latter is of particular interest for synthetic biology since the control of housekeeping genes may be an essential function of cell. This work was funded by RSF grant 14-24-00159.

P.20-006-Wed
Versatile reporter for multiplex analysis of the transcription factor activity in mammalian cells

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Transcription factor (TF) activity plays pivotal roles in the regulation of broad spectrum of signaling pathways in cells. Considering cross-regulation loops between different TFs, it is an important task to perform simultaneous evaluation of the activity of several TFs for the analysis of a given signaling pathway. Not only it is a topical issue for fundamental scientific research, but it also has direct applications in screening for drug discovery. Here we present a multi-reporter system based on qPCR evaluation of the activity of 4 TFs (NFkB, AP1, STAT, IRF) for monitoring of the inflammation-related signaling. It could be used both for transient transfection of cells or for the lentiviral delivery and contains GFP gene for the evaluation of the transfection efficiency as well as for the selection of the reporter-containing cells via FACS-sorting. Each of four reporters contains a TF response element (3–8 consensus sites of the TF) followed with a minimal promoter and two consensus sequences separated with a chimeric intron. Second consensus sequence contains a unique label for each of the TFs. Analysis is performed by qPCR with a pair of primers, common for all the reporters, and a probe, specific for each TF and complement to the TF label. It is possible to analyze four TFs with quadruplex qPCR using probes with different fluorophores. Normalization for the number of the reporter integrations to the genome could be performed with gDNA, a pair of primers, common for all the TFs, and the same probe as is used for the mRNA analysis. The system could be easily modified for the analysis of the activity of the TF of interest via one-step cloning of the corresponding response element. It could be scaled up and used for RNA-seq analysis instead of qPCR. Therefore here we present a useful tool for the routine monitoring of the transcription factor activity in mammalian cells that could be easily modified to meet the needs of a particular research.

P.20-007-Mon
Stress causes the formation of a morphologically new type of *Mycoplasma hominis*

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Mycoplasma hominis (*M. hominis*) is the human pathogen belonging to Mollicute class. *M. hominis'* genome is extremely reduced and contains only 559 protein-encoding genes. In this work we extracted unique *M. hominis* ("miniM") isolates from blood of patients with bronchial asthma, urogenital infection, from synovial fluid of patients with juvenile rheumatoid arthritis and from the cell culture with gentamicin. In contrast with classic "fried-egg" colonies they produce very small colonies, exhibit low growth rate and are capable of growing without arginine and glucose. The miniM's cells have elongated shape and this morphology is permanent – we did not observe reverse transition from miniM to the classic cells ("CC"). We suppose that human organism has a resistant form of *M. hominis*, which escapes the immune system. We've developed a method to produce the miniM cells from laboratory strain H34 using stress factors (UV, blood plasma treatment, antibiotics). We identified 346 proteins for CC and 291 proteins for miniM with shotgun proteomics approach and found several proteins (transcription factor *yebC*, ABC-transporter, lipoproteins, metabolic proteins and others) unique to miniM cells. Quantitative analysis of data by MaxQuant has shown that the amount of proteins, which participate in the glycolysis, translation, transcription and replication, decreases, but levels of lipoproteins (lmp1, p120), elongation factors (fusA, IF-Tu), deoA protein and some proteins without assigned function, increases more than 2-fold. This work was funded by RSF grant 14-24-00159.

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P.20-008-Tue
Small open reading frames of plants: trends in evolution and peptide coding capacity

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Genomes of nearly all organisms contain hundreds of thousands of small open reading frames (sORFs; < 100 codons). However, gene annotation algorithms are generally not suited for dealing with sORFs due to high false discovery rate and low conservation. Nevertheless, recent reports suggest that sORFs may regulate translation of proteins or produce bioactive peptides. Although sORFs have been found in some plant species our knowledge about sORF function and evolution in plants is scarce. Here we used moss, *Physcomitrella patens,* to address questions about trends of sORF evolution between evolutionary diverged plant species and peptide coding capacity of sORF. We identified about 600 000 sORFs with high coding potential in moss genome, 6763 of which were potentially secreted. About 70 000 transcribed sORFs were located on mRNAs and lncRNAs. Using mass spectrometry analysis, we identified hundreds translatable sORFs in moss cell and secretome. Intensive evolutionary analysis demonstrated that peptide coding capacity of sORFs and conservation rate are weakly correlated. We hypothesized that sORFs can be a source of biological novelties used by plant cell for global regulation of proteome and adaptation. This work was supported by grant № 17-14-01189 from Russian Scientific Foundation.

P.20-010-Mon
Specificity of transcriptome response to acute stress in human skeletal muscle depends on adaptation to repeated stress

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Acute stress acting on the cell changes the expression of a specific set of genes. However, the number of stress-dependent genes may significantly differ after the first and subsequent stress actions. The aim of this work was to investigate the specificity of transcriptome response to acute stress in human skeletal muscle depending on the level of adaptation to repeated stress. As acute stress, we used contractile activity: one-legged knee extension for 1 h. This test was performed before and after adaptation to repeated stress (8 wk cycling training, 5/wk, 1 h/day). Transcriptome was investigated in biopsy samples from m. vastus lateralis of 7 males before and at 1 h and 4 h after acute stress (one-legged exercise). RNA-seq was performed using NextSeq 500 (Illumina). In nonadapted muscle the expression of a large number of genes was changed at 1 h and 4 h after acute stress (about 400 and 900 genes, respectively). After adaptation to repeated stress, the acute stress induced significantly less number of DEG (differentially expressed genes): about 100 genes for 1 h and 4 h. In both cases the increase in expression of the genes regulating mitochondrial biogenesis, angiogenesis, carbohydrate and lipid metabolism, i.e. biological processes specific for this stress, was found. Reduced amount of DEG in the adapted muscle was associated with a decrease in nonspecific response to stress: the GO-terms, such as "Cellular response to biotic stimulus", "Cellular response to the external stimulus", "Response to oxygen levels", and "Actin filament-based process" were found only after the first exposure to acute stress. Consequently, as a result of adaptation to repeated stress, transcriptome changes in response to acute stress become much less pronounced, but more specific. This work was supported by the Russian Science Foundation (14-15-00768).

P.20-011-Tue
Transcriptomic and lipidomic profiling reveals a functional interplay between sex steroids and growth hormone in the liver

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Gonadal steroids (GS) and GH are critical regulators of body growth and intermediate metabolism in mammals. The metabolic influences of GS and GH deficiency have been well documented in adult men by the developing of chronic illness (e.g., fatty liver, insulin resistance), a phenotype that can be ameliorated by E2/testosterone and GH replacement. The effects of GS on liver might be direct through their respective nuclear receptors. Indirect mechanisms, related to the influence of sexual steroids on the pituitary GH secretion and/or their influence on GHR signaling pathway in the target tissue, might also play a relevant role to regulate liver physiology. The molecular characterization of the hepatic changes induced by GS and how they influence the liver response to GH deserves to be explored. Hypothyroidism-hypogonadism is accompanied by systemic and hepatic metabolic disturbances with features that mimic deficiencies in GH and GS. In this study, the analyses of lipid and transcriptional profiles in hypothyroid-orchidectomized rats were combined to obtain comprehensive information on the GS and GH crosstalk in liver. Testosterone (T) activated, among others, a metabolic transcriptional program linked to glucose and fatty acids and lipid class metabolism. The overall impact of T on hepatic lipid content and transcriptome differed from the effects of E2. The combined administration of T and GH revealed biological processes related to lipid biosynthesis, oxidation-reduction, unsaturated and long-chain fatty acid metabolism. GH showed permissive, additive, or antagonistic effects on T actions on hepatic lipid content. Protein-protein interactions analysis revealed a close interplay among proteins which are central in the metabolism of steroid and fatty acids. These findings highlight the impact of GS-GH interplay on liver metabolism which is relevant for physiological and therapeutic roles of these hormones in human.

Synthetic biology

P.21-001-Mon
Peculiarities of the stereospecific interactions of catalytic antibodies A5 and A21 with aryl-phosphonate X

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Specificity of biochemical reactions is the cutting-edge field of the modern biochemistry and biotechnology. Many biochemical

reactions leaded by enzymes are stereoselective. Thus, understanding of stereoselectivity mechanism could become a key to the successful development of biocatalysts with desired activity. Catalytic antibodies are promising objects for directed alteration of their stereoselectivity and increase in activity rates. Previously, a set of abzymes that covalently bind aryl-phosphonate X was obtained from semisynthetic library Griffin 1. Here we focused on peculiarities of interactions of catalytic antibodies A5 and A21 with aryl-phosphonate X. It was established that these antibodies bind different enantiomers of the substrate according to the results of kinetic experiments, immunoblotting and mass spectrum. Selected antibodies were produced in methylotrophic yeast system *Pichia pastoris* as Fab-fragments and chromatographically purified. Kinetic parameters and reaction mechanism were determined using steady-state and pre-steady-state kinetic methods. QM/MM approach was applied to define interactions between catalytic antibody A5 and phosphonate X. Computations showed non-covalent stage to be determined in stereoselectivity. Obtained results could be used for a creation of mutant antibodies and proteins with directly changed stereoselectivity. This work was supported by RSF Grant 16-14-00191.

P.21-002-Tue
Kinetic and thermodynamic description of computer-designed mutants of reactibody A17 as improved scavenger of organophosphorus compounds

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Previously, we developed the QM/MM approach for predicting replacement of amino acid residues in active center of reactibodies to improve their functionality (Smirnov et al. Sci.Adv. 2016). Here our experimental steady state analysis revealed that the mutations Leu47Lys and Ser35Arg lead to an increase of two orders of magnitude for the second order rate constant, compared to ReAbA17 (7.2 M-1 sec-1, 3.4 M-1 sec-1 and 0.022 M-1 sec-1, respectively). The similar situation was observed in case of replacement leucine in position-47 with other potential proton donor amino acid arginine. In this case the second order rate constants was lower than in the case of Leu47Lys mutant. According to our theoretical analysis, these facts should be associated with improved of nucleophilicity of reactive Tyr37 that reflects in rate constant of covalent binding step. We failed to find turnover in the case of the Leu47Lys and Leu47Arg mutations. It appears that the positively charged residues of arginine or lysine block the release of p-nitrophenolate in the course of covalent intermediate hydrolysis, thus making impossible the liberation of the phosphate group from the catalytic active center. The thermodynamics parameters were calculated using Arrhenius equation. It was found that the mutants Ser35Arg and Leu47Lys have lowest activation energy. These data are corresponding to results of computer calculation. It is interesting that in contrast with Ser35Lys and Leu47Arg, Gibbs energy of Ser35Arg and Leu47Lys is defined by entropy factor. Our findings prove the hypothesis that combination of type and position of amino acid residue in active center determines an increase in activity. This work was supported by RSF Grant #16-14-00191.

P.21-003-Wed
Comparison of a retroviral protease crystallized as a monomer and a dimer

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Mason-Pfizer Monkey Virus (M-PMV) is a D-type retrovirus that causes simian acquired immunodeficiency syndrome (SAIDS) in rhesus monkeys. Its aspartic protease (PR) processes the retroviral polyproteins to release the final protein products necessary for virion maturation. Functionally, M-PMV PR is a homodimer but in the absence of inhibitors the equilibrium is shifted toward the monomeric state. We have determined three crystal structure of M-PMV PR in its dimeric form, using two protein variants crystallized with or without a substrate-based active-site inhibitor, Pro-Tyr(OMet)-Val-PSA-Ala-Met-Thr (where PSA is a non-standard non-hydrolyzable residue). Both proteins are inactive by virtue of the D26A active site mutation. One of the proteins (D26N/C7A/C106A) has in addition its Cys residues replaced by Ala. Both proteins were crystallized in complex with the inhibitor. In addition, the D26N mutant was also crystallized in the apo form. In the D26NC7A/C106A structure, despite the presence of the inhibitor, the active site contains an unusual water molecule, hydrogen-bonded in a tetrahedral fashion between the two active-site loops of the dimer, with the G28 N-H groups acting as donors and the side-chain O atoms of the N26 residues acting as acceptors. This water is different from the nucleophilic molecule found in the active site of active aspartic proteases or from the water molecule found at the inhibitor-flap interface of inhibitor complexes of retropepsins. Such a coordination of a water molecule has not been seen in any (retro)pepsin structures before. In the apo structure of the D26N protein, one of the flaps is very well defined, while the electron density of the other flap loop is invisible and the L49-N59 residues are missing from the model. While flap disorder is not surprising in apo structures of retropepsins, the perfect order of the other flap is an unusual feature, confirming again the unusual structural properties of the M-PMV enzyme.

Computational biology

P.22-001-Mon
New details of specific site recognition by human single-strand selective monofunctional uracil-DNA glycosylase SMUG1

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Human single-strand selective monofunctional uracil-DNA glycosylase SMUG1 is involved in base excision repair pathway. This enzyme recognizes and hydrolyses the N-glycosidic bond of uridine and uridine lesions bearing oxidized groups at C5: 5-hydroxymethyluracil, 5-formyluracil, and 5-hydroxyuracil. Pre-steady-state kinetic analysis of conformational transitions of hSMUG1 and DNA in the course of the catalytic cycle revealed mutual conformational rearrangements of both the protein and DNA molecules. In the present work, single mutants of hSMUG1 H239A and F98W were analyzed with the aim to elucidate the kinetic mechanism of protein conformational adjustment during

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damaged nucleotide recognition and catalytic complex formation. To gain a deeper insight into the kinetic mechanism the structural model of the enzyme complex with DNA was created by molecular dynamics approach. Because no structure of human SMUG1 is available, we used the high sequence homology among hSMUG1 and xSMUG1 from *X. laevis* (PDB IDs 1OE4 and 1OE5) to obtain a structure of wild type hSMUG1 and its F98W and H239A mutant forms by homology modelling. To obtain model of hSMUG1–DNA complex the set of complexes of structurally homologous uracil-DNA glycosylases with DNA were analyzed. The structure of the hSMUG1–DNA complex was modeled using structural alignment of hSMUG1 with human thymine-DNA glycosylase TDG bound to DNA (PDB ID 5T2W) as it is the nearest homologue with available enzyme-DNA complex structure. The model structure of hSMUG1-DNA complex revealed new details of the mechanism of specific site recognition and allowed to understand more deeply the functional role of some active site amino acids in the key steps of DNA-substrate binding and catalysis. The work was funded by Russian Science Foundation grant 16-14-10038.

P.22-002-Tue
In silico assessment of cardiovascular adverse effects of drug-drug interactions

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Adverse drug effects (ADEs) are one of the leading causes of death in developed countries and the main reason for drug recalls from the market. The ADEs associated with action on the cardiovascular system are the most dangerous and widespread. Treatment of human diseases often requires the intake of several drugs, which can lead to drug-drug interactions (DDIs) causing an increase in the frequency and severity of adverse effects. Evaluation of ADEs, as well as the effect of DDIs on their manifestation, is a non-trivial task and requires numerous experimental and clinical studies. To solve this problem, we developed a computational approach to assess the cardiovascular effects of DDIs. This approach includes: (I) creation of training sets including pairs of drugs showing synergistic and antagonistic effects on cardiovascular ADEs based on the analysis of FDA spontaneous reports; (II) prediction of interaction of individual drugs with human proteins and influence on expression of human genes based on structure-activity relationships; (III) identification of correlations between drug action on proteins and genes with appearance of ADEs, and calculation of interaction scores for each pair of drugs for each cardiovascular ADE; (IV) validation of developed approach based on the cross-validation procedure; (V) assessment of possible molecular mechanisms of synergy and antagonism based on pathway analysis of selected proteins and genes. The developed approach allows predicting the following effects of DDIs: myocardial infarction, ischemic stroke, ventricular tachyarrhythmia, cardiac failure and arterial hypertension. Validation of the approach showed its acceptable accuracy for the studied effects. For each ADE we identified possible mechanism of synergy and antagonism at the level of genes, proteins and signaling pathways. The study was supported by Russian Science Foundation grant 17-75-10168.

P.22-003-Wed
Pharmacophore generation studies for the discovery of potential glycogen synthase kinase 3β enzyme inhibitors

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Glycogen synthase kinase 3 (GSK3) is a member of Ser/Thr protein kinase family. There are two GSK3 isoforms (GSK-3α and GSK-3β) in mammals that show 98% sequence identity within their kinase domain. Both GSK3 isoforms are highly expressed in the nervous system including brain and spinal cord. GSK3 has a critical role for regulation of neuronal functions, including neurotransmission, neurite out-growth, growth-cone dynamics, cytoskeletal dynamics, synaptic plasticity, endocytosis, apoptosis, and neurogenesis. Because of this the activity of this receptor has been linked with several human diseases as diabetes, cancer and Alzheimer's Diseases (AD). In the recent researches GSK-3β activity is especially found linked to increased levels of Aβ production and Aβ deposits, tau hyperphosphorylation, and synaptic damage in AD patients. In this study, we have aimed to develop a novel pharmacophore model for discovering new lead GSK-3β enzyme inhibitors. For this purpose, all 3D structures of GSK-3β was taken from RSCB Protein Databank. Homology modeling was implemented for missing residues in first step. Molecular docking studies were applied for the choosing of best 3D structure for pharmacophore modeling studies. Discovery Studio 3.5 Client and AutoDock Vina v1.5.6 were used for this second step. Structure-Based Pharmacophore Modeling and 3D QSAR Pharmacophore Modeling were applied for improving of hypothesis and discovering of new lead GSK-3β enzyme inhibitors by using Discovery Studio 3.5 Client. In our preliminary studies, 3D structures with PDB ID: 4PTE, 1O9U, 5K5N, 5HLP, 4ACC, 3GB2, 3F7Z, 1Q41 were found appropriate for the further studies and twenty molecules were chosen with their IC_{50} values from the Zinc and BindingDB databases.

P.22-004-Mon
Computational approach to HIV resistance prediction to reverse transcriptase and protease inhibitors

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The problem of resistance to nucleoside-, non-nucleoside HIV reverse transcriptase (RT) inhibitors (NRTIs and NNRTIs) and protease inhibitors (PRIs) is important because these classes of drugs are included in most of highly active antiretroviral therapy (HAART) schemes. The aim of our study is the development of the computational approach to predict the resistance of the particular variant of HIV to NRTIs, NNRTIs and PRs using available data on relationships between the particular mutations in pol gene of HIV and the resistance of RT variant to NRTIs, NNRTIs and PRs. Our computational approach is based on PASS algorithm, which was earlier proposed to apply for prediction of HIV resistance. We propose to use the (1) descriptors as a set of pentapeptides of the amino acid sequence of reverse transcriptase and protease and (2) combined descriptors involving the pentapeptide and a chemical descriptor of a antiretroviral drug. We used over 3000 RT amino acid sequences and over 3400 PR sequences of HIV variants from HIV Drug Resistance Database to build models aiming at predicting the genotype-phenotype relationships providing the interpretation of the HIV variants

that can be resistant to the specific antiretroviral therapy. The average balanced accuracy of prediction when pentapeptides are used as descriptors is 89% and maximum accuracy is 99%. Average accuracy of prediction using combined descriptors is about 95% with maximum accuracy over 99%. Given results allows to conclude that proposed method can be considered as one providing the predictions for its usage in clinical sciences. Advantages and restrictions of the presented method and its applicability to the prediction of other types of relationships will be considered This work was supported by Russian Science Foundation grant No. 17-75-10187.

P.22-005-Tue
Catch me if you can – How can a frameshift mutation help the virus to escape the host immune system?

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Frameshift mutations often lead to massive changes in protein sequence and premature emergence of stop codons. Therefore, they are usually considered to be extremely deleterious. On the other hand, they can help organisms to easily explore very remote areas of protein universe. It could lead to appearance of proteins with novel and unique abilities. Here we present one such mutation allowing Sepik virus (SEPV – *Flaviviridae*, *Flavivirus*) rapidly change sequence in the very immunogenic part of its polyprotein. We screened representative genome sequences of viruses from families *Flaviviridae*, *Togaviridae* and *Picornaviridae* for presence of frameshift mutations. Among all tested viruses we identified only one frameshift mutation in the SEPV polymerase. The mutation is present in 3 of 6 SEPV polymerases sequences which are currently available in GenBank. It leads to massive sequence change in 14 amino acids located quite far from the enzyme active site. To test if this mutation brings some changes in SEPV polymerase, we modeled the structure of the wild type protein and the protein with the frameshift mutation. Resulting protein structures are very similar and the docking experiments did not show any changes in nucleotide binding. Comparison with sequences in the Immune Epitope Database showed that the mutation is located in a part of the polymerase, which is very immunogenic in closely related yellow fever virus. We present here the first evidence of frameshift mutation leading to sequence change in virus protein without effect of overprinting by another protein. Despite large sequence change, the protein still keeps its enzymatic activity. The mutation is lying in an immunogenic domain, which indicates that it may play a role in escape of SEPV to the host immune system. Further biochemical validation of our predictions and screen for more viral proteins for the similar frameshift mutations could show if this is a common phenomenon in virus evolution.

P.22-006-Wed
Correction of force field for drug-like molecules using property map collective variable

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The accuracy of molecular simulations depends on an empirical molecular mechanics potential known as a force field. While force fields designed for proteins or nucleic acids are considered accurate, force fields for drug-like molecules still need many improvements. Here, we present a novel approach for force field correction tailored to a general drug-like compound. Using property map collective variable, it is possible to approximate a certain conformationally dependent property by a weighted average of this property for a series of representative landmark structures. As a value of property we have chosen the difference between potential energies of selected conformers calculated by accurate (force field) and inaccurate potential (quantum chemical methods). To validate this method we used seven AMBER force fields and we performed a set of 20-ns-long metadynamics simulations of Ace-Ala-Nme in water. We generated 144 landmark structures of Ace-Ala-Nme differing in values of torsion phi and psi. And then we tried to transform one force field (e.g. AMBER94) to another one (e.g. AMBER03). The obtained free energy surfaces of the corrected force fields (e.g. AMBER94 corrected to AMBER03) and the intended ones (e.g. AMBER03 without correction) were in good agreement. Furthermore, we used same landmark structures and differences between potential energy obtained using force field and DFT calculation. We also present force field correction for drug Imatinib as a use case example. Our method appears suitable for adjusting force field for general drug-like molecule.

P.22-007-Mon
Structure, mode of action, and binding mode assessment of AhR-HSP90 complex

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AhR (aryl hydrocarbon receptor) is a transcription factor responsible for regulation of detoxifying enzymes such as CYP450. It normally exists in the cytoplasm, bound to multiple chaperones including HSP90. Upon xenobiotic binding, it translocates into the nucleus to begin transcription of target genes. The agonists of AhR include flavonoids, TCDD and other hydrocarbons, which are common pollutants. However AhR not only binds to potentially toxic xenobiotics but also to beneficial drugs, such as flutamide, which are useful in treatment of some cancers. The protein itself is required to maintain healthy nervous system as the lack of AhR leads to Infantile Nystagmus Syndrome as well as demyelination of optic nerve and CNS. The scope of AhR functionality makes it an attractive pharmaceutical target – being able to modulate the expression behaviour could result in development of new treatments against cancer, as well as demyelination diseases. As the protein is able to bind both toxic and beneficial compounds, it is important to be able to distinguish the two classes. In order to be able to do that, understanding of the binding sites and mode of action is necessary. Currently there is no experimental structure available of AhR-PAS-B, most likely due to aggregation. However there is experimental evidence of a section of HSP90 and PAS-B interacting. This led to atomistic

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modelling of the binding mode of HSP90 and AhR guided by experimental data, umbrella sampling of the binding modes and attempting AhR PAS-B expression in insect cells. Dealing with the large system also led to the development of a tool to aid in umbrella sampling preparation. By being able to know how ligands bind, how the complex translocates and when the binding with ARNT occurs, we would be better prepared for thoughtfully designing compounds. This work explores the questions – What actually causes the translocation of the complex? What role do agonists and antagonists play in the translocation?

P.22-008-Tue
Development of suitable computational methods for intrinsically disordered proteins (IDPs) as medicinal chemistry targets

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IDPs, or Intrinsically Disordered Proteins, are a class of proteins which have only partial tertiary structure or no tertiary structure at all. This characteristic gives this class of proteins a high level of molecular plasticity. Also, this natural flexibility creates a high level instability, creating a high level of aggregation and oligomerization to attain thermodynamic stability. The event of oligomerization and aggregation is related to several pathological conditions including Neurodegenerative diseases such as Parkinson's disease, making this set of molecules important targets for the development of small molecules for therapeutic intervention. To develop therapeutical drugs, one of the main methods consists in the use of computational tools, such as Molecular Dynamics softwares, which uses force fields to characterize protein models. These databases are mainly biased to structured proteins, creating a lack of proper parameters for IDPs. So, a iterative MC fitting code had been developed. Using GROMACS, the dihedral parameters for 8 disorder inducing residues (A, R, Q, E, G, K, P and S) are randomly sampled and a comparison between experimental and theoretical SAXS are assessed to check the improvement in the simulation by the changes in the parameters. A second approach was studying the effect of the TIP3P model on the ensemble generated, as we observe a improvement on sampling for the proteins at hand.

P.22-009-Wed
Raman spectroscopy and multidimensional data analysis of biological fluids

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Raman spectroscopy contributes to the various application areas and has advanced in recent years becoming increasingly important for different application areas. This is mainly due to technical improvement, the versatility of sampling methods and the availability of advanced methods that helps in the data analysis. Over the last years, advances in the instrumental design of Raman spectroscopy overcame the problem with undesired fluorescence, low sensitivity and reproducibility. Raman spectroscopic data gives the vibrational characteristics of analyte, which can be understood as "fingerprint" type of information. The impact of chemometrics to the application of Raman spectroscopy for forensic purposes and medical diagnostics directly from body fluids will be discussed in this contribution. Raman data is characterized by high dimensionality. The high complexity of biological systems and large number of variables usually coupled with the relatively low number of observations can make analyses quite challenging. What are some useful techniques that help deal with high-dimensional data effectively or what statistical or modeling methods perform well on high-dimensional data of complex biological samples? Specifically, we will present a great potential of Raman spectroscopy for detection, identification and subsequent analysis of traces of body fluids like blood, saliva, semen etc. found at a crime scene using some novel approaches. In addition, Raman spectroscopy is capable to provide information about the changes in biochemical composition and molecular structure of the body fluids caused by a disease. The application of Raman spectroscopy of blood for Alzheimer's Disease diagnostics will be shown.

P.22-010-Mon
CaverDock: a novel method for the analysis of the ligand transport processes based on iterative docking

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Protein tunnels, ligand access pathways, play an important role in the function, stability or substrate specificity of enzymes. The tunnels enable the transport of ligands from the outside environment into the buried active site inside of the protein. Here we present CaverDock, a software implementing a novel method for the analysis of the ligand transport processes. The input for CaverDock calculations requires tunnel geometry obtained from CAVER and setup for molecular docking. Our method lies in between of geometrical approaches and molecular dynamics simulations. Contrary to geometrical methods, it provides an evaluation of chemical forces and better description of the studied system. On the other hand, it is not as setup-intensive and computationally demanding as the methods based on molecular dynamics. The method uses molecular docking to iteratively place the ligand along the tunnel with preservation of continuous movement of the ligand and minimization of its binding energy. The output of the calculation is the ligand trajectory and the energy profile of the transport process. CaverDock uses a modified version of the algorithm from AutoDock Vina for ligand placement and implements a parallel heuristic algorithm to search the space of possible trajectories. The duration of the simulations takes from several minutes to few hours, allowing parallelization for virtual screening of a large pool of molecules. We demonstrate CaverDock usability by i) comparison of the results with other available tools for the simulation of ligand unbinding, ii) analysis of the ligand trajectories in different tunnels of wild type and engineered proteins and iii) calculation of the energetic profiles for ensembles of substrates. The software CaverDock is freely available at https://loschmidt.chemi.muni.cz/caverdock/.

P.22-011-Tue
Graph-based pipeline for the classification and the marker genes identification of multifactorial skin diseases

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Inflammatory skin diseases such as psoriasis (PS) are complex pathologies involving large groups of interacting proteins. The central mechanism of PS development is still unclear as different researchers suggest different cell types to initiate the disease. Besides, there is a high level of heterogeneity between the patients in the therapy response, thus the development of a classifier capable for the identification of the genetic basis of the disease and the disease subtypes is the topical issue. However, the attempts to identify markers and classify this disease into subtypes with only the expression data tend to work poorly. Thus we have developed an algorithm to perform this task using databases of protein-protein interactions and gene pathways that classifies PS into types better than a list of differentially expressed genes does. Using the significant features in this subgraph and the index of mutual information (MI), it was possible to identify subgraphs that characterize the division of the disease into subtypes better than the seeds themselves. The pipeline requires an expression matrix and a vector of phenotypes. It begins with a generation of a list of the most reliable predictors that divides the disease into subgroups. The resulting features are further used as seeds for the subgraphs based on the KEGG or STRING databases. The neighbor nodes with highest MI are added to the seed greedily till increment function of MI reaches the plateau. The resulting subgraphs are subjected to the permutation tests to validate the obtained networks, as well as to verify the predictive power on the test dataset. This algorithm can be used as a basis for the identification of the markers for many multifactorial skin diseases such as atopic dermatitis, scleroderma to name a few, and for the future development of subtype-targeted therapies for psoriasis.

P.22-012-Wed
Identification of tumors with CpG island methylator phenotype (CIMP) based on TCGA genome-wide methylation profiling

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Tumors of CpG island methylator phenotype (CIMP) represent a distinct subset of colorectal cancers (CRC) with an increased prevalence of DNA hypermethylation in gene promoter regions. Traditional criteria of identifying CIMP tumors include assessing hypermethylation level of 5 genes (*hMLH1*, *p16*, *MINT1*, *MINT2*, and *MINT31*). Using the Cancer Genome Atlas (TCGA) data, we developed an approach to the identification of the CIMP tumors based on genome-wide methylation profiling. This approach is based on evaluation of median methylation level of a specific subset of 'marker' CpG sites. Each 'marker' site should: 1) be annotated in at least 2 of the 6 cell lines as a promoter, according to ENCODE data (ChromHMM/Segway); 2) belong to the top 10% differentially methylated sites, according to TCGA data. About 15–20% CRC tumors as classified as CIMP according to this approach. Then we performed gene set enrichment analysis (GSEA) for genes that are differentially expressed between CIMP and non-CIMP CRCs. We found that hypermethylation is accompanied by alterations in: 1) immune response and inflammation, TNF signaling pathway, sphingolipoid metabolism and sphingolipoidal signaling pathways, glycolysis (increased expression); 2) cysteine, taurine and methionine metabolism, PPAR pathways, peroxisomes (decreased expression); deregulation of the cell cycle, MAPK cascade, p53, Hippo, HIF1A, cAMP-dependent pathways. Positive perturbations in immune-related pathways are consistent with the fact of increased penetration of lymphocytes in CIMP tumors. Transcription factor binding sites enrichment analysis for genes being overexpressed in CIMP-like tumors suggests that the observed transcriptomic changes may be caused by CTNNB1, IRF1, SOX2, KLF4, STAT1/2, SP1, THRB, CREB2. This work and publication costs were funded by the Russian Science Foundation, grant 14–15-01083. The work was performed using the equipment of EIMB RAS "Genome" center.

P.22-013-Mon
Mutual influence of linker histone H1 amino acid sequence and DNA nucleotide sequence on the linker histone H1 position in chromatosome

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Linker histone H1 is a part of chromatosome and plays role in the transcription and replication regulation. Known and experimentally obtained chromatosome models suggest two conformation of chromatosome with different binding types for linker histone H5 from *G. gallus* and H1 from *D. melanogaster*. Our work was based on the hypothesis that amino acid sequence has impact on the binding type and on the fact that linker DNA geometry changes upon binding with the linker histone. To study mutual influence of amino acid sequence and DNA nucleotide sequence on the chromatosome conformation we used several molecular modeling methods such as atom-atom interaction analysis, DNA deformation energy analysis and homology modeling. Firstly, we analyzed known structures and proposed linker DNA sequences preferable for each conformation based on the DNA deformation energy. Different linker histone variants were classified by binding types based on their amino acid sequences. After classification we built chromatosome models for human linker histones variants using homology modeling. Atom-atom interaction analysis showed that linker histones has contacts not only with DNA, but with base pairs. Number of these contacts may differ depending on linker histone sequence. Homology models with binding type opposite to classified types showed decreasing number of contacts. We also built models with the suggested preferable DNA sequences, and interactions analysis of these models showed increasing number of contacts. Taken together our results support the hypothesis that not only linker histone amino acid sequence determines chromatosome conformation, but linker DNA also plays role. Interesting, that one linker histone may show both binding types depending on the linker DNA sequence. This hypothesis may be tested via spFRET. In our further studies we would like also to extend our approach to all linker histones with known amino acid sequences.

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P.22-014-Tue
Type II toxin-antitoxin systems for metagenomic studies

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The study of the microorganism diversity in human gut microbiota (GM) is actual task. More detailed studies revealed that a person's metagenome depends on the country, urban or rural dwelling, age group, diet preferences, general health state or various diseases. Today two main approaches are used to characterize taxonomic diversity: whole metagenome shotgun analysis and analysis of PCR amplicons from the 16S rRNA gene. The most comprehensive information is provided by metagenomic analysis. Most of similar software tools based on the presence of marker genes like MetaPhlAn or MG-RAST have resolution at most at the species level. These approaches based on gene copy number variation, require very deep metagenome sequencing and long analysis. We suppose that toxin-antitoxin systems (TAS) can provide additional markers for metagenomic analysis of species and strain diversity. The objective of this work was to use polymorphism in TA genes to analyze representation of specific species and strains in human GM. TAS are present in genomes of the majority of bacteria and archaea. Previously we have shown that in *Bifidobacterium* and *Lactobacillus* sp. the TAS may be used as functional biomarkers to differentiate these groups of bacteria at the species and strain levels. We expanded the database of TAS of RelBE and MazEF superfamily and included bacteria inhabiting the GM and shown that distribution of toxins and antitoxin genes in these bacteria are species and strain specific too and each strain is associated with the specific set of genetic markers. Thus we created the pipeline TAGMA and tested it on metagenomes samples from healthy people. The results of analysis were compared with results of well-known programs PhymmBL and MetaPhlAn. Based on a limited number of well selected markers TAGMA displays good time performance taking several hours to analyze a single metagenome as compared with several days required for MetaPhlan2 or PhymmBL.

P.22-015-Wed
Quantification of cell cultures by combination of object counting and statistical analysis methods

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We present a universal tool that implements an original semi-automatic cell count approach suitable for while not limited to the analysis of microscopic images of bacterial cells and tissue samples, bacterial colonies, histological images. Our approach consists of two consecutive steps. In the first step, objects of interest, e. g. single cells, are selected from representative sample images. This selection is performed using multi-threshold adjustment followed by size histogram analysis. Each local size histogram maximum corresponds to certain typical object size for a given threshold value. Once the appropriate maximum is found and typical objects are selected, they are used to estimate the statistics of single cell sizes. In the second step, the area of the raw image exceeding the chosen threshold is divided by the typical cell size this way providing the estimate of the cell count in a given sample. The proposed approach is an excellent solution when direct count of cells by flow cytometry is not available, like analyzing the fractions of differentially stained subpopulations in tissue samples and bacterial biofilms. The performance of the suggested tool has been confirmed using examples of eukaryotic and bacterial cells with different morphology. The software is entitled *BioFilmAnalyzer*, designed for Windows 32 bit architecture and can be freely downloaded by the following link https://bitbucket.org/rogex/biofilmanalyzer/downloads/, utilized and redistributed. The financial support of this work was provided by the Ministry of Education and Science of the Russian Federation (assignment 2.5475.2017/6.7) and Program of Competitive Development of Kazan Federal University

P.22-016-Mon
Potential binding site of two noninhibitory chaperons of human glucocerebrosidase

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Gaucher disease (GD) is caused by mutations in the glucocerebrosidase gene, leading to misfolding of the enzyme and substantial reduction of its activity in lysosomes. N370S amino acid substitution in glucocerebrosidase (GBase) is frequent among GD patients. In contrast to enzyme replacement and substrate reduction therapies, development of small chemical chaperons which increase enzyme stability and catalytic activity seems to be a promising approach for disease treatment. Recently, two chemical compounds NCGC00241607 [1] and NCGC00188758 [2] were found to increase an activity of mutant N370S GBase (Aflaki et al. *Sci. trans. med.*, 2014, 6(240), 240ra73-240ra73., Aflaki et al. *J. Neurosci.*, 2016, 36(28), 7441–7452). Here, we used molecular modeling approach to identify potential binding site of compounds [1] and [2] on a surface of mutant N370S GBase. Among four revealed binding sites we chose the one located near N370 residue. N370S amino acid substitution leads to formation of a cavity on the molecular surface of the enzyme. Molecular docking showed that both compounds: i) can partially embed their chemical groups into the cavity ([1] – benzene, [2] – pyrazolopyrimidine), ii) can form up to three hydrogen bonds with amino acid residues of the enzyme. Obtained complexes of two compounds remained stable during 100 ns molecular dynamics simulations in explicit water. The next stage of our work assumes utilization of revealed binding site for virtual screening of high affinity chemical compounds, having potential to act as molecular chaperons of mutant N370S GBase. The results of the work were obtained using computational resources of Peter the Great Sainte-Petersburg Polytechnic University Supercomputing Center (www.scc.spbstu.ru). The work was carried out with funds from the Russian Science Foundation (project No. 17-75-20159).

P.22-017-Tue
Automated segmentation and analysis of DNA and protein electrophoresis gels

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We present a universal tool for the DNA and protein automated electrophoresis gels analysis. Individual lanes are automatically extracted from the raw gel images by edge detection algorithm following by their comparison using cosine similarity statistics. For a better distinction between nearly similar lanes with cosine similarity of raw data approaching one, we suggest a preliminary transformation that selects individual bands by nonlinear peak detection algorithm using gradient estimates calculated as the product of the raw density and its derivative and averaged in a gliding window. For the transformed datasets, the cosine similarity scores reduced from 0.97–0.99 to 0.3–0.7 this way improving the accuracy of distinction between nearly similar gel lanes. For finding the closest lane from several samples, the maximum cosine similarity score criteria that always provides the closest sample to the tested lane is applied. For testing whether one or several samples are statistically indistinguishable from the test lane, the distribution of cosine self- as well as cross-similarity scores is estimated according to an analytical approximation that has been verified independently by numerical treatment. The performance of the suggested tool has been tested using series of crude extracts of treated and untreated cells, immunoprecipitats and amplificats from random primers. The financial support of this work was provided by the Ministry of Education and Science of the Russian Federation (assignment 2.5475.2017/6.7) and the Program of Competitive Development of Kazan Federal University.

P.22-019-Mon
A new, fast method to estimate sublimation entropy and dissociation constants from molecular crystal data

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One of the main problems in prediction of binding free energy (or dissociation constants) consists in a correct evaluation of binding entropy. Such an evaluation can be done from molecular dynamics, but this requires a huge computational time. We have developed an alternative approach which allows a fast calculation of the binding entropy. This approach is based on an evaluation of the mean range of those molecular movements that are restricted in the bound molecule, but are not restricted in its "free" state. As a model, we considered the reversible dissociation of small organic molecules from their molecular crystals to vapor, which allows us to calculate and compare with experiment their sublimation entropies and then their dissociation constants. The range of the movements of molecules in the bound (in-crystal) state can be calculated from known geometrical parameters of the molecules and experimental data on the pressure of their saturated vapor and their sublimation enthalpy. At first, we have calculated the range of movements in individual crystals, and then the averaged range was used for calculation of sublimation entropies and, combined with the rapidly obtainable binding enthalpies, for calculation of dissociation constants. The results of these calculations are in close agreement with the corresponding experimental values. This work has been supported by the Russian Science Foundation Grant No. 14-24-00157.

P.22-020-Tue
Modeling the reaction catalyzed by carboxypeptidase T by combined QM/MM method

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Metallocarboxypeptidases (MCps) are zinc-dependent enzymes that catalyze the removal of the C-terminal amino acid residue from peptide and protein substrates. Carboxypeptidase T (CPT) from *Thermoactinomyces vulgaris* has so far been considered to possess a 'dual' substrate specificity being capable of cleaving off C-terminal hydrophobic and positively charged residues, whereas specificity of mammalian carboxypeptidases CPA and CPB is strongly limited to hydrophobic and positively charged substrates, respectively. The CPT substrate specificity differs significantly from that of CPA and CPB, and this discrepancy cannot be explained by existing enzyme theory, suggesting a more complex mechanism of CPT substrate selectivity determination. In order to analyze CPT substrate selectivity, we modeled CPT-mediated hydrolysis of the peptide bond in dipeptide substrate. We created the initial substrate-enzyme complex by molecular docking and performed metadynamics simulations to refine the initial catalytic site – dipeptide substrate complex. This method allows to sample the conformational space of site-substrate interactions and choose the conformation with the minimal free energy. The selected initial complex was consistent with the theoretical model of MCps mediated hydrolysis. We used hybrid QM/MM (quantum mechanics/molecular mechanics) method to simulate the interaction between the dipeptide and active catalytic protein residues. Modeling was performed within Amber software package. The resulting model allowed us to estimate the substrate-enzyme binding energy and track structural changes, that take place during the reaction. This study gives an insight into the selectivity mechanism of CPT and introduces semi-empirical quantum chemistry approach tailored for this purpose. This work has been supported by the Russian Science Foundation (Project 17-14-01256).

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P.22-021-Wed
Free energy calculation of transition state analogues binding by carboxypeptidase B mutants

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Carboxypeptidase B (CPB) has a high specificity for positively charged C-terminal amino acid residues. CPB is involved in the process of insulin production, so the study of CPB has both scientific and practical significance. This study is devoted to the investigation of a number of CPB mutations by computer simulation methods. The calculation of the free binding energy by the MM_PB/GBSA method implemented in the Amber14 program was used. The analogues of the transition state for the residues of phenylalanine and arginine of N-sulfamoyl-L-phenylalanine (sphe) and N-sulfamoyl-L-arginine (sarg), respectively, were used. Charges for inhibitor molecules were determined by QM optimization in R.E.D. tools. Parameters for atoms are selected from the GAFF force field. The protein and mutant molecules are parametrized in the FF14SB force field. As new, there are four single and two double mutations of CPB: L203Q, A250N, A250S, T268V, T268N, S207G G243N, S207G G243S. For each of the mutants in the complex, binding energies with sphe and sarg were calculated. According to the results of the work, it is shown that mutants A250S and S207G G243N are the most promising for further research. This work has been carried out using computing resources of the federal collective usage center Complex for Simulation and Data Processing for Mega-science Facilities at NRC "Kurchatov Institute" and supported by the Russian Science Foundation (Project 17-14-01256).

P.22-022-Mon
Protein environment influence on the energy transfer and quantum dynamic in LH1-RC complex of *Thermochromatium tepidum*

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Theoretical description of pigment-pigment and pigment-protein interaction is a question of high importance for the understanding of high efficiency of the photosynthetic processes. Pigment-pigment interaction in the supramolecular chromophore-protein complexes provide an effective light absorption and selective interaction with the protein environment ensures excitation energy transfer to the reaction centers where charge separation process occurs. In the current work we report the calculation of spectral properties of circular LH1-RC complexes of purple bacteria *Thermochromatium tepidum* and rates of excitation energy transfer and exciton relaxation. To calculate stationary spectra we used method of exciton Hamiltonians with transition energies calculated by CASSCF/XMCQDPT2 method and exciton couplings calculated by TrCAMM decomposition. To take into account the site heterogeneity we carried out a separate calculation for each of the bacteriochlorophyll. Calculated absorption, circular dichroism and magnetic circular dichroism spectra agree well with the experimental ones. For the absorption and CD spectra lines of the transition to each exciton state were non-empirically broadened taking into account electron-phonon interaction with the protein environment. We used conventional approach in which system was separated on the quantum subsystem (chromophores) and thermal bath (protein). Hamiltonian of system-bath interaction was constructed based on the exciton wave functions of bacteriochlorophyll system and normal modes of the protein. The calculated spectral density of system-bath interaction was used for the calculation of dynamics of excitation energy transfer and exciton states population. We used a theory of quantum dissipative dynamics with non-equilibrium thermal bath which was previously applied by our group to the process of primary charge separation in the reaction center of *Rba. sphaeroides*.

P.22-023-Tue
Identification of target-specific autophagy modulators for therapeutic intervention in cancer

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Modulating autophagy using small-molecule inhibitors or activators represents one of the most promising therapeutic strategies in the fight against cancer and various major human diseases. Until now only a limited number of pharmacological agents suited for this purpose have been identified, hence restricting therapeutic exploitation of autophagy. Given the considerable progress made in the elucidation of crystallographic structures of components core to autophagy regulation, the present study employed several structure-based drug discovery techniques and computational approaches to identify new target-specific autophagy modulators. The aim of the present study was to identify potent inhibitors of mammalian target of rapamycin complex 1 (mTORC1) as lead compounds for the development of next-generation autophagy modulators with potential clinical utility. Structure-based virtual screening (SBVS) was employed for mTORC1 and the South African Natural Compound Database (SANCDB) selected for screening. A total of four hit compounds were identified from virtual screening and further analysed for their autophagy-inducing capacity using in vitro cell-based systems. Autophagy activity was monitored by acridine orange (AO), monodansylcadaverine (MDC), LC3B antibody detection, which identified promising autophagy-modulating effects for selected hit compounds. In conclusion, the present study provides a firm foundation for continued experimental analysis regarding the autophagy-modulating effects of candidate compounds specifically targeting mTORC1.

P.22-024-Wed
Prediction of the binding modes of antidepressants in the active-site gorge of acetylcholinesterase or butyrylcholinesterase: a protein–ligand docking and interaction profiling study

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Categorized into diverse classes of drugs with somewhat different mechanisms of action, antidepressants are used by many clinicians

worldwide in the treatment of major depressive disorder. The ability of antidepressants to readily penetrate the blood–brain barrier means that they may interfere with a number of neurobiochemical processes. Cholinesterases play multiple roles in the brain and periphery, carrying serious implications for human health and disease. In this study, a tricyclic antidepressant (amitriptyline) and two selective serotonin reuptake inhibitors (fluoxetine and sertraline) were separately docked in the active-site gorge of acetylcholinesterase or butyrylcholinesterase based on kinetic measurements previously taken by our laboratory and other groups. Redocking calculations with the bona-fide inhibitors huprine W and tacrine were also performed to evaluate the power of the methodology to correctly recognize the active-site gorge of cholinesterases and reliably predict the experimentally determined structures of cholinesterase–inhibitor complexes. Docking simulations and subsequent calculations of noncovalent interactions between the protein and the ligand revealed that each antidepressant could be accommodated well in the active-site gorge of the tested cholinesterase and that they necessarily interact with at least one of the several aromatic residues from the choline-binding subsite in addition to key residues from various subsites other than the choline-binding subsite (such as the peripheral anionic site or the esteratic subsite) depending on the orientation of the drug in the active-site gorge. These findings highlight the increased likelihood of acquired cholinesterase deficiency due to the long-term use of antidepressants and may also facilitate the design of new reversible cholinesterase inhibitors as treatments for Alzheimer disease.

P.22-025-Mon
Development of a new classification approach for detecting the heterogeneity in cell population

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Biological data is considered to compose heterogeneous populations in usual. To clarify the mechanism in the living cell by computational approach, dividing such heterogeneous data is important. In this study, we developed a new classification algorithm based on Latent Profile Analysis (LPA) combined with Confirmatory Factor Analysis (CFA) to divide mixed data into classes, depending on their heterogeneity. In general cluster analysis, the number of measured points is a constraint, and thereby the data must be classified into fewer groups than the number of samples. By our newly developed method, the measured data can be divided into groups depending on their latent effects, without constraints. Our method is useful to clarify all types of omics data, including transcriptome, proteome and metabolic information.

Structural bioinformatics

P.23-001-Mon
Structural bioinformatics characterization of chitin deacetylases secreted by marine organisms

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The purpose of this work is to use structural bioinformatics tools to characterize and compare the structures of chitin deacetylases (CDAs) belonging to fungi and marine bacteria. This information is valuable for increasing our knowledge concerning the use of chitin deacetylases for the enzymatic conversion of chitin to chitosan, as the currently used chemical procedure is toxic for the environment. Despite the reduced sequences identity for investigated chitin deacetylases, there are amino acids belonging to their active sites that are strongly conserved reflecting the common biological function of these enzymes. Furthermore, the catalytic cavities of investigates enzymes reveal almost similar electrostatic properties, all of them being negatively charged. There are some local properties that are distinct for the bacterial and fungal chitin deacetylases. The hydrophobicity profiles of the catalytic cavities of fungal CDAs are distinct by comparison to bacterial CDAs, fungal CDAs revealing a local region with higher hydrophobicity that seems to be important for the catalytic efficiency. The loops surrounding the catalytic cavities expose distinct flexibility and hydrophilicity indexes. These local variations could be responsible of the distinct catalytic efficiencies of the investigated enzymes.

P.23-002-Tue
Proteogenomics of ADAR-mediated RNA editing in mouse brain

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Adenosine-to-Inosine (A-to-I) editing is a posttranscriptional modification of RNA molecule catalyzed by ADAR enzymes. It occurs mainly in neural tissue, where, as a result of the modification, adenosine is converted to inosine in particular sites of RNA, some of them code amino acid substitutions. We present a bioinformatic study of this phenomenon on publicly available deep shotgun proteomes of brain regions and acutely isolated cell cultures of C57BL/6 mice using proteogenomic approach. The method includes database generation for proteomics search of edited sites based on specialized genome-wide databases of RNA editing events. All datasets were searched using X!Tandem software with a group-specific false discovery rate for edited peptides with MP Score post search validation. For data filtering the Open search strategy was used. In total, 14 edited proteins were identified in 10 proteins, such as Gria2, Gria3, Gria4, Grm4, neural proteins Flna, Flnb, Cyfip2, Cadps and Ube2o. Four identified edited proteins belonged to AMPA glutamate receptor complex which played a significant role in excitatory synaptic transmission. Identification of RNA editing in these proteins was in good agreement with background works. The signal from peptides resulted from A-to-I editing was strongest in early stages of development such as young cell culture of microglia and oligodendrocytes. Therefore, editing can play a significant role in formation of nervous system. It was also noted that the neurons such as cortical and cerebellar granule neurons, are subjected to editing more than glial cells such as astrocytes and oligodendrocytes. The results presented above have a good correspondence with the literature data. Interestingly, both the edited form of the peptide and the wild-type peptide can be presented in one sample, which requires further research. Thus, it was shown that a proteogenomic approach is a potential tool for the analysis of RNA editing at the shotgun proteome level.

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P.23-003-Wed
Selection of bifunctional tyrosyl-DNA phosphodiesterase 1 inhibitors through molecular modeling

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Tyrosyl-DNA phosphodiesterase 1 (TDP1) is a DNA repair enzyme and a promising target for anticancer treatment. It cleaves chemotherapy-induced stalled topoisomerase-DNA complexes, where a catalytic tyrosine is linked to the 3′-terminal phosphate, thus maintaining the DNA structure in cancer cells. There are several compounds known to suppress TDP1 activity, but their inhibitory mechanisms and specific interactions are still to be uncovered. We identified two binding cavities in the active site of the constructed molecular model of human TDP1: one for the phosphotyrosine and the second for the oligonucleotide moiety of the substrate. The binding site of the first (from the 3′-terminus) phosphate group of the substrate is located in the phosphotyrosine cavity and comprises residues Lys265, Asn283, Lys495, Asn516, as well as a conserved water molecule. The second phosphate group binds in the oligonucleotide cavity forming interaction with Ser400, Ser518, and two conserved water molecules. Virtual screening was performed to identify bifunctional inhibitors targeted towards both phosphate binding sites and able to form specific hydrogen bonds with the above-mentioned residues and water molecules. Since sulfo group is a structural analogue and isostere of the phosphate group, we retrieved sulfonates from the ZINC database (2227 compounds) and docked them into the TDP1 active site using the Lead Finder software. Docking poses were then filtered using an in-house script to identify compounds capable of forming the specified interactions. Several potential TDP1 inhibitors containing two sulfo groups joined together via a linker (e.g. dimesna) were selected for experimental evaluation. The flexible linker allowed functional substituents to occupy both the phosphotyrosine and oligonucleotide cavities and form hydrogen-bonding networks with the phosphate binding sites. The work was supported by a grant of the Russian Foundation for Basic Research (18-315-00389).

P.23-004-Mon
An automatically refreshed public list of amyloid and pre-amyloid structures from the PDB

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The Protein Data Bank is the most important resource of protein structural information today, containing 130 000 entries. The proper mathematical analysis of this dataset may yield significant biochemical results. In the present work we constructed the PDB Amyloid List, an automatically updated list of PDB entries, which contain amyloid structures, or substructures, which have the potential of becoming amyloids. Our algorithm examines the geometrical properties of the polypeptide chains of the PDB entries, and finds parallel beta-sheets, satisfying some strict additional properties. The resulting list of PDB entries is publicly available at https://pitgroup.org/amyloid.

P.23-005-Tue
A machine learning approach towards sequence based RNA binding protein sites prediction in human proteome

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The availability of human genome sequence and expansion of structural data in the Protein Data Bank (PDB) has transformed the biomedical research over the past decade. Analysis of the complete human genome sequence has thus far led to the identification of approximately 20,687 protein-coding genes, although the annotation still continues to be refined. RNA-Protein interactions play crucial roles in driving the cellular machinery as evident from their active participation in regulation of gene expression, protein synthesis, mRNA processing, mRNA assembly, ribosome function and eukaryotic spliceosomes. RNA binding proteins (RBPs) are known to regulate all aspects of RNA biogenesis and various post-transcriptional gene regulation processes and their dysfunction can lead to several diseases ranging from neurological disorders to cancer. Thus, accurate prediction of RNA-binding residues (RBRs) in proteins using innovative computational techniques is indispensable to comprehend the time consuming and cost-ineffective experimental methods and to apprehend the functional implications of specific protein-RNA interactions, to perform site-directed mutagenesis and to develop novel targeted drug therapies. We have thereby, developed a method to identify the RBRs from a given protein sequence employing a machine learning approach, using global and local sequence based features derived from known crystal structures of protein-RNA complexes deposited in the PDB. A Random Forest based classifier is used for the prediction. The analysis of the results on the training dataset using leave-one-out cross-validation experiments gave an accuracy of 98.4%, specificity of 99%, sensitivity of 72.7% and MCC of 0.82, performing better than few existing methods. Our prediction model will be further extended to validate the known RBPs and prepare a catalogue of RBRs in entire human proteome. An online accessible RBR prediction server is under development.

P.23-006-Wed
Investigating the heterogeneity of pathogenic bacterial genomes using computational approaches

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Bacterial populations are known to be heterogeneous, which can provide them with evolutionary advantages during changes of their environmental conditions, including development of resistance to antibiotics. In some cases, as it has been already shown for *Mycobacterium tuberculosis* isolates, heterogeneity exists at the genomic level, so that significant variations can be revealed even within a bacterial population seeded by a single cell. Currently, next generation sequencing (NGS) is extensively used for defining the structures of bacterial genomes, so it is very important to ensure that NGS is able to identify minor clonal variants in population, which should be distinguished from sequencing errors. Moreover, it is possible that in some cases the output of NGS data assembler software would represent an "averaged" genome sequence consisting of a mixture of several different bacterial genomes. Obviously, these issues complicate further analysis of such genomes. We have developed a heterogeneity measure

for genomic regions based on nucleotide substitution frequency matrices, read depth and variant frequencies. We applied the heterogeneity estimation procedure to the genomes of pathogenic and opportunistic pathogenic bacteria newly sequenced by us, including 6 genomes of *Klebsiella pneumoniae,* 6 genomes of *Acinetobacter baumannii*, 4 genomes of *Mycoplasma hominis*, 4 genomes of *M. tuberculosis* and 3 genomes of *Staphylococcus aureus*. As a result, we found that the genomes of *M. hominis* are extremely heterogeneous (more than 4 times comparing to *K. pneumoniae*), while *M. tuberculosis* and *S. aureus* are slightly less heterogeneous, and *A. baumannii* and *K. pneumoniae* have substantially homogenous genomes. We plan to extensively study the available pathogenic genomes to re-assess their sequencing quality, reconstruct minor clonal variants based on NGS data, and get insights regarding the origins of their variability.

P.23-007-Mon
Virtual high-throughput screening for identification of *Plasmodium falciparum* protein kinase CK2-alpha inhibitors through targeting the active, allosteric and subunit interface sites

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Malaria remains the deadliest tropical disease caused by protozoan blood parasite *Plasmodium falciparum* and responsible from 216 million cases and half million deaths in 2016 despite many efforts. Clearly, identification of novel drug targets and drugs are urgently needed. Protein kinase CK2 (formerly casein kinase II) is an unspecific protein kinase which has more than 300 substrates to phosphorylate and have role in a wide range of cellular processes including cell cycle progression, survival, differentiation and proliferation. Recent studies show that the plasmodial CK2's one catalytic and two regulatory subunits are essential for the asexual blood stages of parasites and also having role in chromatin dynamics. In this study, we have focused on the protein kinase CK2-alpha subunit of *Plasmodium falciparum* (*Pf*CK2) to identify novel small chemicals and coumarin derivatives as drug candidates by virtual high-throughput screening workflow. Before screening, structural dynamics of the *Pf*CK2 was assessed by 25 ns long molecular dynamics simulations by using NAMD program. Analysing the structural dynamic nature of the protein, three different conformational structures were selected for the further analysis. Close to ten thousand small chemicals and coumarin derivatives from the ZINC and the PubChem databases were screened against one active site, three allosteric and one subunit interface sites of *Pf*CK2-alpha by Schrödinger Glide program. Based on the highest docking scores, outstanding results come from coumarin derivatives docked into the active site, allosteric site 3 that is related with the activation loop and closed conformation of the subunit interface. Taken together, novel inhibitor candidates targeting the allosteric site and subunit interface of highly druggable protein *Pf*CK2 were determined and selected for the further *in vitro* analyses by integrated virtual approaches.

P.23-008-Tue
Evolutionary conserved residues in proteins – looking beyond active site and structural core

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Functionally important compartments of enzymes tends to be preserved during evolution of protein families. Active site residues are one of the most well-known examples. Due to their indisputable role in catalysis process, most of mutations results in unworkable proteins. Recent research shows that residues located on protein surface evolve faster than residues building protein core. Despite active site residues, there is no research concerning on the evolution rates of functionally important residues buried inside enzyme core. Growing evidence of the importance of transportation pathways linking the active site with protein surface raises the question of tunnels conservation. Problem is not trivial since tunnels may be equipped in gates and anchoring residues which contribute to selectivity of enzymes. This leads to opposing statements: i) due to critical role of tunnels residues building access pathways, tunnels might be conserved and ii) residues fine-tuning access to active site might be highly variable. In our research we are proposing a methodological approach for evolution rate analysis of any particular fragments of protein. As a testing system we have chosen epoxide hydrolases – enzymes with buried active site. Using combination of CAVER and molecular dynamics simulations we have identified several potential tunnels connecting active site with surrounding environment. Such long procedure was compared with simplified one based on crystal structure analysis only, which if accurate enough might be widely used in future for similar studies. The R package BALCONY (Better ALignment CONsensus analYsis) was used to facilitate entropy analysis of selected amino acids and comparison of evolutionary rates with other compartments of enzymes. Finally we are providing information about linking the evolution rates of particular residues with their functional analysis which gives insight into the strategy of enzyme adaptation and allows to identify functional hot spots.

P.23-009-Wed
Longevity in euarchontoglires: lost genes as a determinant

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In the 42nd FEBS Congress, we have reported the hypothesis that the loss of genes has notable evolutionary consequences; it has been exactly realized for specified potential genes in an original bioinformatics method based on the definition of a significant synteny disruption (http://lab6.iitp.ru/en/lossgainrsl). The method is applicable to data describing the absence or, vice versa, the presence of ontogenetic characters specific for these consequences. The method generates relatively short lists of genes for various data sets. First, we analyzed the body appendages regeneration and the telencephalon and eye development, and a diverse experimental testing of a gene identified by our method in *Xenopus laevis* called *c-Answer* coupled with the team of Prof. A. Zaraisky (Institute of Bioorganic Chemistry, Moscow, Russia) has confirmed the hypothesis (in press). Then the method was applied to the data on the genetic propensity of certain species for longevity and anti-

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ageing. The species with this ontogenetic property include mammals with a greater lifespan than could be expected from their body size: mole rats and primates (including the naked mole rat, Damaraland mole rat, capuchin monkeys, gibbons, western gorilla, bonobo, chimpanzee, human). The list of lost mouse genes includes *Smpd5, Spint5, Ttc41, Wap,* and *2310003L06Rik* expressed in the tongue, and certain vomeronasal and olfactory receptor genes. Overall, the revealed mouse genes demonstrate specific expression in reproduction-associated tissues, which agrees with the Williams' hypothesis concerning the reallocation of physiological recourses. The loss of some revealed vomeronasal and olfactory receptor genes in human and naked mole rat conforms to their special anatomical features. For this data, we suggest that the loss of certain genes in evolution is a determinant of lifespan elongation and ageing deceleration including neoteny. The research was supported by the Russian Science Foundation, pr.14-50-00150.

P.23-010-Mon
Bioinformatics analysis of a transgenic personalized murine model of refractory epilepsy

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GRIN2A is a protein coding gene, which encoded NR2A subunit of NMDAR. A mutation in the exon 14 of the human GRIN2A gene was found in the patient (chr16: 9857449; G>G/A) and was associated to refractory epilepsy. In this study, we applied a bioinformatics approach to model murine mutated GRIN2A protein and appreciate possibility to create a murine model of this disease. The BLAST was used to find homologous mutation in murine GRIN2A gene. X-ray resolved structural models of the NR2A segments were used as source for M4T Raptor algorithm to generate predicted structure of mutated protein. The position of homologous point mutation in murine GRIN2A gene was revealed by the BLAST. Sequence alignment of the murine and human NR2A had 98% similarity, allowing to operate X-ray resolved structural models of the NR2A segments. It was shown that the replacement of Val440 to Ile440 amino acid via single nucleotide mutation, was located in S1 domain (404–539) region, forming loop called "loop 1" functioning as the NR1 NR2 ABD–ATD junction and heterodimer structural interface. Both residues were aliphatic and it was claimed, that this particular site is conservative mutation site. Nevertheless, observed changes in mutated protein model involve beta strands 2- and 3-elongated regions of beta sheets could be part of the structural compensation due to steric effect of bulkier than natural valine isoleucine residue. It is possible to assume, that NR2A disfunction can possibly lead to NMDAR hypofunction and neuron excitatory properties changes, causing higher nervous functions malfunction and further severe symptoms. It was shown that homologous mutation in human and murine GRIN2A gene led to the same conformational change in the protein structure and formed the mutated NR2A subunit. Thus, it is possible to create murine personalized model of the human refractory epilepsy. This study was supported by Russian Science Foundation (Grant #17-75-20249).

P.23-011-Tue
Application of a generic platform for the discovery and optimization of bio-active molecules

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In today's molecular medicine, information exchange between experimental and computational approaches is vital for the rational study of clinically relevant proteins. The structural knowledge of such proteins provides the rational context that not only leads to a more profound understanding of the activities of such proteins, but also allows exploitation of such knowledge to provide novel means of diagnosis or therapy. We have built a generic platform that combines experimental approaches (i.e. basic biochemistry, molecular biology, peptide chemistry, surface plasmon resonance and ITC) with structural bioinformatics approaches (docking, molecular modeling, virtual ligand screening, molecular dynamics simulations and free energy calculations). With this platform, a team of experimentalists and bioinformaticians jointly address common research questions and adhere to a structure-driven approach to guide and rationalize functional work. This allows the identification and optimization of biologically active molecules that are active in vivo and that may be used as novel therapeutics or form the basis of novel modes of diagnosis. We have successfully identified small molecules and peptides against a variety of protein targets, both intra- and extracellular, including some from the fields of thrombosis, atherosclerosis, inflammation and antibiotic resistance. The variety of targets illustrates the synergistic power of our approach and moreover, we have shown that it is possible to obtain highly active molecules that express their activities also in vivo, via a cost-effective optimized non-traditional route of discovery and optimization.

P.23-012-Wed
The reliability of loop rebuilt by homology modelling in context of protein functionality and active site accessibility

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The reliability of study based on protein structures strongly depends on quality of models and flexibility of particular compartments of macromolecules. Flexible and solvent-exposed regions like loops are prone to be poorly solved what hinders further protein analysis. Homology modelling is one of the widely used approach for reconstruction of missing residues in incomplete structures. The quality of obtained model is determined mostly by sequence similarity between used templates and size of the missing fragment, however scoring functions assessing accuracy of obtained models were not designed for flexible and solvent-exposed fragments of proteins. The structure of *Aspergillus niger* epoxide hydrolase is a good example: each crystal structure deposited at PDB database (Protein Data Bank) is missing a nine-amino-acids-long loop: 320TASAPNGAT328. This missing loop is located at the protein surface adjacent to the entrance of a buried active site in proximity of the catalytic pocket and thus

it can play a major role in regulating access to the active site. The reconstruction of this particular loop is not trivial since in comparison with other members of the epoxide hydrolase family, *Aspergillus niger* epoxide hydrolase has an additional N-terminal meander domain and extended cap domain. The missing loop is located just between the cap domain and the protein core. Therefore it is a perfect model for detailed study of the importance of reconstructed model quality and reliability of the most often used approach. We decided to verify obtained models using molecular dynamics simulations and analyse the water accessibility into the active site using AQUA-DUCT software. The extended analysis suggests that the active site accessibility is quite similar although the pathways used by water molecules depend highly on the loop model used for analysis. We were able to discard poor models easily and propose a general approach which can be applied for loops reconstruction.

P.23-013-Mon
Creation of a personified model of atherosclerosis in genetically modified mice

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According to the WHO CVD are the leading cause of early death worldwide. Atherosclerosis is a chronic artery disease characterized by the accumulation of lipoproteins in the vessel wall. General theory of atherosclerosis is still not developed. 9 mutations in the mitochondrial genome associated with atherosclerosis were found in a group of 190 patients. So, there is an actual problem of creation genetically modified mouse model of atherosclerosis, which has homologous mutations. To solve this problem, we chose mutations located in structural genes. There are MT-ND6 gene (mt:14459; G>A) and MT-CYTB gene (mt:14846; G>A). The MT-ND6 gene encodes the 6th core subunit of the ETC 1st complex – NADH-dehydrogenase, and the MT-CYTB gene encodes the catalytic subunit of the 3rd complex – cytochrome-bc1-oxidase. We studied the spatial structures of these proteins using bioinformatic programs PyMOL and Geneious. In ND6 protein the amino acid alanine72 is replaced by valine72, and in CYTB protein amino acid glycine34 is replaced by serine34. It was shown that mutation in the MT-ND6 gene leads to a change in the ubiquinone-binding site of NADH-dehydrogenase, making it more sensitive to ubiquinone, which is able to inhibit complex I. In the protein MT-CYTB mutant serine is sterically bulkier then native glycine. Apparently, this leads to incorrect assembly and a decrease of the enzymatic function. We assume that in this case, an electron leak occurs during oxidative phosphorylation, resulting in oxidative stress, which is known to correlate with the course of CVD. It was shown that homologous mutations in the appropriate genes of human and mouse lead to the same conformational changes. Thus, a mouse model of mutations in the genes MT-ND6 and MT-CYTB is suitable for the human, and these mutations were chosen to create a genetically modified model of atherosclerosis in mice. This study was supported by Russian Science Foundation (Grant # 17-75-20249).

P.23-014-Tue
Bioinformatic structural analysis of a transgenic personalized murine model of epileptic encephalopathy

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GNAO1 is a Guanine nucleotide-binding protein G(o) subunit α coding gene. Being part of heterotrimeric G proteins, Gα subunit transduce signal from GPCRs to cytoplasmic effectors. Single nucleotide change in GNAO1 cause 203G>R aminoacid replacement. We built structural model of murine mutated Gα protein segment to analyse possible structural-activity change and to substantiate creation of a murine model of this human disease. Homologous mutation in murine GNAO1 gene was found by BLAST. X-ray resolved structural model of the murine Gα were used as template for M4T Raptor algorithm to generate predicted structure of mutated protein. Structural models were visualised and aligned for analysis in PyMol (Schrödinger, Portland, OR). 203G being changed to arginine, which is negatively charged and sterically much bulkier, located in immediate proximity to the ligand binding site, in conserved switch II region predicted to dramatically decrease GTP binding and activation of signal transducing intracellular effector proteins. This mutation, which human-mice homologicity was determined by BLAST, possibly lead to severe physiological neurological disorders, called epileptic encephalopathy. Human/murine GNAO1 203>G replacement mutation homology allows us to claim, that murine epileptic encephalopathy model should be established for further investigations for diseased people treatment search. Structural changes in mutated protein being described could be useful for optimal therapy development. This study was supported by Russian Science Foundation (Grant # 17-75-20249).

P.23-015-Wed
Bioinformatic analysis of a transgenic personalized murine model of Lesch-Nyhan syndrome

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HPRT1 is a Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) coding gene, mutations of which cause, among other diseases, Lesch-Nyhan syndrome. LNS is a severe X-linked recessive neurological disorder. In this study, we built structural model of mutated murine HGPRT segment, with Val8del to compare it with native form of the protein by bioinformatic tools. Alignment shows 98.6% homology of human and murine protein sequences. By this, we assume that murine model of this human disease should be developed. The BLAST was used to find homologous mutation in murine HPRT1 gene comparing to Human one. X-ray crystallographic structural model of the HPRT1 was used as template for M4T Raptor algorithm to generate predicted structure of mutated protein. Models were visualized in PyMol (Schrödinger, Portland, OR). Lack of enzymatic activity of the HGPRT could be caused via troubled homodimerization. Deletion is found to be located in the HGPRT monomer surface close to the dimer junction. Absence of the aliphatic Valine could be the reason of the hindered monomers interaction. Even such small structural changes in this region could be the reason of enzyme disfunction due to folding, functional activity checkpoint in endoplasmatic reticulum by ER quality control

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(ERQC) system. Homologous mutation in human and murine HPRT1 gene resulting into comparable conformational change in the protein model structure was revealed, so murine personalized model of the Lesch-Nyhan syndrome could be developed. Structural changes can be further studied to provide treatment strategy for people suffering from Lesch-Nyhan syndrome. This study was supported by Russian Science Foundation (Grant # 17-75-20249).

P.23-016-Mon
DNA structural alphabets

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DNA is a structurally plastic molecule that enables its biological function by adapting to its binding partners. A possible approach to comprehend the structural base of biomolecular recognition and function is to translate complicated three-dimensional structures into a linear code using so called structural alphabets, employing a limited set of building blocks that can be symbolically represented by alphabet letters. The approach is used fairly routinely for protein structures defining an alphabet using oligopeptide segments, the approach is however new in analysis of DNA structures. The prevailing DNA architecture, a right-handed double helix composed of sequentially complementary antiparallel strands accommodates large conformational changes by forming kinks or bends or undergo radical rearrangements into loops or folded forms such as quadruplexes. These conformational changes cannot be understood without going beyond the "A-B-Z classification" traditionally used to describe the DNA structural diversity. The resulting DNA structural alphabet CANA (Conformational Alphabet of Nucleic Acids) accessible at the web site dnatco.org is successfully used to analyze the structural properties of typical DNA structures. The alphabet also allows quantitative assessment and validation of DNA structures and their subsequent analysis by means of pseudo sequence alignment.

P.23-017-Tue
Structural bioinformatics prediction of autoantibody binding to ADAMTS13

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In most patients with acquired TTP, autoantibodies target a cryptic epitope in the spacer domain of ADAMTS13. Based on the findings, auto-antibody resistant, gain-of-function variant (GoF) of ADAMTS13 was designed on the antibody epitope of spacer domain with the following amino acid substitutions: R568K/F592Y/R660K/Y661F/Y665F. Functional studies revealed that GoF-ADAMTS13 can bypass the binding of autoantibodies while the enzyme gains a higher proteolytic activity than its WT form. Our aim was to employ modeling to identify structural determinants in autoantibody binding and provide a knowledge necessary for the design of small compounds that inhibit autoantibody binding to ADAMTS13. Rosetta Antibody and Bioluminate Antibody modules were employed to model the Fab fragments of ADAMTS13 autoantibodies II-1 and I-9. Autoantibody binding onto the spacer domain was studied by HADDOCK protein-protein docking where the structures were constrained according to major epitope residues in ADAMTS13 Spacer domain and the CDR3 residues of the antibody heavy chain. GoF ADAMTS13 mutations were *in silico* introduced to final poses and both WT- and GoF- ADAMTS13 were subjected to binding free energy calculation with AMBER16 over 100 ns molecular dynamics simulation. Subsequently, these poses were investigated to reveal which antibody residues contribute to ADAMTS13 binding. A pose with relatively higher binding affinity to WT- and lower binding affinity to GoF-ADAMTS13 was used to predict which residues participate in autoantibody binding. These residue predictions will be subjected to *in vitro* mutation studies to test changes on ADAMTS13 conformation, its proteolytic activity and resistance against autoantibodies. We used structural bioinformatics tools to predict the nature of autoantibody binding which renders the human ADAMTS13 protein inactive. This knowledge can be further applied on designing peptide inhibitors of autoantibody binding to ADAMTS13.

P.23-018-Wed
Artifact-resistant bioinformatics pipeline for detection of somatic retroelement insertions in human genome

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The high-throughput sequencing (HTS) technologies opened exceptional opportunities to analyse genomic diversity of somatic cells in health and disease. However, due to the high sensitivity of HTS, the goal to distinguish the true signals from errors became a real challenge for bioinformatics. The main types of errors include single nucleotide errors appearing during HTS, chimeric molecules formed during library preparation and incorrect genome mapping of HTS data. Here we describe the advanced bioinformatics pipeline for error-resistant identification of one of the main source of human genome instability – somatic retroelement (RE) insertions. Our pipeline consists of Python scripts set assembled in Jupyter Notebook interface. The pipeline includes: genome mapping of HTS data, removal of known RE, errors filtration, comparison of target and control HTS libraries and identification of target site duplications – characteristic signs of transposition events. For the genome mapping we tested two widely used programs: BWA and Bowtie2. Our results indicated 4% rate of false-positive somatic RE generated by BWA and absence of these errors in case of Bowtie2. Artifacts filtration step contains removal of chimeras produced during adapter ligation, template switching and incorrect priming in PCR amplification. Thresholds for artifacts filtration were determined by statistical models based on training sets of fixed and polymorphic RE. The accuracy of our pipeline was tested on series of sequenced RE libraries with artificially generated imitations of RE insertions used as spike-in. During this tests, we correctly detected above 80% of added spike-ins. Thereby, our pipeline is one of the first artifact-resistant bioinformatics method for detection of somatic RE insertions. It can by applied for analysis of various type of HTS data including RE enriched libraries and whole-genome data. Funding: RFBR grants № 17-04-01280, № 16-04-00779 and RSF grant № 18-14-00244.

Omics technologies

P.24-001-Mon
Subfractionation of *Cronobacter* membranes and proteins characterization by mass spectrometry and immunoblotting

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Cronobacter spp. are opportunistic foodborne pathogens. These bacteria can cause severe necrotizing enterocolitis and meningitis, especially to immunosuppressed people and newborns with low birth weight with an alarming lethality up to 80%. The pathogenesis processes remains unclear, even though several virulence factors have been already described. Identification of potentially virulent proteins could help to clarify the process of *Cronobacter* pathogenesis. Subsequently, specific proteins could also be used to develop specific immunochemical identification methods, which can help with early identification to prevent the spread of disease. In this work, the method for isolation of membrane fractions and purification of proteins for further mass spectrometry based analyses was established. The isolation of periplasm, outer and inner membrane fraction of 7 representatives of *Cronobacter* spp. and 3 strains of other related genera was performed in triplicates. These fractions were characterised by SDS-PAGE and immunoblotting using antibodies against the whole *Cronobacter* cells and against particular membrane fraction of virulent *Cronobacter* strain. Proteins from membrane fractions were identified using mass spectrometry coupled with liquid chromatography (LC-MS). Altogether over 8000 proteins have been identified and subcellular localization was determined by *in silico* analysis. Finally, the potential virulent function of the identified proteins was determined.

P.24-002-Tue
The impact of ageing on the metabolic profile of rat testicular tissue: from the onset to the senescence

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Delayed parenthood has renewed the interest in age-related decline of male fertility. Ageing is often associated with testicular morphological and functional alterations that result in the declining of its functioning. Still, little is known about the molecular mechanisms associated with testicular senescence. Herein, we evaluated the testicular metabolic alterations associated with ageing. For that, we took use of nuclear magnetic resonance-based metabolomics to determine the testicular metabolic profiles of rats from 3 to 24 months-of-age. Moreover, as testicular function is highly susceptible to oxidative stress levels, due to the high metabolic rates, testicular oxidative damages were also evaluated. Several small metabolites were determined and creatine signals were the most predominant in testis up to 9 months-of-age. There was an age-associated decrease in most antioxidant metabolites, like betaine, creatine and oxidized glutathione. Aminoacid content changed as early as 6 months-of-age, with an increase in branched chain and aromatic amino acids, accompanied by decrease of nucleotide synthesis (IMP, CMP, ATP). On contrast, the testicular content of phosphatidylcholine and phosphatidylethanolamine increased with advanced age, with a concurrent decrease on the levels of their phosphorylated products, illustrating lower phospholipid synthesis. The contents of other phospholipid precursors, myo-inositol and glycerol, also increased in the testis of the same animals, further suggesting compromised spermatogenesis. This is the first metabolomics study in testicular tissue of aged rats. We were able to identify metabolites associated with reproductive maturity from the onset to senescence. Our results provide evidence for an influence of ageing on testicular metabolome, as early as 6 months-of-age, with a profound alteration on several key metabolic pathways associated with the male reproductive potential.

P.24-003-Wed
Identification of potential molecular targets by quantitative phosphoproteomics in sorafenib resistant hepatocellular carcinoma cells

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Hepatocellular carcinoma (HCC) is one of the most common cancer mortality and is often been diagnosed with advanced stages. Many efforts had been made for the development of effective HCC therapy, but sorafenib is to date the first and only FDA approved targeted therapy for advanced HCC. While many patients showed good initial response rate, it was inevitable for the development of drug resistance soon after the treatment. Due to the lack of other novel effective therapeutic agents, sorafenib is still the only option for advanced HCC. Therefore, comprehensive understanding of the underlying mechanism of sorafenib resistance in HCC and trying to identify the potential therapeutic protein targets is of great importance. To elucidating the signaling changes during the formation of drug resistance in HCC, we adopted quantitative phosphoproteomic approach to the analysis of the difference in phosphoproteome between parental HuH-7 and sorafenib resistant HuH-7 (HuH-7^R) cell lines. In current study, we identified and quantified 884 phosphoproteins among which most are up-regulated in serine and threonine sites. Global bioinformatics analysis of the significantly (>1.5-fold) up-regulated 569 phosphoproteins revealed that many signaling pathways involved in cell growth, motility, adhesion, protein synthesis and metabolism, comprised of certain important molecules such as RPS6, 4EBP1, PXN, EPHA2 and PFKP were altered during resistance development. From in silico interaction linkage analysis, we found that an Akt-centered signaling network is highly activated in $HuH7^R$ cells. Among the altered phosphoproteins, some important proteins which are implicated in close cross-talk with Akt. In conclusion, by quantitative phosphoproteomics we figured out a drug-resistance induced Akt signaling activation in HCC cells and also identified some potential targets for further evaluation of the effect on intervention in dysregulated resistant signaling.

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P.24-004-Mon
Identification and subtyping of *Cronobacter* strains using MALDI-TOF MS

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The Genus *Cronobacter* belongs to a gram-negative bacteria which could sporadically cause meningitis, necrotising enterocolitis or sepsis. Beside the immunocompromised elderly, especially neonates with low birth weight are at higher risk, because of premature digestive system and a mode of transmission. The neonatal infections are mainly linked to the consumption of contaminated powdered infant formula. Nevertheless, several infections of breast milk feed neonates have occurred. Moreover, contaminated feeding tubes and the utensils used for PIF preparation has been also linked to several cases. It has been shown, that not all of the *Cronobacter* species are pathogenic and only three of seven species (*C. sakazakii*, *C. malonaticus* and *C. turicensis*) of this genus was implicated in the majority of the infections. And there are obvious consequences between sequence types determined by MLST and caused infection such as neonatal meningitis and *C. sakazakii* clonal complex 4; necrotising enterocolitis and *C. sakazakii* sequence type 12; adult infections and *C. malonaticus* clonal complex 7. Our goal is to determine differences between *Cronobacter* strains already indicated by multi-locus sequence typing using intact cell mass spectrometry technique. We have implemented a procedure, which could be used to extend current Bruker Biotyper database of bacterial fingerprints and we are able to distinguish all the *Cronobacter* species. Moreover, we are focusing on the further subtyping. We use bioinformatics software Bionumerics for looking for such biomarkers, which could help us to determine possible strain association with the infection.

P.24-005-Tue
Proteomic analysis of nicotine response in *Paenarthrobacter nicotinovorans* pAO1

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Paenarthrobacter nicotinovorans is soil bacteria able use the toxic alkaloid nicotine as carbon and energy source. This ability was linked to the presence of the pAO1 megaplasmid and might offer a unique way of exploiting nicotine containing waste for the production of "green" chemicals. In the current study, we investigated the proteins expressed by *P. nicotinovorans* in the presence of nicotine by using shotgun proteomics. For this, *P. nicotinovorans* pAO1+ cells were grown to log phase on citrate, 0.005% nicotine and a combination of the two. The cell-free extracts were separated on 9–16% SDS-PAGE maxi gradient gels and stained by Coomassie Brilliant Blue. The gel lanes for different growth conditions were divided into gel pieces and subjected to in-gel digestion using trypsin. The resulting peptides mixture was analyzed by reversed-phase nanoLC-MS/MS using a NanoAcquity UPLC (Waters, Milford, MA, USA) coupled to a Q-TOF Xevo G2 MS (Waters). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium with the dataset identifier PXD008751. The bottom-up approach based on the MASCOT MS/MS database search algorithm used to identify all the proteins allowed us to identify a total of 792 non-redundant proteins. The differences in protein abundance in the different growth conditions showed that deamination is preferred in the lower nicotine pathway when citrate is present. Several putative genes from the pAO1 megaplasmid have been shown to have a nicotine-dependent expression, including a hypothetical polyketide cyclase and we hypothesize that the enzyme would hydrolyze the N1-C6 bond from the pyridine ring with the formation of alpha-keto- glutaramate. Moreover, two chromosomal proteins, a malate dehydrogenase, and a D-3-phosphoglycerate dehydrogenase were shown to be strongly up-regulated when nicotine is the sole carbon source correlating well with production the alpha-keto-glutarate from the pyridine ring of nicotine.

P.24-006-Wed
Host-specific reorganization of the *Sinorhizobium meliloti* bacteroids proteomes

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Root nodule bacteria belonging to the order *Rhizobiales* comprise species significant for agriculture and biotechnology with unique ability to fix atmospheric nitrogen in the symbiosis with leguminous plants. During symbiotic development, cells of the root nodule bacteria undergo drastic morphological and metabolic changes caused by global reorganization of transcriptomic and proteomic profiles resulting in the formation of so-called "bacteroids" that are terminally differentiated and capable of nitrogen fixation. Recent data suggest that formation of bacteroids is under systemic molecular control coordinated by a host plant species, whose specificity is one of the most intriguing aspects of plant-microbial interactions. In this study, we analyzed host-specific response of the bacteroids of the root nodule bacterium *Sinorhizobium meliloti* at the proteomic level. Using high-performance liquid chromatography coupled with mass-spectrometry (HPLC-MS) we performed a comparative proteomic analysis of the *S. meliloti* bacteroids isolated from two different species of leguminous plants, *Medicago sativa* L. and *Melilotus officinalis* L. We found that *S. meliloti* bacteroids produce various proteins in the host-specific manner, and many of these proteins were previously shown to be involved in symbiosis. We revealed that genes encoding ExoZ (surface lipopolysaccharide production) and MscL (large mechanosensitive channel) proteins show host-specific changes in the expression correlating with the proteomic data. Overall, our data show that bacteroids of the root nodule bacteria are regulated by the host plant in a species-specific manner. The work on proteomic analysis of the *S. meliloti* bacteroids was supported by the Russian Science Foundation (Grant No 14-26-00094); bioinformatic tools for data analysis were developed within the grant of the President of the Russian Federation (MK-3240.2017.4).

P.24-007-Mon
Comparative proteomic analysis of different tick cell lines

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Ticks are hematophagous ectoparasites and vectors for a variety of human and animal pathogens, including bacteria, viruses, and

protozoa. The availability of tick *in vitro* culture systems has facilitated many aspects of tick-borne pathogen research, including proteomics. One of the opportunities is a study of tick tissue-specific responses to an infection, as these cell lines are derived from embryonic cells and can differentiate to the various cell types differing in morphology and behavior. Nevertheless, the nature of tick cell lines remains unclear. Thus, the aim of the present study was the proteomic comparison of different tick cell lines. Three cell lines obtained from the hard tick *Ixodes ricinus*, and two from *I. scapularis* were investigated. An Intact mass spectrometry (IC MS) approach was used, as well as classical proteomic workflows, including 2DE. The IC MS spectra were very similar between all analyzed cell lines. The comparison of IC MS spectra of the cell lines and *I. ricinus* tick's organs revealed 15, 12, 7, and 2 shared peaks between cell lines and ovaries, salivary glands, Malpighian tubes, and gut, respectively. Two cell line peaks at m/z 6154 and 6822 were detected only in the ovaries spectra, and peak at m/z 8451 only in the salivary glands. The peak at 6935 m/z was detected in all analyzed samples. Despite the high similarity of tick cell lines according to the IC MS, some changes in their protein profiles were determined by 2DE. This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic project Postdok_BIOGLOBE (CZ.1.07/2.3.00/30.0032) and the Grant Agency of the Czech Republic (15-03044S, 18-27204S). Access to instruments and other facilities was supported by the Czech research infrastructure for systems biology C4SYS (project no LM2015055).

P.24-008-Tue
A set of serine peptidases from the S1A chymotrypsin family in Tenebrionidae beetles

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The stored product pests *Tenebrio molitor* and *Tribolium castaneum* are model beetles from the family Tenebrionidae. We identified, annotated and compared predicted sets of serine peptidases from the S1A chymotrypsin family (SP) in the gut transcriptomes of T. molitor and T. castaneum larvae and evaluated relative expression levels of their mRNA. In T. molitor larvae we found 183 sequences similar to SP, but only 87 represented active peptidases. The residual 96 sequences had substitutions in the catalytic triad and were classified as inactive homologs of serine peptidases (SPH). 31 sequences of active SP were related to trypsins, two to chymotrypsins A/B, one to chymotrypsin C, 13 were chymotrypsin-like peptidases, 12 pancreatic elastases I, and the specificity of 28 peptidases was uncertain due to unusual residues in the substrate binding site. T. castaneum contained 178 SP sequences, of which 98 represented active peptidases, and 80 SPH. T. castaneum contained many more trypsin sequences – 51, and fewer chymotrypsin sequences than T. molitor: one chymotrypsin A/B, one chymotrypsin C, and only five chymotrypsin-like peptidases. Three sequences were annotated as pancreatic elastases I and 37 peptidases were with uncertain specificity. These data are reflective of a highly complex digestive organization in these larvae, presumably a result of high selection pressure of cereal inhibitors. Analysis of the relative expression levels of SP mRNA revealed that each group of SP usually contained one major SP with the expression level exceeding 50% of the total expression of the whole group, a number of peptidases with medium level of expression, and many SP with minor level of mRNA expression. Usually the major SP from each group of *T. molitor* and *T. castaneum* SP were not orthologous. Some SPH had rather high levels of mRNA expression, and this implies that they have important physiological functions. This work was supported by RFBR grants 17-54-61008 Egypt_a, 18-04-01221_a.

P.24-009-Wed
A digestive system microbiota of medicinal leech *Hirudo medicinalis*

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Recent omics studies have attracted attention to the role of the microbial community for maintaining host organism. This is particularly true for hematophagous due to different molecular mechanisms and evolution adaptations caused by blood feeding. These adaptive mechanisms may include a signal transduction cascades and pathways related to immune response, blood digestion and storage processes. A combination of laser microdissection, sequencing, and bioinformatics allowed us to determine the bacterial composition of morphologically and functionally distinct parts of the leech digestive system. Analysis of 16S rRNA data obtained for different parts of the leech digestive system revealed that bacterial community of crop and coeca differs from intestine. In a *H. medicinalis* metagenome we identified taxonomic groups of bacteria relevant for leeches. Besides the genera of bacteria (*Mucinivorans*, *Aeromonas*, *Niabella*) that have been described for leeches earlier, we determined several bacterial taxa that have not seen in the leech metagenome before. Some of them were also described in other hematophagous. We found that microbiome of the digestive system supports the synthesis of vitamins and water-soluble cofactor, fixation of heme compounds and excessive iron metabolism. Surprisingly we identified genes involved in antibiotic resistance and genes encoding multidrug efflux pumps. Thus, the sequencing data obtained in our study allows for the analysis of the metabolic potential of microbiota and determination of the contribution of individual bacterial species and strains to metabolic pathways. Detailed studies are relevant not only for bloodsucking leeches but other hematophagous to determine the role of microbiota in the host organism adaptation to blood nutrition. This work was supported by the Russian Science Foundation (project № 17-75-20099).

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P.24-010-Mon
A genome-wide association study of muscle fibers composition revealed 5 SNPs associated with slow-twitch fibers

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Biochemical properties of muscle tissue are linked to a specific muscle fibers type. Predominance of slow-twitch muscle fibers predispose to more efficient endurance performance while fast-twitch fibers are crucial for sprinting activity. Since the composition of muscle fibers is known to be influenced by heredity up to 45%, we aimed to identify genetic factors associated with both muscle fibers structure and athletic performance. We compared genome-wide genotyping profiles (HumanOmni1-Quad) of 89 Russian sprinters and 103 endurance athletes. High throughput genotyping (OmniExpressExome-8) and investigation of muscle fibers composition using immunohistochemical analysis were performed for another group of 45 male athletes. To validate the results, we used a Japanese athletes sample (54 sprinters, 60 endurance athletes). Data analysis, processing and annotation were done using Plink, R, and Ensembl VEP. Russian sprinters and endurance athletes genotypes were compared using a case-control design: all together and after excluding sub-elite athletes. The results were intersected with a study of similar design that included Japanese athletes and resulted in 201 common SNPs ($P < 0.05$). After performing regression analysis of muscle fibers composition (percentage), we matched the findings and identified 5 SNPs associated with both predominance of slow-twitch fibers in males and endurance athlete status. Two SNPs (rs4561252, rs7080650) belong to phosphodiesterase superfamily and are involved in cardiovascular function while other three (rs6789275, rs7525663, rs992297) are intergenic and require further functional investigation. None of the 5 SNPs were described in the NHGRI-EBI GWAS Catalog earlier. This study has revealed 5 SNPs that connect the slow-twitch muscle fibers profile with the predisposition to endurance sport performance. The work was supported by the Russian Science Foundation, Grant No. 17-15-01436.

P.24-011-Tue
Proteomics for the detection of protein interactors of vascular endothelial growth factor in age-related benign prostatic hyperplasia rats

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Benign prostatic hyperplasia (BPH) is an age-related disease affecting the majority of elderly men worldwide. BPH involves an inflammatory process that can later lead to the development of prostate tumors. Inflammation is mediated by pro-inflammatory cytokines, chemokines and prostanoids, and growth factors such as vascular endothelial growth factor (VEGF). VEGF stimulates the proliferation and survival of endothelial cells and promotes angiogenesis and vascular permeability. VEGF participates in tumor metastasis and intraocular neo-vascular syndromes. Here we combined the Surface Acoustic Wave (SAW) technique with proteomic approaches for the direct characterization of interacting proteins related to the same metabolic pathways in biological samples. This novel combination was used to study the pathway of VEGF protein and its receptor. Briefly, the antibody against VEGF was immobilized on the surface of a SAW biosensor chip. Prostate homogenates of 12-month-old rats with age-related hyperplasia were injected and passed through the chip surface. Affinity binding phenomena of prostatic homogenate proteins with the immobilized anti-VEGF antibody and also with its receptor were first measured via the SAW biosensor. Interacting proteins were then eluted from the chip surface, collected and analysed by classical bottom-up proteomics. All the proteins identified were related to VEGF and VEGFR metabolic pathways and were previously reported to play crucial roles in inflammation diseases, cancer cell proliferation and metastasis when they are overexpressed in cells. The biosensor was also coupled online to MS using a dedicated interface (MSI). Proteins of 19–23 kDa corresponding to various isoforms of rat VEGF-A and VEGF-B were detected by online combination SAW-MS. This approach allows the detection, quantification and mass spectrometric structural characterization of affinity-bound biopolymers. PRB3 (IPT17/0019-ISCIII-SGEFI/ERDF), BMBS COST Action BM 1403 and FBG309273.

P.24-012-Wed
Adaptation of *Acholeplasma laidlawii* to stressors: changes in genome, cell and vesicular proteomes and mutagenicity to human cells

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Acholeplasma laidlawii (class Mollicutes) is widespread in nature (it can be found in humans, animals, plants); is the main contaminants of cell cultures and vaccine preparations, being a danger

to human health. Solution of the problem controlling the mollicute infections turns out to be connected with elucidating the molecular bases of adaptation of these microorganisms to adverse environments that determine a broad distribution of the bacteria in nature, formation of the "parasite-host" system *in vivo* and *in vitro*, and realization of virulence. The omics technologies have opened new opportunities for carrying out the appropriate studies. To select *A. laidlawii* PG8B strains with differential sensitivity to adverse environments (long-term exposure to low temperature, substrate limitation), to antimicrobials (ciprofloxacin, tetracycline, melittin) used for suppressing mollicutes, and to perform the comparative analysis of the complete genomes, cell and vesicular proteomes and virulence of the strains was the object of the study. Adaptation to low temperature, substrate limitation and antimicrobials in *A. laidlawii* proved to be accompanied by multiple changes in genomic profile, cell and vesicular proteomes as well as level of extracellular vesicles production. In all cases the processes proved to be accompanied by mutations in genes for targets of antimicrobials of different classes, including those indifferent for these bacteria. The mutant genes were detected in the *A. laidlawii* extracellular vesicles, which can provide gene distribution in bacterial populations via horizontal transfer. A significant part of the stress-reactive genes and proteins proved to be related to virulence. It was found that the strains adapted to the stressors exhibit mutagenicity to human cells – induce hypoploidy and total premature centromere separation in peripheral blood lymphocytes *in vitro*. Work was supported by grant MK-1099.2017.4; RFBR 18-04-00660.

P.24-013-Mon
Comparison of TLR3- and TLR4-mediated signaling cascades in glial cells

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Toll-like receptor (TLR) mediated signaling cascades play crucial role in neuroinflammation and therefore may be targets for manipulation with therapy purposes. Although many efforts have made, molecular mechanisms of TLR-mediated signaling in astrocytes is largely unknown, especially relative to regulation of signaling lipids release. Many of signaling lipids belong to a family of resolution of inflammation processes factors, and low molecular weight substances can regulate their synthesis, therefore the aim of our study was comparison of signaling lipids synthesis in course of stimulation by lipopolysaccharide (LPS, a TLR4 agonist) and poly I:C (PIC, a TLR3 agonist). Primary astrocytes from newborn rats were cultured 12 days before experiments; Western blot was used for detection of TLR signaling pathway proteins and enzymes of lipid synthesis. Cell-free culture media were taken for solid-phase lipid extraction; lipid mediators (25 lipids) were analyzed by 8040 series UPLC-MS/MS (Shimadzu) with 4 MS standards. Time-course cellular responses (1, 2, 4, 6, 8, 12, 24 h) for stimulation with LPS and PIC were compared. We obtained that three pathways of polyunsaturated acids (PUFAs) transformation (COXs, 12/15 LOX, cytP450) are involved in astrocytes responses. 13 substances are released in significant amounts. Releases of PUFAs or their non-enzymatic derivatives are similar for both tested agonists. TLR3 activation significantly induces enzyme-mediated metabolism in comparison with TLR4 activation. Both agonists induce shift of PUFAs metabolism from proinflammatory to resolution substances. Supported by RSF (№ 16-15-10298).

P.24-014-Tue
Metabolic profile analysis of a *Drosophila* model of Parkinson's disease

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Parkinson's disease (PD) is the second most common neurodegenerative disease in the world. Its symptomatology is due to a selective loss of dopaminergic (DA) neurons in the substantia nigra pars compacta. Currently, diagnosis of PD is mainly based on the presence of motor alterations, which appear at late stages when there is up to 70% DA neuron loss. Therefore, it is important to find new biomarkers that will allow early diagnosis and treatment of PD patients. Recent studies have revealed links between glucose metabolism and alterations in cellular bioenergetics, redox homeostasis and cell death progression induced by PD-related risk factors. To study the possible link between altered metabolism and PD, we used a *Drosophila* model of PD based on inactivation of the *DJ-1β* gene (ortholog of the *DJ-1* human gene). *DJ-1* is involved in early-onset recessive PD and encodes a multifunctional protein that plays an important role in antioxidant responses but also regulates central metabolism. Consistent with this, studies performed in our laboratory have shown that several proteins related with metabolic pathways are highly carbonylated in mutant flies. To further investigate the impact of *DJ-1β* loss on cellular metabolism, we characterized the metabolic profile of polar and non-polar extracts from 15-day-old *DJ-1β* mutant and control flies by high resolution nuclear magnetic resonance (NMR) spectroscopy. These analyses have revealed the presence of metabolic changes in PD model flies that could be relevant to understand the pathophysiology of PD. We will present the results obtained in these studies, which have allowed to the identification of metabolic pathways altered in mutant flies. Our results will be discussed in the context of how these metabolic alterations could help to understand the pathophysiology of PD and may lead to the discovery of biomarkers for PD diagnosis.

P.24-015-Wed
Restoration of the NEPHGE two-dimensional electrophoresis method

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Two-dimensional gel electrophoresis (2DE) is one of the most widely used techniques for the global protein separation. NEPHGE (non-equilibrium pH gradient electrophoresis) based 2DE is a carrier ampholytes-based isoelectric focusing (IEF) technique that failed to achieve widespread application due to its labor intensiveness but is used in more specialized research. It provides better separation of highly basic proteins, has a higher protein capacity with good reproducibility and quality of spots at high protein load when compared with a widespread IPG (immobilized pH gradient) 2DE technique. The equipment and solutions needed for performing NEPHGE technique were commercialized by WITA GmbH as a "WITAvision" 2DE

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system. Unfortunately, the company suspended its activities and left no options for equipment holders to obtain their commercial solutions. Since the NEPHGE broad range (pH 3–10) 2DE technique was customized by "WITAvision" from publication by J. Klose in 1995, there was enough data for restoring the original solutions needed to perform NEPHGE based 2DE. In this work, we recreated the mix of carrier ampholytes for IEF using currently available ampholyte solutions with diverse pH ranges as most of the ones used in the publication by J. Klose in 1995 are no longer available. After analyzing various mixtures of the carrier ampholytes we selected one that has shown the highest spot count, best reproducibility, and its composition was the most similar to that described by J. Klose. We then compared our recreated carrier ampholyte IEF gel solution with the commercial solution and found that despite slight discrepancies in overall protein pattern the spot count and their reproducibility was highly uniform in both gels. To conclude, our restored mixture of carrier ampholytes resembles that used in the commercial version produced by "WITAvision" and unavailability of commercial solutions for NEPHGE based 2DE is no longer a reason to stop using this method.

P.24-016-Mon
Autoantigens produced by transformed thyroid cells expressing frequent oncogenic proteins

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The diagnostics of oncological diseases is the key factor to their successful treatment. The process of tumor development is often accompanied by the change of autoantibody repertoire that can serve as a diagnostic marker of the oncological process but is rarely used in clinical practice. Apparently, most autoantibodies result from various nonspecific cellular and tissue changes which are caused by the tumor growth or other pathologic processes. We are developing an approach to search for autoantibody biomarkers produced in response to the activation of a few key oncogenes. This approach is based on the comparative immunoproteomic analysis including Difference gel electrophoresis (DIGE) followed by Western-blot analysis and tandem mass-spectrometry identification of the detected antigenic targets. To search for the thyroid cancer autoantigens we sequentially compared the proteome of normal cells of human thyroid follicular epithelium (Nthy-ori 3-1) with the proteomes of three variants of this cell line stably transfected by lentiviral constructs expressing the key oncogenes of thyroid cancer, BRAF(V600E), NRAS (Q61R) or chimeric protein CCDC6-RET. In order to determine the autoantigens we estimated the ability of the differentially expressed proteins to react with sera of the cancer patients expressing the respective oncogenes, in comparison to sera from cancer-free controls. Our preliminary results suggest that it may be possible to detect antibodies to specific oncogene-induced autoantigens produced by the tumor tissue. This work is supported by grant 16-15-10423 from Russian Science Foundation.

P.24-017-Tue
Redox proteomics analysis in *Drosophila* DJ-1β mutants: relevance to Parkinson's disease

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Parkinson's disease (PD) is the most common movement disorder in the world and is caused by the selective loss of dopaminergic neurons (DA) in the substantia nigra pars compacta, leading to a decrease of striatal dopamine levels. Functional studies of genes involved in familial PD forms have shown that oxidative stress (OS) and mitochondrial alterations play a central role in the pathogenesis of the disease. One of them is *DJ-1*, whose mutations cause an early-onset recessive form of PD. Indeed, it has been demonstrated that the DJ-1 protein plays an important role in the cellular defense against OS. In this scenario, we are working with a *Drosophila* model of PD that harbors a mutation in the *DJ-1β* gene (ortholog of human *DJ-1*). *DJ-1β* mutant flies show motor defects, hypersensitivity to OS-inducing toxins and high levels of OS markers like protein carbonylation, one of the most widely studied OS-induced protein modification. To determine how increased OS is affecting protein function in PD model flies, we performed redox proteomics analysis in 15-day-old *DJ-1β* mutants. This analysis led to the identification of several proteins with higher carbonylation levels in mutant than in control flies. Among them, proteins involved in cellular metabolism were selected for further studies. Phenotypic analyses of mutant flies for the genes encoding these enzymes as well as functional studies to analyze their activity in *DJ-1β* mutant flies were performed. We found that there is a correlation between oxidative modification and protein dysfunction in the nervous system of PD model flies, which results in phenotypic defects. We will present these findings and discuss the potential of the oxidized enzymes as PD biomarkers after validating them in human cell models. The discovery of new biomarkers to early diagnose PD is essential to develop therapies that could stop or delay its progression.

P.24-018-Wed
Toxins from MazEF and PemIK-Sa1 toxin-antitoxin systems modulate proteome of *Staphylococcus aureus*

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Toxin-antitoxin (TA) systems are commonly found in bacteria, particularly abundantly in pathogens. TA systems are known to be responsible for maintenance of mobile genetic elements as well as for helping bacteria to survive adverse environmental conditions by inducing persister and biofilm-forming phenotypes. A regulatory role for the plasmid-encoded PemIK-Sa1 was demonstrated, as a result of RNase activity of the PemK-Sa1 toxin towards UAUU sequence. Moreover, in Staphylococcus aureus the MazF toxin targeting UACAU sequence is present. However, the reflection of their activity in protein profiles has not been studied. Here we explore a potential link between the activity of both toxins and changes in S. aureus cellular and extracellular proteome. In order to achieve this goal the following four recombinant S. aureus strains were used in the study: RN4220/pCN51,

RN4220/pCN51PemK, RN4220deltaMazEF/pCN51 and RN4220 deltaMazEF/pCN51MazF. At three time points after induction the toxin's expression cellular and secretory proteins were isolated, labeled with fluorescent dyes and subjected to two-dimensional difference gel electrophoresis (2D-DIGE). The number of differentiating protein spots was substantially higher in the proteome profiles for PemK toxin than for MazF toxin. Comparison of RN4220/pCN51 versus RN4220/pCN51PemK revealed 293 and 390 differences in intra- and extracellular proteomes, respectively. In contrast, in RN4220deltaMazEF/pCN51 versus RN4220deltaMazEF/pCN51MazF only 27 and 12 differentiating protein spots were found. The obtained results indicate that toxins from PemIK-Sa1 and MazEF TA systems modulate both pools of the proteome, however the number of changes negatively correlates with the length of toxin target sequence. Thus, post-transcriptional regulatory role of these TA systems entailed changes in the proteome of S. aureus. The study was supported by the National Science Centre, Poland, grant no.: UMO-2014/13/B/NZ1/00043 (to B.W.).

P.24-019-Mon
Molecular response of flax (Linum usitatissimum L.) to zinc deficiency

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Flax is an important crop, which is grown for seeds and fiber. Unfavorable conditions decrease quantity and quality of flax products, however, cultivation of adaptive varieties can significantly reduce the effects of stress. Studying the mechanisms of flax response to stresses allow identification of probable resistance genes and will help in breeding of improved cultivars. We examined the response of flax varieties Norlin (resistant) and Mogilevsky (sensitive) to zinc deficiency. Flax seeds were grown in control conditions or in 1000-fold reduced zinc content for three weeks. Total RNA was isolated from root tips (pools of 10–12 plants) and evaluated on Agilent 2100 Bioanalyzer. Illumina TruSeq Stranded Total RNA Sample Prep Kit was used for library preparation for high-throughput sequencing. A total of 8 cDNA libraries were obtained – two varieties in control and zinc deficiency conditions, in duplicate. The libraries were sequenced on Illumina NextSeq 500 with 80 nucleotide read length. From 30 to 40 million reads were obtained for each sample. Transcriptome assembly was performed using Trinity 2.4.0. Transcripts and their predicted proteins were annotated using Trinotate. Transcripts were analyzed for the presence of ORF by TransDecoder. Transcripts and proteins were annotated using Gene Ontology. Reads were mapped to the assembled transcripts and quantified using Bowtie2 and RSEM. Expression alterations of each transcript for resistant and sensitive varieties were evaluated. The most significant expression alterations under zinc deficiency were identified for genes involved in transmembrane transport, vitamin synthesis, oxidoreductase activity, and photosynthesis. Besides, differences in expression of particular genes were revealed for resistant and sensitive flax genotypes. Identified genes could play an important role in flax response to zinc deficiency. This work was financially supported by the Russian Science Foundation, grant 16-16-00114.

P.24-020-Tue
Assessment of genetic variation for SAD and FAD genes in flax by high-throughput sequencing

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Flax (*Linum usitatissimum* L.) is emerging as one of the richest functional food because of its high content of health-promoting compounds such as alpha-linolenic acid (ALA) and lignans. In high-linolenic acid flax varieties, α-linolenic acid constitutes up to 73% of the total fatty acids, thus making them the richest source of plant-based omega-3 fatty acids (FAs). Meanwhile, low-linolenic acid varieties are rich in omega-6 FAs. The FA composition, with potential for the high and low content of ALA, is that in the end determines the applications of flaxseed. Moreover, different health benefits, such as reduction of cholesterol levels and prevention of cardiovascular diseases, are associated with the composition of polyunsaturated FAs in flax. Given the importance of FAs, many of the genes involved in FA synthesis have been identified. The FA biosynthetic pathway involves the family of fatty acid desaturases (FADs) genes and the family of stearoyl-ACP desaturase (SAD) genes. So, we know the genes but little is known about their genetic variability and polymorphism. The aim of this study was to analyze the genetic variation for SAD and FAD genes in flax by sequencing these genes from 96 flax genotypes with different proportion of FAs, obtained from the All-Russian Research Institute for Flax. Before the sequencing, primers were designed by MethyMer, DNA libraries were prepared by a two-stage PCR, with the concentration analyzed on Qubit 2.0 fluorometer (Life Technologies) and quality – on Agilent 2100 Bioanalyzer (Agilent Technologies). DNA sequences were obtained with high coverage using an Illumina platform, which therefore enables the accurate assessment of levels of genetic variations and the identification of single nucleotide polymorphisms (SNPs). This work was performed within the framework of the Program of fundamental research for state academies for 2013–2020 years (№ 01201363819) and was funded by RFBR according to the research project 17-29-08036.

P.24-021-Wed
Proteomic analysis of host-cell proteins in Mason-Pfizer monkey virus

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Mason-Pfizer monkey virus (M-PMV) belongs to the *Retroviridae* family, similarly to the human immunodeficiency virus (HIV). Although, M-PMV causes AIDS-like disease in its host organism, it differs from HIV in the genome and virion structure, as well as in its morphogenesis. Different morphogenesis points to the fact that the M-PMV most likely uses other cellular pathways during its life cycle than HIV-1, whose lifecycle is described in more details. LC-MS/MS-based proteomic approaches has already been used analyze the composition of purified viral particles. In this study,

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similar proteomic analysis of M-PMV virions was performed to identify host cell proteins that are incorporated into newly formed particles. Identification of reproducibly incorporated proteins into the virions could help to clarify the mechanisms of morphogenesis or virulence of M-PMV. About sixty of host cell proteins were stably identified in whole M-PMV particles produced in human embryonic kidney cells. From those, the proteins participating in clathrin-mediated endocytosis, which has been found to be used by HIV-1 when entering the host cell, or proteins of the ESCRT complex, which HIV-1 uses to bud from the host cell, were identified. Additionally, another identified proteins are involved in COPI and COPII mediated endosomal transport, which could be important for transport of M-PMV proteins within the host cell.

P.24-022-Mon
Discriminating sub-population responses of a mixture of human cell lines by proteogenomics

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Monitoring proteome dynamics from different human cell types present concomitantly in a given sample is of great interest and could be applied to ultra-precise molecular characterization of complex tissues. Here, we propose a proteogenomics-based strategy to point at cell line molecular signatures. For this, the proteome is analyzed by high-throughput shotgun mass spectrometry and specific bioinformatics search are performed. First, mRNA from chondrosarcoma cells (SW1353 cell line) and immortalized chondrocytes (T/C28A2 cell line) were sequenced by RNAseq for establishing the most appropriate protein sequence database. For this an innovative cascade search allows to conciliate *de novo* and mapping RNAseq assemblies and the Human SwissProt database. A set of 2 million of discriminating peptide sequences of the two cell lines are then identified. From them, 480 peptide sequences were detected and monitored based on extracted ion chromatogram (XIC) signals recorded by tandem mass spectrometry. A list of 55 peptides was used for quantitating the ratio of each cell type in a given co-culture sample with high precision, selected with cell lines mixed at 2:1, 1:1; and 1:2 ratio. This new methodology was used to analyze the bystander effect generated by irradiated chondrosarcoma cells (SW1353 cell line) on immortalized chondrocytes (T/C28A2 cell line) in co-culture conditions. Such strategy could be applied to investigate intercellular interactions between different cell types, paving the way to new insights into the molecular mechanisms of crosstalk between human cells.

P.24-023-Tue
Common miRNA signatures in a group of rare neuromuscular disorders

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Neuromuscular disorders (NMD) are heterogeneous group of genetic diseases that encompasses many different syndromes and diseases that either directly or indirectly impairs the function of skeletal muscle. However, there are currently no effective and common therapeutic approaches to prevent or delay the progression of these diseases. Recent studies revealed important regulatory roles for small noncoding RNAs, called microRNAs (miRNAs), in skeletal muscle function under physiological and pathological conditions. In this study, we aim to identify common miRNA signatures associated with etiopathogenesis of different neuromuscular diseases (Duchenne Muscular Dystrophy, Megaconial Congenital Muscular Dystrophy (CMD), Ullrich CMD and alpha-dystroglycanopathy), each caused by mutations in different nuclear genes encoding proteins with distinct roles. For this purpose, skeletal muscle biopsies from selected NMDs presenting mitochondrial damage (n = 12, 3 from each group) and control individuals (n = 3) were analyzed by using Affymetrix GeneChip miRNA 4.0 Array. To identify differentially expressed miRNAs in patients, raw data was analyzed by two different programs, MeV-SAM and Affymetrix TAC. Differentially expressed miRNAs whose expression were found to be statistically significant by both programs (miRNAs that showed an increase/decrease by 2 fold in patient samples compared to the control group) were identified as candidates. We then identified potential target genes of these candidate miRNAs by using miRWalk and classified them by using GENE ONTOLOGY-PANTHER databases. Our results revealed that 17 miRNAs were differentially expressed in patients and 5 of these miRNAs are likely involved in skeletal muscle differentiation. Our commonality approach will provide contribution to the literature by identifying common potential therapeutic targets and/or biomarkers related to different rare NMDs.

P.24-024-Wed
Quantitation of drugs using for treatment of essential hypertension in dried blood spots by LC-MS/MS and correlation with dried serum spots

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Mortality from cardiovascular diseases in Russia is in the first place (Russian Statistical Yearbook, 2015). As a rule, the cause of this is complications of coronary heart disease leading to angina, myocardial infarction, cardiac arrhythmia, sudden cardiac death (McMurray J. J 2012), slowing of blood flow, formation of intravascular thrombus (Uster V 1997). In about half the cases, the disease begins without previous symptoms with unstable angina or myocardial infarction and immediately changes into a chronic form (RI Litvinenko, 2014). Often the complications lead to increased physical and psychological stress. The development of new tests for determining the pathologies of the cardiovascular system consists in finding new biomarkers and improving existing methods by increasing the sensitivity and accuracy of the analysis. The ease of collection and storage of samples makes the choice of dry blood spots (DBS) and dry blood plasma spots (DPS) attractive as a source of biological material collection for biomedical research and analysis, especially outside the traditional medical environment (for example, at home, in remote locations, limited resources) (McDade TW, 2007). Unlike standard blood plasma samples, samples on dry spots do not require freezing, can be stored and transported in simple folders or envelopes at room temperature or with cooling to −4°C. If the stability of the substances is proved, then such samples can be sent by regular mail. In this study, we propose to develop methods for quantitation of drugs using for treatment of essential hypertension in dried blood spots

by LC-MS/MS and correlation with dried serum spots. The research was supported by Program of RAS "Fundamental research for biomedical technology" 2018–2020.

P.24-025-Mon
MS-key to unlocked bacteria resistant to beta-lactam antibiotics

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In recent years, multidrug resistant strains are actively spreading, which are resistant to several or even all antibiotics. Resistance to antibiotics is a big botnet in the selection of personalized therapy. In this regard, there is a problem of identifying bacteria that are resistant to beta-lactam antibiotics. The main mechanism of this type of resistance is the production of beta-lactamase enzymes. They are extremely different in structure and substrate specificity. In this work several approaches have been developed for the MS profiling of beta-lactamases to determine the family and type, depending on which antibiotic therapy is selected. From the UniProt and literary sources amino acid sequences belonging to mutant forms of beta-lactamases (1) of different types (2)were loaded. Using Skyline virtual trypsinolysis was performed and using BLAST-search proteotypic peptides (specific for each form) were determined for subsequent mass spectrometric analysis. Proteotypic peptides for several TEM, SHV, CTX-M families were chosen. Methods for their detection using SRM technology were developed. It was shown that this peptides can be used for mass-spectrometric identification basing on more than 50 experiments in mass-spectromic repositories. At the same time, it was not possible to select specific peptides that distinguish mutant forms TEM-1 from TEM-84. Bioinformatic analysis of mutant forms of beta-lactamases allowed to determine specific peptides, taking into account the changes in the nucleotide sequence for the subsequent creation of a set of peptides and their mass spectrometric identification in the samples. An experimental verification of the proposed approach presupposes a directional mass spectrometric analysis of beta-lactamases in the periplasmic fraction. Ek.I. acknowledge the Leading Scientific School of Prof. Andrey Lisitsa (№ NSh6313.2018.4).

P.24-026-Tue
Potential prognostic markers for locally advanced prostate cancer without lymphatic dissemination

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Prostate cancer (PC) is a major cause of cancer-related deaths in men worldwide. Locally advanced prostate cancer (LAPC) is characterized by invasion of the prostatic capsule without evidence of nodular or distant metastatic spread. Patients within this clinical category have different risks of recurrence. Our study is aimed at identifying novel prognostic biomarkers for LAPC, which will lead to optimization of treatment and development of appropriate clinical recommendations. We performed bioinformatics analysis of The Cancer Genome Atlas (TCGA) project RNA-Seq data using the computational resources of EIMB RAS "Genome" center (http://www.eimb.ru/rus/ckp/ccu_genome_c.php); 130 samples (only derived from Caucasian patients) of LAPC without lymphatic dissemination were divided into two groups depending on the cancer prognosis (favorable or unfavorable). In the analysis, the genes previously identified by published data as potential prognostic biomarkers were considered. Additional eight genes, that are characterized by differential expression, were found. Increased expression of *TWIST1, TUBB3,* and *CHAT* and decreased expression of *CYP1B1, IGSF1, EDN3*, *MSMB,* and *SERPINA3* were detected in the group of patients with poor prognosis in comparison to one with favorable prognosis. These genes are involved in the key processes of carcinogenesis, such as angiogenesis, proliferation and migration of tumor cells (*CYP1B1, EDN3, TWIST1, TUBB3*, and *CHAT*), as well as with disturbance of regulation of signaling cascades (*IGSF1, SERPINA3,* and *MSMB*). Thus, we identified a number of genes as potential prognostic biomarkers associated with poor prognosis of LAPC without lymphatic dissemination. This work was funded by the Program of fundamental research for state academies for 2013–2020 years (№ 01201363819) and by RFBR according to the research project no. 17-29-06083.

P.24-027-Wed
Empowering shotgun analysis with 2DE data: story of HepG2 proteome exploration

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Proteome heterogeneity is unavoidable during proteomic studies. Investigation of alternative splicing, single amino acid variations and posttranslational modifications is crucial because of significance of these events in expression and functional properties of proteins. Our research is devoted to studying proteome of HepG2 cell line. Basing on the results of RNASeq analysis of HepG2 cell line, we created a transcriptome-specific library, containing 52 thousand protein sequences, encoded by 12 thousand genes. Such library allows to take into account individual variations and helps to avoid uncontrolled extension of search area by populational data and reduce FDR. One of the most popular methods of proteomic analysis – shotgun mass spectrometry – is characterized by low (ca. 20%) coverage of protein sequence. Short peptides, detected with shotgun MS, do not allow to distinguish highly homologous proteoforms. To empower shotgun approach, we added two dimensional electrophoresis (2DE). After 2DE fractioning we cut the gel in 96 cells and analysed every cell by MS. Fractioning of protein mixture before MS allows to enrich the results with coordinates of proteoform on the gel (p*I* and MW). 2DE profiling with further MS analysis allowed to discover over 2614 proteoforms (1972 of which aberrant) encoded by 2326 genes. Without 2DE we identified only 925 proteoforms because of sample heterogeneity and lack of knowledge of physical–chemical parameters of proteoforms, used for specification of certain proteoform. Effective tandem of 2DE/MS allows us to forecast modifications, which can change physical–chemical parameters (and the location of protein spot on the gel, consequently). Obtained results consist not only of evaluation of proteoforms implemented at the protein level, but also of improvement of experimental approaches to cell proteotyping. O.K. and Ek.P. acknowledge the Leading Scientific School of Prof. Andrey Lisitsa (NoNSh-6313.2018.4).

P.24-028-Mon
Polymorphism of cellulose synthase genes in flax (*Linum usitatissimum* L.)

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Flax (*Linum usitatissimum* L.) is an important agricultural plant that has been used for fiber production since ancient times. Nowadays, linen fabrics are also in demand due to their high quality. High cellulose content is typical for flax fibers, however, cultivars are different in this characteristic. It has been shown that cellulose synthase genes (*CesA*) play a key role in biosynthesis of cellulose. In the present work, we performed high-throughput sequencing of *CesA* genes in flax cultivars and lines with varied cellulose content and assessed their polymorphism. Primers for amplification of *CesA* genes were designed using MethyMer. Two-stage PCR was used for DNA library preparation. First-stage PCR primers included sequences of overhang adaptor and target region. Second-stage PCR primers included sequences of Illumina adapter, multiplexing index, and a part of overhand Illumina adaptor. For library preparation for high-throughput sequencing, DNA from 48 flax cultivars and lines with a known cellulose content was used. Amplification was performed on GeneAmp PCR System 9700 (Applied Biosystems). Obtained DNA libraries were quantified on Qubit 2.0 fluorometer (Life Technologies), evaluated on Agilent 2100 Bioanalyzer (Agilent Technologies), normalized and pooled. The pool of DNA libraries was sequenced on Illumina platform. Thus, we first analyze the genetic diversity of *CesA* genes in flax plants with varied cellulose content using high-throughput sequencing of amplicons. Our results enable identification of associations between particular *CesA* alleles and cellulose content that is essential for the development of flax marker-assisted selection. This study was funded by the Presidium of the Russian academy of sciences, Program № 41 «Biodiversity of natural systems and biological resources of Russia».

P.24-029-Tue
Polymorphism of glycosyltransferase UGT74S1 gene in flax (*Linum usitatissimum* L.)

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Flax (*Linum usitatissimum* L.) is one of the promising functional food. Flaxseed is the richest known source of mammalian lignans, enterolactone and enterodiol, as it is rich in their precursors – secoisolariciresinol diglucoside (SDG) lignans. SDG lignans are polyphenolic phytoestrogens with antitumor, antimitotic, antiviral and antidiabetic properties. Biosynthesis of these lignans in flaxseeds includes a number of enzymatic steps, and uridine diphosphate glycosyltransferases (UGTs) have been identified as key enzymes, along with other biosynthetic enzymes such as pinoresinol-lariciresinol reductases (PLRs). Despite the knowledge of UGT genes in lignans' biosynthesis, the genetic diversity of these genes in cultivars has not been investigated. The goal of the present study was to assess the polymorphism of a UGT gene, that have been proven to be involved in SDG glycosylation – UGT74S1, in a collection of 96 flax cultivars and lines from the All-Russian Research Institute for Flax in Torzhok. Our objective was achieved using high-throughput sequencing of UGT74S1 gene in these flax collection. MethyMer was used to design primers for amplification, and a two-stage PCR – to prepare DNA libraries. The concentration and quality of obtained DNA libraries were analyzed on Qubit 2.0 fluorometer (Life Technologies) and Agilent 2100 Bioanalyzer (Agilent Technologies) respectively. Sequencing was performed on an Illumina platform. This work allowed us to assess the genetic diversity of UGT74S1 gene in flax cultivars and lines using high-throughput sequencing of amplicons, that therefore enables the application of marker-assisted selection in development of lignan-rich flax genotypes. This work was supported by the Program of fundamental research for state academies for 2013–2020 years (№ 01201363819).

P.24-030-Wed
Establishment of first reliable predictors of disease association of nonsynonymous amino acid substitutions

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Recent progress in genomics and proteomics is associated with the prolific development of numerous computational algorithms that focus on the prediction of the functionality of affected proteins or other molecules. Despite these algorithms are designed in order to predict the changes at the molecular level, they are often used to predict, whether the respective variations are disease-associated or not. For example, the Ensembl genome browser, which is widely used by clinical geneticists, has two integrated algorithms, SIFT and PolyPhen, and provides their predictions of effects of each of the nonsynonymous substitutions listed. Many more-advanced computational algorithms are available. But all of them, ranging from the old generation of predictors, such as the above-named SIFT and PolyPhen, to the most recent ones, such as the EVmutation, and the combined approaches, such as REVEL, suffer from a simple, but very important problem – the lack of specificity. This lack of specificity is particularly obvious when attempting to predict the effects that are manifested at the clinical level – thus, such effects, which the organism cannot compensate by some of its intrinsic regulatory pathways. We will present here the recently developed distinct improvement in prediction outcomes, which is mediated by the adjustment of settings of a number of a number of state-of-art computational algorithms. We first designed our model based on a dataset of 7,178 missense variations in 44 genes that are associated with Mendelian diseases. We further validated the newly proposed approach using another independent set of genes and their variations. Concluded, we present the first approach, which allows getting rid of a burden of the systemic error that affects the predictions of clinical effects of nonsynonymous amino acid substitutions and allows trusted evidence-based predictions of a large part of the nonsynonymous amino acid substitutions that result from the omics screens.

P.24-031-Mon
Immunometabolic effects of peritoneal alanyl-glutamine in clinical peritoneal dialysis detected by a multi-omics biomarker approach

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Peritoneal dialysis (PD) is a renal replacement therapy using a glucose-based hyperosmotic solution and the peritoneal wall as semi-permeable membrane. PD-effluent represents a rich source of proteins, metabolites and cells for predicting outcome, developing novel PD-fluids that provide patient-tailored benefits, and understanding peritoneal biology, such as interaction of the secretome and transcellular transport of plasma proteins and metabolites with peritoneal mesothelial and immune cells. A multi-omics approach was established in samples from 2 randomized clinical trials, based on enrichment of low abundance proteins and highly sensitive identification methods analysing the proteome with multiplex mass spectrometry, RNAseq based transcriptome analysis of effluent cells and targeted analysis of the metabolome. PD patients were treated either with standard PD fluid or with added alanyl-glutamine (AG). The presented workflow identified more than 2500 unique proteins in PD-effluents. Identified proteins provided novel information on overrepresentation of proteins linked to membrane remodeling and fibrosis, whereas underrepresented proteins indicate decreased immunocompetence. Addition of AG resulted in changes of markers reflecting peritoneal immune-modulation in accordance with transcriptome analysis. Integration of transcript data from almost 10000 genes indicated reduction of fibrosis and increased host defense as candidate mechanisms. Co-regulated biological processes such as oxidative stress responses and regulation of immune responses were identified also in the metabolome. Our data demonstrates feasibility of a multi-omics approach to investigate pathomechanisms relevant in PD. Treatment with AG was associated with restoration of biological processes involved in important immunometabolic pathways as well as reduction of fibrosis. The identified molecular targets may also serve as surrogate for monitoring PD therapy and evaluation of novel interventions in PD.

P.24-032-Tue
Insights into potential underlying causes of vascular calcification in humans

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Vascular calcification (VC) is a pathological accumulation of calcium phosphate crystals in the medial and intimal layers of vascular walls. However, underlying causes of pathology progression remain unknown. Several mechanisms of VC were proposed based on in vitro experiments and animal studies. In order to examine processes underlying VC in humans we collected carotid and aorta plaques of the 16 patients underwent vascular surgery. To confirm calcification we performed alizarin red (ARS) and hematoxylin-eosin staining (HES) of plaques. Despite HES is widely used in clinical histopathology routine for the detection of calcium deposits, it failed to detect presence of mild and diffuse calcification in our samples. Based on ARS, 5 of the patients had severe cases of calcification (>30% of plaque area is calcified), 3 had mild calcification (< 30%) and 8 showed no signs of calcification. In order to investigate potential underlying cause of VC in humans we examined mRNA level of 2277 genes previously proposed to participate in CVD and calcification development. Analysis of differentially expressed genes in the calcified plaques revealed 58 down-regulated and 55 up-regulated genes. Most of the genes associated with osteogenesis and previously suggested to be drivers of vascular calcification (for instance, BMP family, RANK and RANKL) remained unchanged in calcified plaques. At the same time, bioinformatic analysis revealed two negative regulators of osteogenesis (HDAC7, NOTCH1) to be down-regulated and a few positive regulators of osteogenesis to be up-regulated. Among them are IFT80, three subunits of the fibrinogen – FNA, FNB, FNG, lamin A – LMNA, and transcription factor EBF2 that activates RANKL. We conclude that disbalance of osteogenesis regulators could contribute to VC in humans, but gene expression of previously reported potential drivers of VC from in vitro and animal studies are not affected in human calcified plaques.

P.24-033-Wed
Transcription-level regulation of muscle atrophy and recovery in rats

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It is well-known that muscle disuse is associated with a muscle atrophy, which leads to negative consequences for the locomotor system in mammals. However, knowledge of regulation of muscle atrophy and subsequent recovery, particularly transformation between muscle fibers, remains insufficient. In current study we aimed to identify transcription initiation sites (TSS), and evaluate full-genome RNA expression at the level of individual promoters and enhancers during hindlimb unloading and subsequent recovery in rats. Two types of muscles, "slow" (m. Soleus) and "fast" (m. EDL), were examined in rats in normal conditions, after 1, 3 and 7 days of antiorthostatic suspension (AOS) and after subsequent 1, 3 and 7 days of recovery using CAGE (Cap Analysis of Gene Expression) method followed Illumina HiSeq 2500 sequencing. In the issue 9971 expressed CAGE clusters and 5766 associated genes in two muscle types were determined. Differential expression of genes and their promoter activity were strongly varied in m. EDL and m. soleus within AOS-recovery time course: "slow" m. soleus has no significant changes of transcriptional activity up to 7 days of suspension, but drastically shifted during recovery, while "fast" m. EDL shows quick and stable response to the stress and fast recovery after placing in normal conditions. First atrophic changes were marked after 3 days of AOS in soleus muscle and associated with upregulation of proteasome and peptidase complex genes. After the first day of recovery genes involved to transition between fast and slow fibers were activated in m. EDL. This study provides the first systematic annotation of promoters landscape and genes activated in "fast" and "slow" muscle types under induced atrophy and following recovery in rats. The reported study was funded by RFBR according to the research project № 17-00-00243 and is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

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P.24-034-Mon
Genetic and physiological characteristics of the abscisic acid metabolizing rhizobacteria

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The fundamental investigations carried out previously showed that plant-growth-promoting rhizobacteria can stimulate plant growth by producing or metabolizing plant hormones. Utilization of auxins by bacteria are well studied both in terms of the biochemical processes and physiological action. In recent work we showed the ability of rhizosphere bacteria metabolize the phytohormone abscisic acid (ABA), which is the main stress hormone in plants. Nevertheless biochemical pathways of the ABA conversion in bacteria remain unknown as well as there are no information about genetic determinants of this process, the mechanisms of their regulation and their prevalence between soil phyto-associated microorganisms. In this studies cytological and physiological characteristics of two strains of ABA-utilizing rhizospheric bacteria *Novosphingobium* sp. P6W and *Rhodococcus* sp. P1Y was carried out. The sequencing of the total DNA of the two strains studied was performed. The resulting genome P6W consists of 6,556,287 pairs of nucleotides and includes 6009 genes encoding proteins and 54 non-coding RNA genes. The results are presented on the NCBI website, project PRJNA274225. The sequencing and assembly of the draft genome of *Rhodococcus* sp. P1Y was performed. The genome consists of 6,722,294 pairs of nucleotides and includes 6411 genes encoding proteins and 65 genes of non-coding RNA. The results obtained made it possible to carry out a functional annotation of the main genes necessary for the further transcriptomic analysis of ABA-utilizing cultures P1Y. The level of identity of P6W genomes and the nearest *N. barchaimii* LL02 strain was 90–90.9%. The result indicates that the P6W strain is a new species of the genus *Novosphingobium*. Species that are closely related to *Rhodococcus* P1Y strain have not been found. The identity of closest species genomes does not exceed 77% (*Rhodococcus kyotonensis*). This study was supported by the Russian Science Foundation, project 17-14-01363.

P.24-035-Tue
Transcriptome analysis of the abscisic acid metabolizing rhizobacteria

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Many plant growth-promoting rhizobacteria (PGPR) produce phytohormones such as auxins, gibberellins and cytokinins, and these traits were repeatedly described as important mechanisms by which bacteria stimulate plant growth. The role of microbial utilization of phytohormones in mediating hormone status of plants and in plant-microbe interactions was not studied. The ability to synthesise the phytohormone abscisic acid (ABA) was found in several phytopathogenic fungi as well as in different PGPR species and endophytic bacteria. It is possible that along with processes of chemical destruction of ABA in soil, the biodegradation of this substance is an inherent property of soil microorganisms, but the microbial contribution to the ABA conversion in nature remains unclear. The answer to this question as well as elucidation of the mechanisms of microbial catabolism of ABA will help to develop more effective growth-stimulating and protective bacterial strains. Characteristics of two strains of ABA-utilizing rhizospheric bacteria *Novosphingobium* sp. P6W and *Rhodococcus* sp. P1Y was carried out. To identify bacterial genes involved in biodegradation of ABA, we used transcriptome analysis by high-throughput RNA sequencing. 62 genes of *Novosphingobium* sp. P6W activated by the growth of bacteria on the medium with ABA. In this group, the genes encoding proteins involved in iron transport, oxidative metabolism enzymes, fatty acid metabolism enzymes, DNA-binding and regulatory proteins were annotated. It was revealed that many ABA-activated genes are assembled into compact clusters on chromosomal or plasmid DNA. The resulting list of differentially activated genes was used to reveal putative enzymes involved to ABA degradation. We selected for cloning three genes encoding dioxygenases and one of the deacylases. This study was supported by the Russian Science Foundation, project 17-14-01363.

P.24-036-Wed
Metabolic profiling of desiccation – rehydration cycle in anhydrobiotic chironomid *Polypedilum vanderplanki*

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Larvae of an African chironomid *Polypedilum vanderplanki* have the unique ability to survive under extreme dehydration (anhydrobiosis), losing up to 97% of the water content of their total body mass. The question of existence of metabolites, which can play an important role in protection of cells from adverse impacts, remains open. It is known that activation of biochemical processes takes place in the first minutes of rehydration, which emphasizes the role of non-genetic processes to successfully maintain a viability of cellular organelles and proteins in a dry state. To get more detailed understanding of anhydrobiotic machinery we obtained metabolic profiling of *P. vanderplanki* larvae after 48 h of desiccation and after 3 h of rehydration process. In total, 266 compounds (124 metabolites in Cation mode and 142 metabolites in Anion mode) were detected. Statistical analysis demonstrated significant changes in concentration level of 193 compounds (72%). Principal Component Analysis revealed groups of metabolites with a similar pattern of action during desiccation-rehydration cycle. The highest increase in content was observed in trehalose-6-phosphate (47-fold) that has been determined as one of a key factor contributing to desiccation tolerance in *P. vanderplanki* midges. The distribution of basic metabolic processes was differed in desiccated and rehydrated larvae. Level of citric acid cycle intermediates was kept low during all stages of anhydrobiosis, while glycolysis seemed to become predominant catabolic pathway. Also, metabolome of desiccated larvae was enriched with biomarkers of ongoing stress and pathological processes, as well as accumulation of antioxidants and putative protectants were observed. This study was supported by Russian Foundation for Basic Research (18-34-00094 mol_a) and Russian Government Program of Competitive Growth of Kazan University.

Nanoworld

P.25-001-Mon
Mushroom derived glycan as capping and reducing agent for pH-dependent growth of gold nanoparticles

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Mixed nanocomposites combined both organic and inorganic compounds extend our capabilities to form nanostructured architectures of advanced functionality. Such architectures can be used in sensors and systems of medical diagnostics, environmental monitoring etc. Here we describe the synthesis and characterization of gold nanoparticles under different pH conditions using the Tremella mesenterica's glycan glucuronoxylomannan (GXM) as reducing and stabilizing agent. Three GXM solutions with different pH values (acidic, neutral and basic) were used for the synthesis. Aqueous solution of HAuCl4 was added to each sample, stirred and heated. Obtained nanoparticles were characterized by optical spectrometry and transmission electron microscopy. It was demonstrated, that the efficiency of the synthesis strongly depends on the pH of the glycan solution. Namely the spectrum of the solution did not change under the acidic conditions even after heating. In the neutral and basic medium the characteristic band at ca. 550 nm appears after heating of the mixture. It was found that the maximum was more intensive under neutral conditions. The morphology of the obtained nanoparticles also differs depending on the pH conditions. In neutral solution small seed-like nanoparticles were mainly formed, the morphology of nanoparticles obtained under basic conditions was much more various including polymorphous particles. Due to the presence of acute tips and sharp edges, which sustain large electromagnetic fields upon excitation with light of appropriate energy, these nanoparticles demonstrated their usefulness as SERS substrates, transducers or plasmon-active labels like contrast-enhancement agents etc. The possible applications of these functionalized noble metal nanoparticles in catalysis, sensors, diagnostics, biomedical imaging and photo thermal therapy are envisaged.

P.25-002-Tue
Supramolecular compounds interaction with chemotherapeutic drugs, antibodies and carbon nanotubes – hybrid nanocomplexes with potential drug delivery systems applications

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The presented studies concern combinations of supramolecular compounds of the Congo red type (SC) with model drug doxorubicin (DOX), antibodies (Ab) and carbon nanotubes (CNT) as possible complexes for delivering drugs to affected areas. The hypothesis underpinning the proposed research is that SC, which are capable of binding drugs as well as interacting with CNT, might efficiently deliver drugs to areas affected by pathological processes. This hypothesis was based on a number of to-date results, indicating: (1) the capability of SC to associate into supramolecular ribbon-like complexes in aqueous solution; (2) the ability to intercalate various organic molecules (drugs) in such ribbon-like structure; (3) *in vivo* interactions between SC and antigen-antibody complexes (Ag-Ab) followed by their progressive elimination from the organism; (4) the ability of SC to disperse hydrophobic CNT in aqueous solution producing capacious drug carriers. The goal of the research was to describe the properties of complexes formed by SC-DOX mixed supramolecular structures with immunoglobulin L-chain (a model system) and with Ag-Ab complexes. It is known that antigen-bound antibodies selectively bind SC, but their interaction with SC-drug complexes has not been studied so far. The properties, and drug capacity of Ab-conjugated CNT coated by SC-DOX complexes has also been studied. In such a system antibody provides tissue specificity (e.g. to cancer cells) of the carrier, while CNT increase the capacity. Research methods: electrophoresis, gel filtration, synthetic antigen-linked beads affinity chromatography, microscopy and Dynamic Light Scattering. We can conclude that supramolecular properties and protein-binding capabilities of SC and SC-DOX differ. This is presumably caused by lowering of the supramolecular ribbon plasticity, which can no longer adapt to the binding site within the L-chain domain.

P.25-003-Wed
Mitochondrial fullerene C60 accumulation in human leukemic cells for LED-mediated photodynamic therapy

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Recent progress in nanobiotechnology has arisen interest to biomedical application of the carbon nanostructure – fullerene C60. Fullerene C60 possesses unique structure, versatile biological activity and a significant potential as a photosensitizer for anticancer therapy since under ultraviolet and visible light irradiation fullerene C60 produces cytotoxic reactive oxygen species (ROS) with almost 100% efficiency. Fullerene C60 application in the frame of cancer photodynamic therapy (PDT) relies on rapid development of new light sources as well as on better understanding of its interaction with cancer cells. The aim of this study was to analyze fullerene C60 PDT efficiency against human leukemic cell line (CCRF-CEM) with the use of ultraviolet (UV, 365 nm), violet (405 nm), green (515 nm) and red (632 nm) high power single chip light-emitting diodes (LEDs). The time-dependent accumulation of fullerene C60 in CCRF-CEM cells up to 247,18 ng/106 cells at 24 h with predominant localization within mitochondria (70 of the content in the whole cell extract) was demonstrated with immunocytochemical staining and mass spectrometry. Cell viability assay showed that photoexcitation of accumulated nanostructure with ultraviolet or violet LEDs was followed by significant phototoxic effect. Less pronounced fullerene C60 phototoxic effect was observed after irradiation with green LED and no effect was detected with red one. No changes in viability of CCRF-CEM cells, incubated with C60 and maintained in the dark were detected. It was shown that fullerene C60 photoactivation with violet LED induced substantial ROS generation and apoptotic cell death, confirmed by caspase3/7 activation and phosphatidylserine translocation. Our work proved fullerene C60 ability to induce apoptosis of leukemic cells after photoexcitation with high power single chip 405 nm LED as a light source and testified the perspective of nanostructure application as a photosensitizer for anticancer therapy.

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P.25-004-Mon
Highly sensitive detection of circulating tumor DNA using rolling-circle amplification and FRET

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Circulating tumor DNA (ctDNA) in liquid biopsies can be exploited as important biomarkers for various types of cancer. Detection of ctDNA allows early cancer diagnosis, control of treatment response, and survival prognostics. One of the most common signatures of the disease is a single point mutation in protein coding genes, mainly in the KRAS, BRAF and PIK3CA. The primary goal of our research is detection and quantification of the following mutations – E545E, H1047R, V600E, G12D, and G13D as the most widespread in cancer patients. We propose a novel ctDNA assay that integrates time-gated Förster resonance energy transfer (TG-FRET) from Tb-complexes to dyes into target-primed rolling circle amplification (RCA). The proposed method demonstrates high specificity for simultaneous quantification of wild-type and mutated ctDNA in solution. Limits of detection in the femtomolar concentration range corresponds to the level of ctDNA in blood of patients in case of early stage of cancer. In addition, color multiplexing using various dye acceptors can be applied for simultaneous detection of several mutations in one liquid sample. Our approach is applicable to different kinds of liquid biopsies (e.g., blood, urine) and we believe that RCA-FRET will become a valuable tool for ctDNA quantification in fundamental research as well as applied clinical diagnostics.

P.25-005-Tue
Nanoemulsions of lipid droplets – a new model lipid system

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In this study, we introduce nanoemulsion-based lipid droplets and their use in studying protein-lipid interactions as a new biomimetic lipid model, complementary to the lipid bilayers, and monolayers formed at the water/air interfaces (Langmuir monolayers). Specifically, stable lipid nanodroplets, i. e. nanoemulsions of lipid droplets (LDs) composed of trioleoylglycerol core covered by a monolayer of sphingomyelin (SM) and cholesterol (Chol), have been prepared by our recently developed combined reverse-phase evaporation/ultrasonication method. Prepared LDs were examined for lipid composition, polydispersity, stability, and interaction with sensor chips by using several (bio)chemical and (bio)physical techniques. To our knowledge, this is the first in-depth characterization of LDs covered by a SM/Chol monolayer. In parallel, Langmuir monolayers and large unilamellar vesicles (LUVs) composed of SM and Chol have been prepared. All three lipid membrane models with SM/Chol molar ratios of 1/1 and 4/1 were used to study interaction with a Chol-binding protein, perfringolysin O (PFO). We found that PFO binds comparably well to monolayers and bilayers containing Chol above 20 mole-%. Moreover, PFO binding to LDs appeared similar to that observed with LUVs and Langmuir monolayers. In conclusion, our findings suggest that artificial LDs are a suitable biomimetic lipid system for studying protein-lipid interactions complementary to lipid vesicles and Langmuir monolayers.

P.25-006-Wed
Preparation and characterization of bitter vetch (*Vicia ervilia*) seed protein films reinforced by nanoparticles and transglutaminase-catalyzed crosslinks

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Since the environmental impact of plastic wastes is escalating rising widespread global concern, it is crucial to find enduring plastic alternatives. Among the biodegradable polymers useful to substitute the oil-based polymers, some plant proteins may represent a possible renewable raw source. Although the limited mechanical and barrier properties of hydrocolloid films so far produced, their use might be extended by adding reinforcement agents as nanoparticles (NPs) as well as chemical or enzymatic matrix cross-linkers. We suggest here a new strategy to produce nano-reinforced biomaterials by using as polymer matrix a protein mixture extracted from bitter vetch (BV) seeds and, as filler, mesoporous silica NPs functionalized or not with (3-aminopropyl)-triethoxysilane. Hence, nanostructured edible films were prepared from BV seed proteins, before and after their crosslinking by microbial transglutaminase (mTG), and characterized for their physicochemical, morphological and bioactive properties. Film tensile strength and elongation at break significantly increased in the presence of both kinds of NPs, even though the amino-functionalized ones resulted more effective, determining a two-fold increase of the mechanical properties. mTG-catalyzed protein crosslinking counteracted these NP-induced effects while, conversely, it further increased film barrier properties to gases and water vapour observed with NP alone. AFM and SEM analyses indicated a more compact structure of the nanocomposite film matrix with more evident continuous zones compared to control films, as well as an effect of mTG in including more homogenously both NPs into the crosslinked protein network. Finally, the antimicrobial and antifungal activities of the obtained biomaterials, possibly increased by nisin addition, suggest their potential application as a bio-preservative active packaging able to improve the shelf life of a variety of different food products.

P.25-007-Mon
Electrospun polymer-protein blends based on biodegradable aliphatic polyesters – from preparation to functional properties

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Aliphatic polyesters are thermoplastic polymers widely used in biomedical applications. Some properties of the aliphatic polyesters (e.g. strong hydrophobicity and poor biocompatibility) can be improved by blending them with proteins. Here we regard the properties of polymer-protein blends prepared by mixing the components in the common solvent (hexafluoroisopropanol) and electrospinning. We investigate three systems: a blend of poly(e-

caprolactone) (PCL) with gelatin, a blend of polylactide (PLA) with gelatin and a blend of poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) with bovine serum albumin (BSA). Eukaryotic cells can be grown on the blend films, and it proves their cytocompatibility. Scanning electron microscopy shows that electrospun proteins tend to form ribbons rather than ordinary round fibers. Contact angle measurements show that the blends are more hydrophilic than the pure polyesters. When incubated in water, the electrospun blend films degrade faster than the electrospun polyester films due to the protein dissolution. When incubated in water, an electrospun PHBV-based film with 30% BSA loses 6% of its mass over 11 days, which does not exceed 20% of the initial BSA mass. For comparison, electrospun BSA film dissolves completely for 1–2 h. Thus, the blend protects the protein from the quick dissolution. In addition, the electrospun blend films degrade faster than the electrospun polyester films due to the protein dissolution. The electrospun blends prepared from PLA and gelatin demonstrate 2–5 times higher adsorption capacity towards many water-soluble dyes than the electrospun films prepared from PLA only. It could be explained by the diversity of the amino acid side chains exposed at the fiber surface. We regard the electrospun polymer-protein blends as a novel class of biodegradable materials for biomedical applications. The work was supported by Russian Science Foundation, project № 17-75-30064.

P.25-008-Tue
Atomic force microscopy visualization of metalloprotein complexes

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Metalloproteins such as myeloperoxidase (MPO), ceruloplasmin (CP) and lactoferrin (LF) play an important role in regulation of oxidation stress and inflammation in vertebrates. Moreover, they form complexes that modulate their activity. For example, anionic CP may inhibit peroxidase activity of cationic MPO by electrostatic binding to the latter. Though the interaction of MPO, CP and LF has been studied by different physicochemical methods, it has not been characterized at a single-molecule scale. The aim of this work was to investigate the morphology of single MPO, CP and LF proteins and their interaction using high resolution atomic force microscopy (AFM). We have demonstrated that MPO, CP and LF molecules adsorbed onto a highly oriented pyrolytic graphite surface modified with oligoglycine hydrocarbon graphite modifier retain their nativelike dimensions. For the first time, we have visualized CP-MPO complexes formed at different CP:MPO molar ratio and revealed the presence of the supercomplexes, consisted of several single CP-MPO complexes. AFM has also confirmed the decreased ability of formation of CP-MPO complexes for partially proteolyzed CP and inability of LF to form complexes with MPO. The authors acknowledge funding from the Russian Science Foundation [17-75-30064 to D.V.K.].

P.25-009-Wed
Titanium dioxide nanoparticles inactivate p53-mediated cell death in MRC-5 fibroblasts

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Titanium dioxide nanoparticles (TiO_2 NPs) are widely used in many industrial and biomedical applications, and for this reason the increasing human exposure has raised serious concerns. There is already evidence that TiO_2 powder commonly used as E171 food additive contains up to 35% of nano-sized particles which may persist in the lung of exposed workers several years after the end of exposure. Recently, the risk assessment committee of the European Chemicals Agency (ECHA) has declared TiO_2 as suspected of causing cancer through the inhalation route (category 2) in lack of sufficient evidence to classify it in the more severe category for carcinogenicity (category 1B). Therefore, our research aimed to highlight the ability of photocatalytic TiO_2 NPs to alter the normal proliferation of human lung fibroblasts (MRC-5 cells). In this way, we focused on the modulation of tumor suppressor p53 protein and nuclear factor-kappa B (NF-kB) expressions, two of the key proteins that regulate the apoptotic response. Our results showed that although cell viability did not record any significant changes, TiO_2 NPs induced a decrease of p53 expression even after the first 24 h of incubation, leading to a total inhibition after 72 h of exposure to 25 μg/ml TiO_2 NPs. The same pattern was observed for NF-kB expression, indicating that this nuclear factor is essential in p53-mediated cell death. Taken together, our data suggest that through the inhibition of p53 expression, cells that undergo DNA damages following the oxidative stress induced by TiO_2 NPs, are no longer eliminated by apoptosis, developing, probably, a tumoral potential. In conclusion, TiO_2 NPs can disturb the homeostasis of cell cycle, promoting a possible uncontrolled proliferation, but further research is needed to establish the complete biochemical mechanism. Acknowledgments: This work was supported by the projects NANO-BIO-INT (PN-III-P1-1.1-PD-2016-1562) and NanoToxClass (ERA-NET SIINN 13/2015).

P.25-010-Mon
Anti-vascular effects of 4 nm and 20 nm PMA coated gold nanoparticles on CAM model

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Angiogenesis, the formation of new blood vessels from existing ones, plays an important role in the growth and spread of cancer. New blood vessels "feed" the cancer cells with required oxygen and nutrients, allowing these cells to grow rapidly, expand nearby tissue, spread to other parts of the body eventually. Nanostructure materials have attracted a great deal of attention because of their potential for providing selectivity in specific processes, especially in biomedical and pharmaceutical applications. Among from their counterparts, gold nanoparticles (AuNPs) exhibit unique physical, chemical, optical and electronic properties for the design of nano-sized biocompatible materials, which pose higher sensibility, selectivity and specificity than conventional methods for early recognition and treatment of many diseases. This study aims to investigate anti-angiogenic effects of different size (4 and 20 nm) of PMA coated AuNPs on the chorioallantoic membrane (CAM) assay. The spherical AuNPs with sizes of 4 and 20 nm in diameter were synthesized and then were evaluated for inhibitory activity to new vessel formations *in ovo*. TEM images confirm the size distribution of gold

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nanostructures. Our data suggest that nanostructured gold can inhibit vessel density, total vessels network length, total branching point and total vessel segment in VEGF induced angiogenesis on CAM. More importantly, we found that anti-angiogenic effects of AuNPs are size dependent and 4 nm AuNPs had comparable higher anti-angiogenic effects than 20 nm sized ones. Thus, these materials could be potential therapeutic platforms for treatment of pathological angiogenesis.

P.25-011-Tue
Surface modification with polyallylamines for immobilization of biopolymers and cells

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Surface modification for bio-medical, environmental, agricultural and security applications can be performed physically and chemically by surface coverage or grafting with polymers and conjugating ligands. The immobilized substances must retain their structures and not desorb from the functionalized surfaces. Hydrophobic, electrostatic and van-der-Waals interactions, coordination and covalent binding are involved in the adsorption. Our aim was the development of the functionalization method for immobilization of native proteins and viable cells. Cationic polymers with NH_2-groups were used for functionalization of charged and uncharged surfaces of planar slides, wells of plates and spherical nanoparticles. Poly(allylamine)s (PAA) and polylysines (pLys) of different molecular weights formed interfaces of the thicknesses ~1.5-2 nm for PAA 65 kDa as measured by: (1) the atomic force microscopy on mica slides; (2) the label-free biosensor with registration of the critical angle of total internal reflection on photonic crystal surface covered with SiO_2. Sorption capacity of 0.1 mg/ml PAA 65 kDa exceeded the values of other polyamines with different concentrations. Physisorption of proteins on PAA-layer is reversible and up to 70% of attached proteins can be removed. Glutaraldehyde provides stable chemical cross-linking of the compounds containing primary NH_2-groups with the aminated surfaces. The proteins immobilized on the pAA-covered surface retained their ability to bind with specific monoclonal and polyclonal antibodies. Bacterial cells attached to PAA65-covered surfaces could express the green fluorescent protein (GFP) gene under control of the inducible *lac* promoter. Eukaryotic cells also remained alive on PAA-treated slides as shown by fluorescent staining. Thus, treatment of plain and spherical surfaces with PAA is convenient for attachment of biopolymers and viable cells. The authors acknowledge funding from the Russian Science Foundation [17-75-30064 to D.V.K.].

P.25-012-Wed
Cytotoxic activity evaluation of metalloporphyrins in binary catalyst system

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Binary catalyst therapy is a promising method in cancer treatment that based on the production of reactive oxygen species stimulated by catalyst (transition metal complex) and oxidation substrate (e.g. ascorbic acid (AA). Metalloporphyrins contained a transition metal firstly were investigated as components for binary catalytic therapy. However, poor water-solubility of metalloporphyrins limits its use in binary catalyst therapy. Thus, biodegradable metalloporphyrins-loaded nanoparticles based on poly(D,L-lactide-co-glycolide) (PLGA) were obtained. Here, nanoparticles of two transition metal, tetraphenyl porphyrins, (Fe^3ClTPP, Mn^3ClTPP), were prepared by one-step emulsification method and were optimized for particle size and entrapment efficiency. The results showed that size of optimized nanoparticles were in the range of 190–230 nm and the loading contents were 7.9% and 5.2% for Fe^3ClTPP and Mn^3ClTPP, respectively. The results of MTS assays showed that nanoparticles in combination with AA possess cytotoxicity in chronic myelogenous leukemia (K562) cell line. It was found that such combination exhibits antitumor properties against K562 cell lines, due to the interaction of metalloporphyrins and AA, which led to the formation of reactive oxygen species. The results revealed that free substance and AA in this concentration range has no effect on the viability of the cells. As a control, K562 cell line was treated with 3-amino-1,2,4-triazole, a catalase inhibitor, which increased the cytotoxic activity of the Fe^3ClTPP and Mn^3ClTPP with AA against K562 cell line.

P.25-013-Mon
Intrathecal injection of Foxp3 encoded plasmids-encapsulated PLGA nanoparticles attenuate spinal nerve ligation-induced neuropathic pain behavior

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Neuropathic pain from damage or dysfunction of the nervous system is a highly debilitating chronic pain state and is often resistant to currently available treatments. It has become clear that neuroinflammation mediated by proinflammatory cytokines and chemokines from microglia plays an essential role in the development of central sensitization during the establishment and maintenance of neuropathic pain. Here, we succeeded in alleviating spinal nerve ligation (SNL)-induced neuropathic pain by inhibiting the microglia activation through Foxp3 overexpression with PLGA nanoparticles. To relieve SNL-induced pain behavior, plasmids encoding Foxp3-loaded PLGA nanoparticles (Foxp3-PLGA-NP) were prepared and characterized. We revealed that the Foxp3-PLGA-NP decreased the expression of proinflammatory genes such as TNF-alpha, IL-1beta and iNOS in BV2 cells incubated with ATP. Moreover, neuropathic pain generated by SNL was effectively diminished in rats administrated intrathecally with the Foxp3-PLGA-NP in behavioral test. Similarly, the loss of microglial activity was also confirmed during histological and cytokine analysis. Taken together, these data suggest that Foxp3 overexpression in spinal microglia with PLGA nanoparticles efficiently relieves SNL-induced neuropathic pain, and it would be therapeutic value for treating neuropathic pain.

P.25-014-Tue
Poly(OEGMA)-b-Poly(4-VP) block copolymer nanocarriers for anticancer agent release

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Today, the most common approach to treating cancer is to transport the active substances with nanocarriers, which is more advantageous than systemic administration. One of the major advantages of nanocarriers is that they can be transferred to tumors (EPR effect) through passive targeting due to their small size and with lower toxicity than systemic administration for higher drug concentration in the tumor locus. For this purpose, micelles have been widely studied because of the advantages such as increasing the solubility of the active substance or masking its toxicity. In our study, we have synthesized a block copolymer based on PEG and 4-VP by RAFT polymerization in order to obtain uniform polymer chains that will allow forming micelles in better characteristics. Block copolymers were characterized by FTIR and ^{1}H-NMR. We confirmed RAFT-mediated polymer formation by following molecular weight with GPC. Following that, polymers were tested on SKBR3 breast cancer cells in terms of toxicity by MTS using Cell Titer 96® Aqueous One Solution Cell Proliferation Assay. Polymers which are not loaded with drug revealed good cell viability (above %80). Then, micelles containing DOX as a model drug were prepared by the emulsion-solvent evaporation method. Micelles were characterized by Zeta Sizer in terms of size and size distribution. Afterward, resulted drug-loaded micelles were applied to breast cancer cells and toxicity was evaluated by MTS.

P.25-015-Wed
Silicon nanowire sensor with independent channels for cancer biomarker detection

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Nowadays the number of oncological diseases is growing rapidly around all the world and therefore the early prevent diagnostic of specific biomarkers come into the forefront of research interest. We present a label-free highly sensitive biosensor based on a silicon nanowire field effect transistor (Si NW FET) with two independent channels, allowing simultaneous detection of various analytes. Traditional "top-down" approach was used for sensor fabrication. The process started from rigorously patterning of positive e-beam resist in a high-resolution electron lithography system, next the design was transferred into the silicon layer through a thin aluminum mask by reactive-ion etching in a fluorine plasma. Afterwards, the titanium metallic contact leads were additionally insulated by a sputtered silicon oxide layer to avoid a current leakage into analyte. In addition to "lab on chip" application design, the construction of NW sensors for local measurements was also developed. For the nanowire surface modification we used an original method of antibodies immobilization on gold nanoparticles. The developed biosensor was successfully applied for a detection of a prostate specific antigen (PSA) in real serum samples with different concentrations. The sensitivity estimated to be ~23 fg/ml and was two orders of magnitude better than for well-established ELISA methods. The construction of novel sensor allows the separate modification of nanowire surfaces with different specific antibodies and independent measurement of the analytes. We tested the sensor towards simultaneous determination of thyroglobulin (Tg) and thyroid stimulating hormone (TSH) which is important for post-thyroidectomy patients with differentiated thyroid cancer. Both analytes were determined with high selectivity and sensitivity. The work was supported by the Russian Foundation for Basic Research (RFBR grant ofi-m № 16-29-03266).

P.25-016-Mon
In vitro cytotoxicity of surface functionalized iron oxide (II, III) nanoparticles

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Iron oxide (II, III) nanoparticles (ION) functionalized with different chemical groups or biomolecules provide a promising tool for biomedical applications. They are intensively studied and have been used in gene and drug delivery therapies, cell tracking, magnetic resonance imaging, as well as in a variety of *in vitro* diagnostic strategies. The aim of the present work was to investigate and compare the influence of different type of surface functionalization on the cytotoxicity of iron oxide (II, III) nanoparticles *in vitro*. Three human cell lines (A549, HeLa and normal dermal fibroblasts) were treated with various concentrations of amine functionalized, carboxylic acid functionalized, peptide coated amine functionalized, peptide coated carboxylic acid functionalized, protein coated amine functionalized and protein coated carboxylic acid functionalized ION. The cytotoxic potential was determined by MTT and Neutral red assays following 2, 15, 48 and 72 h exposure with ION. Our results demonstrated significantly higher cytotoxicity of amine functionalized iron oxide (II, III) nanoparticles compared to ION functionalized with carboxylic acid. Biofunctionalization with peptides or proteins reduced the level of toxicity of amine-functionalized NP. Carboxylic acid functionalized ION coated with peptides or proteins did not induce pronounced toxic effect on the three tested cell lines. These data suggest that peptide/protein biofunctionalization improves the properties of ION and their potential for therapeutic application.

P.25-017-Tue
Water-soluble fullerene C60 derivatives have antioxidant activity due to activation of expression of NRF2

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Water-soluble fullerene C60 derivatives potentially have a wide range of applications in the field of biomedicine. Fullerenes can act as radical sponges due to their chemical structure. Thus, they can possibly be used as effective antioxidants and radioprotectors. We assessed antioxidant activity of 6 different water-soluble fullerene C60 derivatives on HELFs. All of the investigated substances effectively neutralize ROS in water in the absence of cells. 5 out of 6 derivatives cause activation of NOX4 upon addition

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to HELFs. NOX4 is an enzyme responsible for ROS synthesis, thus, its activation causes a secondary response that is characterized by increased levels of ROS synthesis. The mechanism of ROS elevation via NOX4 protein caused by fullerene derivatives was proven with help of plumbagin. In this case the master-regulator of antioxidant response – NRF2 – isn't activated (neither on gene expression level, nor on protein level). One of the 6 investigated substances (F6) didn't cause a secondary response (increase in ROS level) in HELFs in vitro. Two concentrations of F6 and three times of incubation were assessed: early response (1 and 3 h) and late response (24 h). After 1 h of incubation with 4 nM and 4 mkM of F6 the level of ROS in the cells is decreased. NOX4 enzyme activity is blocked and activity of transcription factor NRF2 is increased. In 3 h the level of ROS decreases and so does the activity of NOX4. The level of NRF2 is increased both on gene and protein levels. Moreover, NRF2 translocates to the nucleus. In 24 h after start of incubation of HELFs with F6 (4 nM and 4 mkM) the level of ROS stays decreased, although NOX4 expression is increased. The activity of NRF2 is increased. Thus, derivative F6 is a potential antioxidant. This work was supported by RFBR grant № 16-04-01099_A.

P.25-018-Wed
The influence of surface nanotopography on mammalian cells in vitro

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Research in the field of nanotechnology opens up many ways of application of nanomaterials in the biomedicine. There are a number of reports that prove the influence of nanoparticles on the differentiation and properties of cells. Here we report about the influence of the glass surface modified by magnetic nanoparticles (MNPs) on the cell differentiation. The adipose-derived stem cells (ADSC) of a rat were seeded at concentrations 10^5 cells into each well of the six-well plate containing 2 ml of culture medium. MNPs were synthesized by the method of chemical co-precipitation. Rod- and sphere- shaped nanoparticles were used to modify glass surfaces by the method based on the self-assembly of nanostructures. The size and zeta-potential of MNPs was observed on Zetasizer Nano ZS. Cells were cultured with modified glass for 48 h. Morphological appearances were observed using various types of microscopy. The hydrodynamic size of nanoparticles was 20 nm. Biocompatible nanoparticles were plated either on the whole surface of glass or on certain areas depending on the experiment. The surface of the glass was studied by the AFM, SEM and bright-field microscopy. The attachment and distribution of ADSC cells were observed after 24 and 48 h of treatment. There was no difference between cell growth in areas with modification and without it according to the results. In this work, we developed an effective method of modification of glass surface by MNPs. The lack of the influence of MNPs on cell growth is an indicator of biocompatibility of nanoparticles but additional studies are required. The work was performed according to the Russian Government Program of Competitive Growth of Kazan Federal University and funded by the Russian Presidential grant MK-4498.2018.4

P.25-019-Mon
Investigation of anti-cancer activity of hesperetin and hesperetin loaded polymeric nanoparticles on C6 glioma cells

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Hesperetin (HSP), a bioflavonoid, has received considerable attention in cancer prevention due to its antiproliferative, antioxidant and apoptosis inducing activities. High permeability of HSP for blood brain barrier makes it an attractive molecule for treatment of brain tumours disease. Poor water solubility limits the potential of HSP to be used as an active cancer agent. Nanoparticles (NPs) could be used to enhance the efficacy and solubility of low soluble therapeutic agents. We aimed to analyze anti-cancer activity of HSP and HSP loaded PLGA nanoparticles (HSPNPs) on C6 glioma cells for the first time. HSP was encapsulated to PLGA nanoparticles by single emulsion solvent evaporation method. Particle size, encapsulation efficiency of HSPNPs were determined. C6 Glioma cells were treated with 0.01–200 μg/ml concentrations of free HSP and HSPNPs for 24, 48, 72 h. The cytotoxic activity was determined by MTT assay. PCNA antibody was examined as proliferation marker. Apoptosis was detected with TUNEL assay. Measurement of intracellular antioxidant activity and oxidative stress were assessed by measuring ROS, SOD activity and GSH levels. Characterization studies showed that synthesis of HSPNPs was carried out with %80.5 encapsulation efficiency 260.2 nm average particle size. IC_{50} values of HSPNPs of 24, 48 h of 28 and 21 μg/ml, respectively. The percentage of PCNA positive cells decreased to 20% and 10% respectively (100 μg/ml) for both HSP and HSPNPs treated cells. Treatment with 25, 50, 75, 100 μg/ml concentrations of HSPNPs resulted in 9.1, 7, 12.5, 12.7 fold in increase in apoptotic cell number. According to MDA, SOD and GSH levels the optimum doses of HSP and HSPNPs were found to inhibit oxidative damage in C6 gliomas. The results showed biocompatible polymeric nanoparticle systems has great advantages to enhance solubility of low soluble therapeutic agents. HSPNPs showed significant anticancer activity and could be used as promising novel anti-cancer agent.

P.25-020-Tue
Different surface modification of detonation nanodiamonds leads to significant differences in their protein corona composition

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Detonation nanodiamonds (DNDs) are a promising platform for biomedical application thanks to their high biocompatibility, relatively easy and low cost synthesis, chemical inertness of diamond core and distinct surface chemistry. Currently, very little is known about ultra-small detonation nanodiamonds (under 5 nm) especially in the interaction with biomolecules. The understanding of these interactions is however crucial for future bio-application of this material. The presented work is focused on identification of proteins originating from biological medium that are spontaneously adsorbed on surface of different DNDs. Nanodiamonds of different size (2 or 4 nm) and surface termination (hydrogenated or oxygenated) were incubated in complete biological medium supplemented with foetal bovine serum and their interaction with proteins was analyzed. After several washing steps, proteins remaining on DND were identified and quantified by data independent acquisition liquid chromatography – mass spectrometry analysis (SWATH). DNDs with protein corona were also analysed by TEM and other biochemical methods. Mass spectrometry results clearly showed differences in bound proteins and their abundance, depending on surface termination. Overall, oxygenated DNDs attract more proteins, but there is also clear statistical enrichment of different proteins on hydrogenated termination. The elucidation of DNDs termination influence on protein corona could lead to design of effective cargo delivery carrier.

P.25-021-Wed
Characterization of biological effects induced by functionalization of multi-walled carbon nanotubes in breast cancer 3D in vitro models

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The aim of present study was to evaluate the influence of different functionalization of multi-walled carbon nanotubes (MWCNT) on biological characteristics of breast cancer 3D in vitro models. MWCNT produced via chemical vapor deposition were functionalized with amino and carboxy groups on the nanotubes' surface. Breast cancer spheroids were generated in low-attachment 6-well plates and incubated for 24 and 48 h with different concentrations (0.1–4 μg/ml) of non- and functionalized MWCNT. Untreated spheroids were used as control. Cell viability and MWCNT cytotoxicity was evaluated by MTT and LDH assays. The proliferation level was estimated in spheroids incubated with 1 and 2 μg/ml MWCNT by analyzing PCNA expression through immunoblotting and variation of spheroids' diameter over time. Oxidative stress markers as reduced glutathione (GSH) content, level of malondialdehyde (MDA) and nitric oxide (NO) production were also assessed. The results showed a significant time-dependent decrease of cell viability at doses higher than 2 μg/ml accompanied by a decrease in spheroids' diameter and an increase of LDH release after 48 h of incubation, more pronounced for carboxyl-functionalized MWCNT in comparison with non- and amino-functionalized MWCNT. A decrease in PCNA protein expression was noticed after 48 h of incubation with both amino and carboxyl-functionalized MWCNT compared with control. Starting with 48 h, the GSH intracellular concentration was reduced in a dose-dependently by incubation with all three types of MWCNT in accordance with an increase of MDA level and NO production in spheroids incubated with 2 μg/ml MWCNT. Overall, even the changes were more pronounced for carboxyl-functionalized MWCNT possible to their better dispersion in culture media and higher interaction with cells, oxidative stress was initiated by all types of MWCNT after 48 h for doses higher than 2 μg/ml. Acknowledgements to PN-III-P2-2.1-PED-2016-0904 project.

P.25-022-Mon
On the nature of nanoparticle-enzyme interactions and their application in nucleic acid detection

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There has been an explosion of interest in the co-employment of functional enzymes and nanoparticles for ultrasensitive detection of nucleic acids. Combining these with advances in DNA nanotechnology, we can envision new assays with excellent figures of merit. Further, unique phenomena, such as increased reaction kinetics and altered specificities, can occur when enzymes or substrates are immobilized on particles. To fully exploit these in new high performance assays, it is imperative that a deep understanding of such systems is developed. In this work, the behaviours of various nuclease enzymes, including exonucleases I and III, nicking endonucleases Nt.BspQI and Nt.BstNBI, and duplex specific nuclease (DSN) have been investigated, using fluorescent, plasmonic and magnetic particles. DNA-particle conjugates were prepared using a variety of conjugation chemistries. The reaction kinetics were assessed using fluorescence spectroscopy, and enzyme specificities explored with a range of DNA templates. We discuss methods developed to directly compare free versus particle-bound probes, and which factors influence enzyme kinetics. For example, we observe that enzyme activity is extremely sensitive to the length of the surface-bound DNA probes. We discuss the nature of the surface-bulk interface, and molecular composition of the surface, and their effects on the action of the nuclease enzymes. To contextualize these points, we discuss their application in assays for RNA detection, co-employing functional nanoparticles, enzymes, target recycling and/or isothermal amplification. These include a quantum dot-based assay with a ca. 40 fM limit of detection for miR-148 using target recycling, and a magnetic particle-assisted assay with a ca. 2 fM limit of detection for miR-21, co-employing rolling circle amplification and target recycling.

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P.25-023-Tue
Label-free, multiplexed, single-molecule characterization of protein-DNA complexes with biological nanopores

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Protein-DNA interactions are key regulators of gene expression and replication. Therefore, they are good targets in cancer therapy and against antibiotic resistance. Beyond its importance, the study of the interaction between these two macromolecules is challenging. Nanopore technology measures the ionic currents passing through a single nanopore embedded in a lipid bilayer. It detects single molecules passing through the pore by the decrease in the ionic current. The measurements have high temporal resolution and sensitivity. Indeed, it is used for next-generation DNA sequencing, as each nucleotide of a single-stranded DNA causes a different ionic current drop when passing through the nanopore. Here we present an approach that allows to measure single molecules of protein-DNA complexes and to obtain the kinetic and affinity constants of the interaction. It works with an oligonucleotide reporter that self-hybridizes in the 3′-end forming the binding site of a target protein, followed by a single-stranded DNA that pulls the reporter through the nanopore detector. We take advantage of the sequencing ability of the pore to distinguish different DNA sequences and using different reporters we multiplex the measurements. We use two oligonucleotides with distinct ssDNA tails and protein-binding sites that allow us to identify which protein-DNA complex molecule is passing the nanopore detector. We also show that it is possible to analyze the inhibitory effect of small compounds on each protein-DNA complex. Our current work aims to expand the multiplexing capability of our approach by generating a single-molecule barcoding system. This would allow simultaneous measurements of hundreds of different protein-DNA interactions within the same sample.

P.25-024-Wed
The evaluation of gold nanoparticles and carbon dots as vaccine nanocarrier system

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Recently, different antigen carrier systems are being explored to increase the effectiveness of vaccines to fight infectious diseases and cancer. Especially nanoparticle-based delivery systems are being widely investigated. Biocompatible gold nanoparticles and carbon dots have been commonly used as potential delivery vehicles. Recently, gold nanocages (AuNCs), a special design with ultra thin porous walls and hollow interiors, have successfully been used in the fields of image-guided delivery systems. Here, our aim is to use the freshly synthesized AuNCs and carbon dots extracted from sugar beet molasses for the delivery of specific cargo and to compare their toxicological and inflammatory potential in innate immune cells (i.e. macrophages) *in vitro*. Here, Au nanocages and carbon dots were used to explore their effect on the metabolic activity, uptake efficiency and immune responses of antigen presenting macrophage cells by different techniques such as Real-Time Cell Analysis System, Flow Cytometry and ELISA. The *in vitro* tracking of AuNC and carbon dot-based nanocarrier systems was performed by confocal microscopy imaging. We believe that construction, characterization and biotesting of different nanocarrier systems might increase their promising potential in nanomedicine more.

P.25-025-Mon
Biophysical aspects of fullerenols interaction with peripheral blood mononuclear cells subjected to ionizing radiation

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Exposure to ionizing radiation, for example during radiotherapy and accidental exposures, causes damage to normal tissues. In order to reduce dangerous radiation effects on healthy tissues and organs the radioprotective compounds are searched for. Fullerenols C60(OH)x, the water-soluble derivatives of fullerenes, are being intensively studied in the context of the possibility of their application in the biomedicine. The aim of this study is to examine the mechanism of the influence of fullerenol C60(OH)x, x>30 on peripheral blood mononuclear cells (PBMCs) subjected to ionizing radiation. Studies on PBMCs will also assess the possibility of intravenous administration of this compound. Peripheral blood mononuclear cells (PBMCs) were obtained from leukocyte platelet concentrates from healthy donors. Leukocyte platelet concentrates were purchased from Regional Blood Donation and Transfusion Center in Lodz. PBMCs were separated from erythrocytes and granulocytes by density gradient centrifugation. The impact of fullerenol on the level of radiation damage to PBMCs was analyzed inside the cell and within plasma membrane. The level of oxidative stress inside the cells was measured by fluorescent label H2DCF-DA and DNA fragmentation by TUNEL assay. The lipid peroxidation in plasma membrane was assessed by fluorescent label C11-BODI-PY581/591. The obtained results will be discussed in the context of the influence of cellular localization of fullerenol on its radioprotective properties. In addition, the genotoxicity of fullerenol to PBMCs will be discussed.

P.25-026-Tue
Biochemical study of selenite bioconversion by *Azospirillum brasilense*

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Bacteria play an important environmental role in transforming selenium compounds, particularly in reducing selenium oxyanions into less toxic and insoluble elementary selenium. The exact mechanisms of this phenomenon are yet not fully clear. Different cellular biochemical processes can be involved: anaerobic respiration, denitrification, detoxification, *etc*. We present here the results of a study of selenite reduction by rhizobacteria of the species *A. brasilense* with the formation of Se nanoparticles (SeNPs). A mutant unable to synthesise nitrite reductase showed a higher resistance to selenite as compared to the parent strain (*A. brasilense* Sp245) but was still capable of reducing SeO_3^{2-}. This indicates that both the denitrification system and other routes are involved. We optimised the synthesis conditions for these bacteria to produce extracellular homogeneous SeNPs. The involvement of proton motive force-dependent transport in the extracellular synthesis of SeNPs was demonstrated: after treatment with the efflux pump inhibitor carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), TEM images showed the formation of intracellular Se crystallites only. Extracellular SeNPs were isolated and characterised. Using FTIR spectroscopy it was shown that SeNPs are associated with proteins, polysaccharides and lipids, while TEM images showed a thin layer over the NPs. SDS-PAGE study of proteins associated with SeNPs showed the

presence of a dominant protein with a mass of 95–110 kDa. These macromolecules participate in the SeNPs assembly, providing for their stability and unique properties, fundamentally different from those of chemically synthesised nanoparticles. Microbial reduction of Se oxyanions can be useful in water and soil bioremediation, production of bioactive food additives and in green synthesis of SeNPs. This work was supported by in part by RFBR Grant 16-08-01302-a.

P.25-027-Wed
Influence of silver nanoparticles (SNPs) with different size and shape on copper status in mice

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SNPs, due to their antibacterial properties, have been widely used during the last decades. However, there are few studies devoted to the effect of SNPs on mammals. While SNPs can be corroded in biological environment with formation of Ag^+ that are isoelectronic to Cu^+, so could be involved into disturbance of Cu homeostasis. The aim of this work was to study the relations between size, shape, antibacterial activity of SNPs and their ability to interfere in murine Cu metabolism. Four types of SNPs fabricated by chemical reduction of $AgNO_3$ and characterizes by laser diffractometry, UV/Vis, AAS, SEM, TEM were used. Analysis have shown spherical SNPs with linear size 10-, 20- and 75-nm as well as 75-nm SNPs with non-spherical shape. All SNPs demonstrated dose- and time-dependent antibacterial activity against *E. coli*. In mice, intraperitoneal injections of SNPs for 7 days have no toxic effect and do not affect Cu content in the body organs. Ag accumulate mainly in the liver and is excreted through the bile within a month after the withdrawal of injections. In the blood serum of animals receiving SNPs during a week, Ag according to gel-filtration data is contained in the peak belonging to ceruloplasmin, Cp, the main Cu-transporting protein, as well as in the peak, eluted earlier, while Cu is associated only with the Cp. In addition, there is a significant decrease in the Cu concentration and oxidase activity of Cp in serum. Interestingly, that the injection of SNPs with a larger size leads to a more significant decrease of oxidase activity. At the same time, the Cp protein content according to WB does not change in animals of all groups. The obtained data show that fabricated SNPs have antibacterial properties and significant impact on the Cu status in the blood serum of mice. The effect of SNPs depended on their shape and size. These results should be considered for further SNPs application. The work was supported by RFBR grant 16-34-60219 and grant MK-2718.2018.4.

P.25-028-Mon
Nanopipette navigation system as a new tool for nanoscale investigation of living cells

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Recently we have developed the new system for nanopipette navigation with feedback control. The ability to precisely move the nanopipette and to measure simultaneously an ion current allows an unprecedented level of nanoscale imaging of living cells – scanning ion conductance microscopy (SICM). The speed of data acquisition positions this as a technology which may be suited to relatively high-speed scanning of cell membrane during various biological processes in real time. This nanopipette navigation system can be used in combination with other techniques such as confocal and fluorescence microscopy, microinjection, electrochemical measurement, and patch-clamp recording. This has the potential to open new horizons in medicine and biology and could be of particular value to the pharmaceutical industry. We have demonstrated unique application of SICM and nanopipette biosensing as new methods to study action mechanism of magnetic hyperthermia. It was shown that mechanical properties of cancer cell membrane were increased after incubation with cobalt ferrite nanoparticles and decrease after incubation with the same nanoparticles in altering magnetic field. This fact proves a supposed acting mechanism that an apoptosis followed by destruction of an intracellular machinery during magnetic hyperthermia. Intracellular level of ROS was measured by nanopipette based biosensors for cancer cells incubated with cobalt ferrite nanoparticles in altering magnetic field. We have demonstrated other examples of nanopipette techniques implementations for *in vitro* drug discovery which prove a good potential of these methods. The research was carried out at the expense of the Russian Science Foundation grant (project No. 17-75-10065) - Skolkovo.

P.25-029-Tue
Smart nanopipette for single cell analysis

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Nanopipettes have been used in different applications with integration into Scanning Ion Conductance Microscopy (SICM): high resolution topographical imaging of living cells, quantitative delivery of molecules to the surface of living cells. Additionally, nanopipette probes still hold great promises as intracellular biosensors. Here we describe the fabrication, characterization, and tailoring of carbon nanoelectrodes based on nanopipette for intracellular electrochemical recordings. We demonstrate the fabrication of disk-shaped nanoelectrodes whose radius can be precisely tuned within the range 5–200 nm. The functionalization of the nanoelectrode with platinum allowed the monitoring of oxygen consumption outside and inside of melanoma cell. We applied the nanoelectrode to perform intracellular measurement

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in cultured melanoma cells, HEK293 and LNCap cancer cell. Upon penetration of the cells the anodic current quickly increases followed by equilibration to a level above the one measured in the cell media. A cell can withstand multiple penetrations and we measured a substantial difference between the electrochemical signal measured inside and outside the cell. We believe these results show the potential of functional nanoelectrode to probe endogenous species into cells and with further improvements they may allow the study of oxidative stress under influence of different drugs. The research was carried out at the expense of the Russian Science Foundation grant (project No. 17-75-10065) - Skolkovo.

P.25-030-Wed
Electrospinning p-tert-butylcalix[4]arene scaffolds manufacture for three-dimensional colon cancer cell culture and their applications in drug discovery

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A growing body of evidence has suggested that 3D cell culture systems, in contrast to the 2D systems, represent more accurately the actual microenvironment where cells reside in tissues. Therefore, researchers have concentrated on developing realistic *in vitro* 3D cell culture models that actually resemble the complex environment of native tissues. *p-tert*-butylcalix[4]arene, similar to a ring basket, represent a third generation of supramolecular hosts. Synthesis of calix[4]arene nanofibers by electrospinning will arise innovative approaches in biomedical applications to establish in vitro cell-based systems that can more realistically mimic the *in vivo* cell behaviors and provide more predictable results to *in vivo* tests. 5,11,17,23-Tetra-tert-butyl-25,27-bis (3-aminomethylpyridineamido)-26,28-dihydroxycalix[4]arene functionalized with a pyridinium group on position 3 were synthesized by appropriate procedures and the nanofibers were withdrawn by electrospinning. Surface characterization of the nanofibers was done by SEM, TEM and AFM analysis. Caco-2 cells (1×10^5) were cultured on nanofibers and after 48 h incubation, cells were treated with 240 μM irinotecan for 24 h. Cell growth/proliferation analysis were done by XTT assay and fluorescent microscopy analysis with DAPI stain. SEM/EDS measurement was used to characterize the morphology of the attached cells. Cell attachment kinetics revealed that the Caco-2 cells attached to the nanofibers of *p-tert*-butylcalix[4]arene at the same rate as to tissue culture plates. 3D tumors on *p-tert*-butylcalix[4]arene scaffolds, it was found that 3D Caco-2 cells were significantly more sensitive to irinotecan than 2D Caco-2 cells, with a 10-fold disparity in the IC_{50} values. In the present study, p-tert-butylcalix[4]arene 3D cell culture systems for anticancer drug screening systems have been developed as suitable platforms for drug screening and are served as more reliable models for *in vitro* testing, compared to 2D.

Chemistry of food and environment

P.26-001-Mon
Bioactive molecules isolated from olive pomace extract protect cells from calcium mediated damages

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In this study we show that molecules extracted from olive pomace prevent cell death induced by Ca^{2+}-overloading. Exposure of human neuroblastoma SKNBE, mouse brain endothelioma bEnd5 and human peripheral blood mononuclear cells to these molecules counteracts the Ca^{2+}-induced cell damages by reducing the activation of the Ca^{2+}-dependent protease calpain. Indeed, this proteolytic enzyme is involved in key cellular processes including apoptosis. In order to characterize this cell protective effect, we purified the olive pomace extract and concentrated the phenolic fraction, which contained mainly tyrosol, caffeic acid, oleuropein and apigenin. However, none of these polyphenolic compounds, separately tested as purified molecules, showed the protective effect on cell viability following Ca^{2+}-overloading. Hence, to identify the relevant bioactive molecules in the purified olive pomace extract, we performed a further fractionation by RP-HPLC. Only one of the fractions obtained, maintained the ability to protect the cells from damages induced by Ca^{2+}-overloading. This finding indicates that the cell protective effect depends of molecules different from the main polyphenols present in the purified olive pomace extract. We hypothesize that the molecular characterization of these molecules would eventually define new strategies for therapeutic applications in pathologies characterized by alterations of the intracellular Ca^{2+}-homeostasis. These promising starting points prompted us to plan further investigations aimed to identify the chemical nature of the bioactive molecules contained in this extract and the specific cell target sites.

P.26-002-Tue
Effects of gluten-free buckwheat cookies on plasma phospholipid fatty acid profile in healthy women

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Balanced and healthy diet, regular physical activity and promotion of healthy dietary habits with quality intake of carbohydrates, fat and fatty acid, is very important not only for saving health care costs, but also for preventing many chronic pathological conditions such as obesity, atherosclerosis, insulin resistance, diabetes type 2 and cancer. In the present study we investigated whether gluten-free buckwheat cookies with added blueberry and raspberry powder can modulate plasma phospholipids fatty acid (FA) composition in healthy subjects. In this study we included 20 healthy woman from 30 to 50 years old, which consumed gluten-free buckwheat cookies during 4 weeks. Formulation of gluten-free buckwheat cookies mixture, substituted with blueberry and raspberry, was made in Institute for Food Technology. Plasma lipids were extracted by a solvent mixture chloroform/methanol (2:1). Plasma phospholipid fatty acid methyl esters were analyzed by gas–liquid chromatography. Our results have

shown a significant decrease ($P < 0.001$) in percentage of palmitic (16:0) and palmitoleic acid (16:1n-7) after consumption of cookies. Also, percentage of total saturated FA was significantly decreased ($P < 0.05$) at the end of the study. On the other hand, percentages of docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) were significantly increased ($P < 0.05$) after intervention period. Accordingly, percentage of total n-3 fatty acid was significantly increased ($P < 0.01$), while the ratio of n-6/n-3 was significantly decreased ($p < 0.05$) at the end of study. Consumption of gluten free cookies during 4 weeks lead to beneficial changes in plasma phospholipids FA composition, especially due to rising level of total and individual n-3 polyunsaturated FA, suggesting that these products may have a beneficial effect on health and reduce the risk of developing many chronic non-communicable diseases.

P.26-003-Wed
Authorization of identification and quantification methods for new GMO lines in the Russian Federation

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The presence of recombinant DNA (rDNA) in food obtained by genetic engineering does not affect the food chain, since rDNA and natural DNA are absolutely identical. The genetic modification brings about the nucleotide sequence rearrangement while the chemical structure remains intact. Meanwhile the genetically engineered organisms (GMO) control system harnesses the possibility of rDNA detection in food and feed by using polymerase chain reaction (PCR). The authorization of identification and quantification methods for the new GMO lines, that are currently undergo state registration in Russia, is carried out in accordance with the standard procedure. This procedure includes an expert assessment aimed at confirming adequacy methods for the instrumental and methodological base applied in the institutions of the Federal Service For Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor), as well as the development of domestic test systems for the laboratory detection of these lines. In accordance with the above procedure there should be estimated (1) the specificity of the primer systems for identifying the corresponding transformational events using the BLAST analysis (Basic Local Alignment Search Tool); (2) the probability of false positive signals during analysis using this primer system (determined by PCR results with DNA of other GM lines); (3) compliance of methods with the minimum criteria established by the European Network of GMO Laboratories (ENGL) for transformation events quantification methods. As of March 2018, the control methods were evaluated for more than 30 plant-derived GMO lines (soybean, maize, sugar beet, rice, potatoes). Currently, the research algorithms tend to focus on developing molecular-biological methods for identification and quantification of GM potatoes in seed material, feed and food. This work was supported by Russian Science Foundation grant No. 16-16-04123.

P.26-004-Mon
The effects of natural pseurotins on selected immune cell functions

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Pseurotin A is a secondary metabolite produced by many species of fungi, mainly by *Aspergillus sp.* and *Penicillium sp.* During the pseurotin A biosynthesis, a large number of closely related bioactive compounds, such as pseurotin D or synerazol is also formed. Natural pseurotins have antimicrobial and antiparasitic activity. Interestingly, a few studies suggested effects of pseurotins in eukaryotes, such as antiangiogenic activity. In this study, we focused on effects of natural pseurotins on physiological functions of immune cells. Our results employing endotoxin-activated myeloid RAW264.7 cells (murine peritoneal macrophages) show that pseurotins (Pseurotin A, Pseurotin D and some structure analogs) were able to significantly reduce NO production in a concentration-dependent manner, both at the level of nitric oxide (NO) production and at the level of inducible NO synthase expression. These pseurotins also inhibited expression of early response cytokine interleukin (IL)-6 but not tumor necrosis factor α. Moreover, pseurotins were able to inhibit proliferation of RAW264.7. Other tested immune cells were mouse B-lymphocytes. They were isolated by sorter Aria II based on CD19 positivity. Interestingly, we show that pseurotins inhibited immunoglobulin E production of B-lymphocytes activated by a combination of E. coli endotoxin and IL-4. These effects were also related to changes in proliferation of B-lymphocytes via inhibition of JAK/STAT signaling pathway. We did not see any cytotoxic effects of pseurotins on these cells. It can be concluded that natural pseurotins are able to reduce oxidative stress, inhibit production of cytokines, NO and are able to modulate B-lymphocyte immune response. The study was supported by the GACR of the Czech Republic (17-18858S).

P.26-005-Tue
Fatty acids spectrum in parr and smolts brown trout (*Salmo trutta* L.) inhabiting the northern rivers (Kola Peninsula)

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Fatty acids (FAs) are one of the most multifunctional constituents of lipids. Their spectrum is closely related to both the trophic and ecological conditions of aquatic organisms and the ability of organisms to modify these acids with regard to environment. A significant part of the FAs of fish lipids is derived directly from food. Brown trout is a very plastic species that has a complex population genetic structure represented by anadromous, lake and stream forms. Although the ecology and biology of brown trout have been actively studied, little research exists on the features of the dynamic and functional role of the adaptation and use of important metabolic compounds in the life cycle of young brown trout during growth and development. The FA status of juvenile of trout of

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different ages (0+, 1+, and 2+) and smolts (4+) exhibited differences in the levels of the essential 18:2ω-6 and 18:3ω-3, with a higher proportion of 18:2ω-6 and a higher 18:2ω-6/18:3ω-3 ratio among fish in all of the age groups studied. This is connected to the abundance, availability and digestion effectiveness of certain food types that are, for the most part, rich in 18:2ω-6. The high content of 22:6ω-3 among juvenile (at 0+ and 1+) is a mechanism of biochemical adaptation related to the features of habitat use and environment (mainly, the current flow and rate): the motor activity of fish increases and the processes of elongation and desaturation of ω-3PUFAs derived from food are accelerated. In tributaries, juvenile trout (at 0+ and 1+) have active life styles (inhabit places with rapid currents) and actively feed at night, in contrast to older age groups. It was supposed that the content of 22:6ω-3 in structural phospholipids reflects the degree of metabolic and physiological activity; in particular, the correlation of 22:6ω-3 to the degree of fish mobility, i.e., their functional activity, was demonstrated. The research was supported by the Russian Science Foundation № 14-24-00102.

P.26-006-Wed
"Lipid sac": physiological and biochemical adaptation of daubed shanny *Leptoclinus maculatus* (Stichaeidae family) in Svalbard waters

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Fatty acids (FAs) of lipids are the most important structural and functional components of all living systems. Individual FAs and their combinations represent as biomarkers and as indicators reflecting the nutritional needs and physiological condition respectively of fish during ontogeny. The reserve lipids (triacylglycerols (TAG)) have in their structure mainly dietary FAs that allow consider them as trophic biomarkers. Daubed shanny, *Leptoclinus maculatus,* is a circumpolar fish and ecologically important as intermediate link in the Arctic trophic food webs. A unique feature of its postlarvae is the provisional organ – a «lipid sac» that accumulates a large amount of reserve lipids and maintains the buoyancy of pelagic postlarvae. FAs profile of lipid sac and flesh of the daubed shanny during early ontogenesis were analyzed using the Equipment Sharing Centre KarRC RAS. Monounsaturated FAs (MUFA) were dominating in TAG in the lipid sac. During early ontogenesis, it was determined the shifts in major individual MUFAs: hatched postlarvae had abundant amount of 18:1(n-9) due to feeding on phytoplankton while older developmental stages inhabiting pelagic zone showed large amounts of 20:1(n-9) and 22:1 (n-11) FAs – biomarkers for zooplankton *Calanus*. These FAs due to their physical and chemical properties maintain phase of lipids in the lipid sac and it is appropriate for developing pelagic larvae faced such severe conditions as low temperatures, starvation and change in mode of life from pelagic to bottom during development. Interesting, the lipid sac in adults, which descent to the bottom, resolve and in muscles of adult *L. maculatus*, certain 18:1(n-9), 18:1 (n-7) and 16:1(n-7) FAs dominated showing the feeding by benthos. The research was made in the frame of the budgetary theme № 0221-2017-0050 and was supported by RFBR No. 17-04-00466.

Biochemical education

P.27-006-Wed
The necessity of biochemistry and molecular biology for being pharmacist – a students' opinion

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Without any doubt, the knowledge in biochemistry and molecular biology (BMB) is a prerequisite for understanding expert knowledge in pharmaceutical sciences. Yet do Pharmacy students shear that opinion? This research aimed to explore Pharmacy students' opinions regarding the necessity of the knowledge in BMB for understanding of professional subjects. We also examined whether pharmacy students find the knowledge in BMB necessary for their future jobs and beneficial for their future career. This survey was conducted among 100 pharmacy students of the 4th year, at the University of Zagreb Faculty of Pharmacy and Biochemistry. As expected, the survey confirmed that most students agree that the knowledge in BMB is necessary for understanding pharmacodynamics, pharmacokinetics, and mechanism of action of drugs and that this fundamental knowledge is a prerequisite for apprehension of drug metabolism and biotransformation, and molecular basis of disease. The vast majority of the respondents also indicated that it is essential to combine the fundamental knowledge in BMB with the expert knowledge fields. Around one-third of students agreed that the acquired knowledge in BMB is necessary for their future job and that this knowledge will be beneficial for their future career. The conclusion of this survey is that the students consider the knowledge in BMB necessary for understanding of the expert knowledge in general and that these two should be combined. Moreover, it is very important that the basic subjects contain in addition to fundamental principles their practical applications so the students could testify the importance of basic knowledge for understanding professional subjects. This could make the process of studying easier and basic subject more interesting and their usefulness and applicability clearly visible.

Miscellaneous

P.28-001-Mon
Remodelling of the bacterial ribosome during transition into stationary growth phase

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Ribosomes are essential enzymes that participate in gene expression by conducting protein synthesis in every cell. Ribosomes consist of two subunits both comprising ribosomal RNA and proteins. In general, ribosomes are considered as macromolecular complexes with homogeneous and stable composition. This implies that all ribosomes are functionally equivalent and their structure is rather insensitive to environmental changes. However, growing experimental evidence indicates ribosome heterogeneity, i.e. that there are structurally different ribosome subpopulations in eukaryotic and bacterial cells. This notion has motivated the study of ribosomes from the perspective of potential translation

regulator rather than passive protein factory. This study investigates changes in bacterial ribosomal protein composition during cell growth focussing on Escherichia coli ribosomes. Quantitative mass spectrometry analysis of ribosomes from different growth phases has shown that bacterial ribosomal protein composition is generally stable. However, both paralogs of a ribosomal protein (named bL31 and encoded by two paralogous genes with 45% identity) are simultaneously present in E. coli ribosomes in vivo demonstrating ribosome heterogeneity. Interestingly, their abundance changes remarkably as cells transition from fast growth to stationary growth phase. The abundancy of bL31A paralog decreases remarkably concurrent with the respective increase of bL31B. Next, in vitro assays demonstrated that under acidic pH bL31A preferentially dissociates from ribosomes and that bL31B replaces bL31A on ribosomes. In conclusion, we have demonstrated on the example of bL31 paralogs that E. coli ribosomes are heterogeneous at ribosomal protein level, their protein composition changes at the end on fast growth and it is partially caused by protein exchange on ribosomes.

P.28-002-Tue
Profile of mitochondrial biogenesis markers and acrosomal reaction are disturbed in spermatozoa from stressed adult rats

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Although psychophysical stress is the most common stress in human society and major contributor to wide variety of pathological conditions, the molecular adaptation of spermatozoa from stressed males were not described well. The aim of this study was to determine the functionality and molecular adaptation of spermatozoa from stressed rat by applying *in vivo* and *in vitro* approach. For *in vivo* experimental model, psychophysiological stress by immobilization (IMO), was performed for 3 h in different time during the day (03:00 h, 07:00 h, 15:00 h), for one (1xIMO) or ten (10xIMO) consecutive days. For *in vitro* approach, epididymal spermatozoa from undisturbed rats were stimulated with stress hormones adrenaline and cortisol for 30 min. Results showed that number of spermatozoa significantly decreased in all 10xIMO rats comparing to control. Acrosomal status (response to acrosome-reaction-inducer progesterone) significantly decreased in spermatozoa from 1xIMO and 10xIMO rats comparing to control. The same effect was observed in spermatozoa stimulated *in vitro* with stress hormones. Preliminary RQ-PCR results revealed that transcription of the main mitochondrial biogenesis markers *Nrf1*, *Ppara* and *Ppard* decreased in spermatozoa from 10xIMO rats. In the same spermatozoa samples the similar effect was registered for *Ucp2*, the mediator of regulated proton leak. Oppositely, the significant increase of *Cytc* transcription was registered in spermatozoa from 10xIMO rats. Incubation of spermatozoa with adrenaline decreased level of *Ppargc1a* and *Nrf2a* transcripts, while cortisol decreased expression of mitochondrial transcription factor TFAM. Repeated psychophysical stress decreased the number and functionality of spermatozoa and disturbed transcriptional profile of their mitochondrial biogenesis markers.

P.28-003-Wed
Ribosome-associated quality control (RQC) in *Saccharomyces cerevisiae* upon oxidative stress

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Over senescence number of processes in the living cell become dysfunctional and lead to protein homeostasis imbalance. One of the key impairments is mitochondria dysfunction that may result in reactive oxygen species (ROS) production. It is hypothesized that increased levels of ROS lead to production of aberrant proteins resulting in the ribosome stalling. With the use of model substrates it has been shown that ribosome-associated quality control (RQC) is a crucial mechanism for clearance of non-stop or no-go proteins. Here, we investigate the influence of RQC on the protein homeostasis upon oxidative stress. We have confirmed that cells lacking the RQC components (ΔLtn1, ΔRqc2), show growth defect under translation inhibiting conditions (hygromycin B or cycloheximide treatment). Interestingly, deletion of Ltn1 or Rqc2 in yeast causes accumulation of dysfunctional proteins synthesized upon oxidative stress, which was mimicked by H_2O_2 treatment. Furthermore, the oxidative stress increases number of ubiquitinated proteins. Taken together, these results suggest that under conditions of increased ROS production aberrant nascent proteins are released from the ribosome through the RQC pathway and are further ubiquitinated by Ltn1 for proteasomal degradation. The work was founded by National Science Centre, Poland 2015/18/A/NZ1/00025.

P.28-004-Mon
Deletion of insulin and IGF1 receptors genes disturbs expression of the key mitochondrial biogenesis regulators in mouse prepubertal steroidogenic cells of testes but not ovaries

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Controlled changes in mitochondrial biogenesis and morphology are required for cell survival and homeostasis, but the molecular mechanisms are largely unknown. Here, the male and female knock out prepubertal mice (P21), with insulin and IGF1 receptors deletion in steroidogenic tissues (*Insr*/*Igf1r*-DKO), were used to investigate transcription of key regulators of mitochondrial biogenesis (*Ppargc1a, Ppargc1b, Pparg, Nrf1, Tfam*) in Leydig cells, ovaries and adrenals as well as mitochondrial architecture (*Mfn1, Mfn2, Opa1*) in Leydig cells. Accordingly, gene expression was followed employing RQ PCR, proteins expression detected by western blot, while hormones level was measured by radioimmunoassay. Results showed that expression of PGC1, the master regulator of mitochondrial biogenesis and integrator of environmental signals, as well as its downstream target *Tfam*, significantly decreased in testosterone-producing Leydig cells from *Insr*/*Igf1r*-DKO animals. This was followed with reduction of *Mtnd1*, the core subunit belong to the minimal assembly required for catalysis. Transcription of mitochondrial biogenesis markers remained unchanged in ovaries. Differently, in adrenals, the pattern of mitochondrial markers transcripts was the same in both sexes and, besides *Pparg* and *Tfam*, opposite from Leydig cells. The transcription level of mitochondrial architecture markers (*Mfn1, Mfn2*) was significantly increased in Leydig cells from

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Insr/*Igf1r*-DKO mice suggesting that the mitochondrial architecture and mitochondrial phase of steroidogenesis was affected in males. Our results are the first to show that insulin and IGF1 receptors are important for mitochondrial biogenesis in gonadal steroidogenic cells of prepubertal males, but not females, as important regulators of both mitochondrial biogenesis and architecture markers transcription.

P.28-005-Tue
Autophagy in corpus luteum as a possibly critical mechanism for rodent pregnancy and parturition

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Ovarian corpus luteum (CL) is an ephemeral tissue whose regulated secretion of progesterone is essential for maintenance and/or timely termination of pregnancy in rodents. Our previous finding that CL of pregnant rats without Fas/FasL system (an authentic apoptotic system) suggests that this tissue may alternatively undergo autophagic cell death during regression. Here we investigated the presence of autophagic system in CL and its any implications in rodent pregnancy and parturition. LC3 (–I and –II) expression in CL was estimated by Western blot analysis in comparison with progesterone secretion and luteal mass throughout pregnancy. LC3 distribution was tested by immunocytochemistry. While the expression of cytosol-associated LC3-I was not significantly altered throughout pregnancy, that of autophagosome-associated LC3-II increased significantly from day 15, showed a peak on day 21, and decreased on day 23 (day of normal parturition). LC3-II/I ratio had positive correlations with steroidogenic activity and cell size. Immunoreactive LC3 was found to be present in the cytosol of steroidogenic cells and its aggregation was marked on day 21. Functional implication of autophagy in CL tissue was examined by local treatment in vivo with Bafilomycin A1 (autophagy and V-ATPase inhibitor, 6.23 pg/0.1 ml/ovary) on day 15 or 19. The drug treatment on day 15 resulted in altered timing of delivery associated with significant reduction in steroidogenic cell size, but not progesterone secretion, compared to vehicle-treated control groups. We conclude that the autophagy parameter is temporally matched with further structural and functional activation of CL and that Cl autophagy may contribute to activation, but not regression, of rodent CL and thus their female reproduction.

P.28-006-Wed
JAB1 regulates CPNE1 function via direct binding to CPNE1

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Copine1 (CPNE1), has tandem C2 domains and an A domain. We previously demonstrated that CPNE1 directly induces neuronal differentiation via Protein kinase B (AKT) phosphorylation in the hippocampal progenitor cell line, HiB5. To better understand its cellular function, we carried out a yeast two-hybrid screening to find CPNE1 binding partners. Among the identified proteins, Jun activation domain-binding protein 1 (JAB1) appears to directly interact with CPNE1. Between CPNE1 and JAB1, the physical interaction was confirmed *in vitro* and *in vivo*. In addition the specific binding regions of CPNE1 and JAB1 was confirmed with truncated mutant assay. Furthermore, our results also demonstrate that AKT phosphorylation and expression of the neuronal marker protein are increased when JAB1 is overexpressed in CPNE1 high expressed cells. Moreover, overexpression of both CPNE1 and JAB1 effectively increased neurite outgrowth. Collectively, our findings suggest that JAB1 activates the neuronal differentiation ability of CPNE1 through the binding of C2A domain in CPNE1 with MPN domain in JAB1.

P.28-007-Mon
Insertion-deletion mutations of a single nucleotide in the rRNA large subunit methyltransferase gene rlmAII sways the susceptibility of the honeybee pathogen *Melissococcus plutonius* to mirosamicin

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American foulbrood (AFB) and European foulbrood (EFB) caused by *Paenibacillus larvae* and *Melissococcus plutonius*, respectively, are two major bacterial infectious diseases of honey bee broods. Although the 16-membered ring macrolide antibiotics, mirosamicin and tylosin, have been used to prevent AFB in Japan, macrolide-resistant *P. larvae* has yet to be found. *M. plutonius* is not the target pathogen of these drugs; however, since these macrolides are used as prophylactic medicines, *M. plutonius* may have also been exposed to these drugs in the field. In the present study, we revealed for the first time the presence of mirosamicin-specific macrolide-resistant strains in Japanese *M. plutonius* and that the gene encoding the rRNA large subunit methyltransferase A (*rlmA*^{2}) was disrupted exclusively in mirosamicin-susceptible strains due to the same single nucleotide insertion. Primer extension assays demonstrated that *M. plutonius* RlmA^2 methylates G748 in hairpin 35 in domain II of 23S rRNA, and the deletion of *rlmA*^{2} resulted in increased susceptibility to mirosamicin and the loss of methylation at G748 of 23S rRNA, suggesting that methylation at G748 by RlmA^2 confers mirosamicin-specific resistance in *M. plutonius*. The single nucleotide mutation in *rlmA*^{2} of mirosamicin-susceptible strains was easily repaired by spontaneous deletion of the inserted nucleotide under laboratory conditions, resulting in the acquisition of resistance to mirosamicin; nevertheless, intact *rlmA*^{2} was only found in Japanese *M. plutonius*. In a Paraguayan strain tested and all of the whole-genome sequenced European strains in the database, *rlmA*^{2} was disrupted due to the same point mutation, suggesting that these strains are mirosamicin-susceptible. Since mirosamicin has been used in apiculture only in Japan, its use as a prophylactic medicine for AFB may have influenced the macrolide susceptibility of the causative agent of EFB in this country.

P.28-008-Tue
Nicotine metabolic genes in *Arthrobacter*: pAO1 vs AK-YN10

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The accumulation of sequence data through various sequencing projects allows us nowadays to screen and identify metabolic pathways in bacterial genomes. We are particularly interested in

the molecular evolution of the bacterial nicotine pathways and we are using the well characterized pAO1 encoded *nic*-genes from *Paenarthrobacter nicotinovorans* (GenBank GI: AJ507836) as a model for comparative genomics. A rather similar *nic*-gene arrangement was identified in the draft genome of *Arthrobacter* AK-YN10, a bacterial strain isolated for its ability to degrade atrazine. In the current approach, we aimed to investigate the location of these nic-genes and to establish whether these genes are functional or not. For this, total and plasmidial DNA was extracted from *Arthrobacter* AK-YN10 and the presence of the nicotine catabolism genes was assessed by PCR using specific primers. The nicotine consumption during the growth of pAO1 and AK-YN10 strains on citrate medium was monitored by HPLC. We showed that the AK-YN10 strain contains at least one plasmid and that the 6-hydroxy-L-nicotine oxidase gene involved in the nicotine metabolism on pAO1 is placed on one of these plasmids. The AK-YN10 strain is both resistant to and can grow on nicotine containing *media,* but it is not able to degrade this alkaloid.

P.28-009-Wed
The ethnic characteristics of distribution of EDNRA H323H polymorphic genetic marker of cardiovascular diseases

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The Endothelin receptor type A (ETA) is a G protein-coupled receptor that binds endothelin-1 and mediates its vasoconstrictor effect. Many studies suggest that genetic variations in the *EDNRA* gene coding for the ETA receptor are associated with different cardiovascular diseases, however, others could not find this association, probably due to ethnic characteristics of studied populations. In our previous study of the *EDNRA* H323H (T>C) polymorphism (rs5333) we found a significant difference in allelic and genotypic frequencies between Arab Syrians and Russian populations. In the present study, we investigated for the first time the distribution of this polymorphism among 36 Lebanese with the mean age of 25.37 ± 7.57 years. The genotypes were determined using PCR with restriction fragment length polymorphism. The frequency of C allele of EDNRA gene was 19%. The genotype frequencies were in Hardy-Weinberg equilibrium ($P > 0.05$). The TT, TC and CC genotype frequencies were 63.89%, 33.3%, and 2.81%, respectively. A significant difference was revealed in genotype distribution between Lebanese and Russians from Central Russia ($P = 0.00096$), as well as in allelic distribution ($\chi^2 = 4.281$, $P = 0.03852$), whereas genotypic frequencies tended to be different between Arab Syrians and Lebanese ($P = 0.05708$). The results suggest that the analyzed polymorphism seems to have an ethnic distribution; therefore, the allelic and genotypic frequencies of the H323H *EDNRA* polymorphism should be investigated in larger samples and compared with clinical implications through additional research within studied populations. The publication was prepared with the support of the "RUDN University Program 5-100".

P.28-010-Mon
A novel synthetic flavonoid with potent antibacterial properties: in vitro activity and proposed mode of action

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The emergence of pathogenic multidrug-resistant bacteria demands new approaches in finding effective antibacterial agents. Synthetic flavonoids could be a reliable solution due to their important antimicrobial activity. We report here the potent *in vitro* antibacterial activity of ClCl-flav – a novel synthetic tricyclic flavonoid. The antimicrobial effects were tested using the minimum inhibitory concentration (MIC), time kill and biofilm formation assays. Fluorescence microscopy and scanning electron microscopy were employed to study the mechanism of action. MTT test was used to assess the cytotoxicity of ClCl-flav. Our results showed that Gram positive bacteria were more sensitive (MIC = 0.24 μg/ml) to ClCl-flav compared to the Gram negative ones (MIC = 3.9 μg/ml). We found that our compound showed significantly enhanced antibacterial activities, 32 to 72-folds more active than other synthetic flavonoids. ClCl-flav showed a bactericidal activity at concentrations ranging from 0.48 to 15.62 μg/ml. At twice the MIC, all *Escherichia coli* and *Klebsiella pneumoniae* cells were killed within 1 h. Also ClCl-flav presented a good anti-biofilm activity. The mechanism of action is related to the impairment of the cell membrane integrity. No or very low cytotoxicity was evidenced at effective concentrations against Vero cells. Based on the strong antibacterial activity and cytotoxicity assessment, ClCl-flav has a good potential for the design of new antimicrobial agents.

P.28-011-Tue
Low molecular weight metabolites secreted through an ABC-type efflux pump MacAB protect *Serratia marcescens* against hydrogen peroxide

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The emergence of bacterial multi-drug resistance is a growing problem of public health worldwide. Bacterial drug efflux systems are membrane protein complexes that function to expulse drugs from the cell. In addition to well established role of multidrug efflux pumps in antibiotic resistance, efflux pumps also play important additional roles in biology of bacteria that are independent of their role in drug efflux. Macrolide-specific ABC-type drug efflux pump MacAB first identified in *E. coli* has been linked to virulence of *Salmonella enterica* serotype Typhimurium in mice. Here we show that MacAB is essential for survival of *Serratia marcescens* SM6 in the presence of hydrogen peroxide. We further show that the growth of *S. marcescens* Δ*macAB* mutant cells in the peroxide-containing media could be restored by co-culture with wild type cells. This protection is mediated by heat- and proteinase K-sensitive metabolites present in the media used for growth of wild type *S. marcescens* SM6 cells but not in the media used for growth of its isogenic Δ*macAB* mutant cells. Moreover, the synthesis of these metabolites does not require active ribosomes. Fractionation of the conditioned media showed

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that protective antioxidant molecules are present in the fraction containing low molecular weight metabolites (under 10 kDa). Additional HPLC analysis resulted in the identification of five fractions with anti H_2O_2 protective properties which are currently evaluated for their metabolite composition. This work was supported by the Russian Science Foundation project 16-14-10200 and performed in accordance with the Russian Government Program of Competitive Growth of Kazan Federal University.

P.28-012-Wed
Identification of fucoidan sulfatases using bioinformatics and functional screening approaches

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Sulfatases play a key role in the catabolism of various sulfated polysaccharides (ulvans, carrageenans, agarans, fucoidans, etc.) of marine organisms. The variety of sulfated polysaccharide structures implies a large amount of sulfatases with different substrate specificity. Despite advances in processing and annotating the genomic and metagenomic data of marine microorganisms, the correct annotation of carbohydrate sulfatases is still difficult. Due to the fact that sulfatases acting on many marine polysaccharides have been poorly characterized, and some have not yet been discovered. To date, only a few carrageenan sulfatases and agaran sulfatases have been biochemically characterized. There are only fragmentary data about fucoidan sulfatases. Amino acid sequences, specificity, mode and mechanism of action of fucoidan sulfatase are still unknown. We analyzed the genome of the fucoidan degrading marine bacterium *Wenyingzhuangia fucanilytica* CZ1127 and identified 80 hypothetical sulfatases genes. Six hypothetical sulfatases genes, named by us as swf1-6 were located in close proximity to genes of fucoidanases (107 family of glycoside hydrolases CAZy). We assumed that the presence of sulfatases coding genes in the same locus with fucoidanases indicates their participation in the catabolism of fucoidans. To confirm their function, genes of sulfatases were cloned and proteins were produced in Escherichia coli cells. Functional screening among hypothetical sulfatases using sulfated fucooligosaccharides and fucoidans resulted to identification of two fucoidan sulfatases SWF1 and SWF4. Specificity and some catalytic features of sulfatases were determined using various sulfated fucooligosaccharides. Based on the substrate specificity, the enzymes are classified as fucoidan exo-2O-sulfatase (SWF1) and fucoidan exo-3O-sulfatase (SWF4). This work was supported by the Russian Science Foundation (Project No. 18-04-00905).

P.28-013-Mon
ATP-synthase inhibition by semi-synthetic oligomycin A derivatives

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Macrolide antibiotic oligomycin A (OlgA) is a high-active ATPase inhibitor, which is widely used for biochemical studies of mitochondrial F_oF_1 ATP synthase. In micromolar concentrations, OlgA binds to F_o c-subunit and blocks proton translocation, resulting in disruption of bioenergetic metabolism. It has been proposed that hydroxyl group at C33 position of the OlgA side chain might have an influence on its binding with the target. Also, recently we have found that modifications of OlgA macrolactone core led to significant changes in its biological properties. In order to investigate mitochondrial ATP-synthase inhibition by oligomycins more closely, a series of semi synthetic OlgA derivatives with site-selective modifications were synthesized and their inhibitory activity on the F_oF_1 ATP synthase were determined on inverted membrane vesicles obtained from cells of *Streptomyces fradiae* ATCC 19609 (strain, supersensitive to OlgA). It has been found that nitron-oligomycin, modified at positions C7 and C3 into intramolecular heterocycle and 2,3,16,17,18,19-hexahydrooligomycin A with reduced double C-C bonds didn't inhibit ATP-synthesis in the vesicles, probably due to significant change of macrolactone geometry and, consequently, decreasing the affinity to the target. These data were in agreement with results of molecular modelling of binding of OlgA and its derivatives to the intracellular target. In striking contrast, 33-azido-33-deoxy-oligomycin and 33-*O*-mesyl-oligomycin inhibited ATP synthesis more potently than parent antibiotic. Finally, 33-dehydrooligomycin and 33-deoxy-33-thiocyanato-oligomycin were slightly less active than OlgA. Thus, we can conclude that structural changes in macrolactone cycle can be critical for binding affinity of OlgA to ATP synthase and functional groups at C33 position indeed play the important role in ATP-synthase inhibition by OlgA. This work was supported in part by Russian Science Foundation (agreement № 15-15-00141).

P.28-014-Tue
Relationship between iFGF23, iPTH and phosphorus in renal transplant patients

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IFGF23, a bone-produced hormone, plays a key role in phosphorus metabolism. The aim of this study is to investigate the relationship between iFGF23, iPTH and Phosphorus in renal transplant patients. 40 males (age 39.28 ± 11.01) and 20 female (age 40.05 ± 12.09) renal transplant patients who are followed in the transplantation polyclinic of Gazi University Medical Faculty Hospital were included in the study. The control group consisted of 18 males (age 41.22 ± 6.34) and 16 healthy females (age 41.88 ± 8.24) were included in the study. The mean transplant year was 6.95 ± 5.44. The median (min–max) values of serum iFGF23 in renal transplant patients were found to be 219.67 (93.7–652.83) pg/ml respectively, while serum iPTH levels were 83.6 (16.52–278.3) pg/ml, and serum Phosphorus was found to be 3.195 (1.6–5.7) mg/dl. Serum iFGF23 levels were found to be 119.67 (52.39–361.74) pg/ml in the control group, while serum iPTH levels were 38.05 (3.41–109.1) pg/ml serum Phosphorus 3.52 (2.54–4.62) mg/dl respectively. Serum iFGF23 and iPTH levels were statistically significantly higher in the patient group than the control group ($P < 0.05$). Whereas serum Phosphorus levels were statistically significantly lower in the patient group than the control group ($P < 0.05$). Correlation study of transplant patients showed a strong positive correlation between serum iFGF23 and serum iPTH ($P < 0.01$) but no correlation between iFGF23 and Phosphorus ($P > 0.05$). There was no correlation between iFGF23 and iPTH and Phosphorus in the correlation study performed in the control group ($P > 0.05$).

P.28-015-Wed
Application of enzyme treatment and NMR spectroscopy for the research of *Sargassum horneri* fucoidan

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Structure and anticancer activity of fucoidan from *Sargassum horneri* and of products of its enzymatic transformation were investigated. The purificated fucoidan's monosaccharide composition includes fucose (90%), galactose (9%) and sulfate groups (23%). The fucoidan's NMR spectrum was unclear. The enzyme treatment gave the mixture of oligosaccharides (LMP, 40%) and fucoidan fragments of high molecular weight (HMP, 52%). The LMP fraction was divided by ion-exchange chromatography and structures of 6 sulfated oligosaccharides with polymerization degrees 4–10 were established by NMR-spectroscopy. The HMP fraction was treated by fucoidanase from marine bacteria *Wenyingzhuangia fucanilytica*. Structure of the resulting polysaccharide was determined by NMR spectroscopy. The main chain of the fucoidan is established to consist mostly of the repeating →3-α-L-Fuc*p*(2SO_3^-)-1→4-α-L-Fuc*p*(2,3SO_3^-)-1→ fragment, with an irregular →3-α-L-Fuc*p*(2,4SO_3^-)-1→ fragment. Unsulfated side chains with the α-L-Fuc*p*-1→2-α-L-Fuc*p*-1→ structure connect to the main one at C4. Human colorectal DLD-1 cell line (8000 cells) was treated with the native fucoidan, HMP and four oligosaccharides. The investigated saccharides were non-cytotoxic at concentrations less than 200 μg/ml for the investigated cell line. ScF and HMP suppressed colony formation of DLD-1 cells by 50%, while oligosaccharides were non-effective on the inhibition of cancer cells colony formation. Some differences in the anticancer activities of fucoidans and oligosaccharides can be explained by their structural features. The degree of polymerization of fucoidan's fragments apparently influences antitumor activity heavily. Short fragments of fucoidan with 4–10 polymerization degree are apparently unable to interact with the target tumor cells effectively. This work was supported by RFBR grant (18-04-00905).

P.28-016-Mon
Bioengineered analytical system to study potassium channel–ligand binding

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Voltage-gated potassium (Kv) channels are found in most cell types and represent a vast class of potential drug targets. Peptide toxins from scorpion venoms, high-affinity blockers of Kv channels, are considered as prospective templates to design new drugs targeting Kv channels. We developed a bioengineered analytical system, in which Kv channel–ligand interactions are studied using a confocal microscopy-based approach. In this system, hybrid channels KcsA-Kv1.X (X = 1, 2, 3, and 6) are used. The pore domains of these hybrids are highly homologous to those of corresponding eukaryotic Kv1 channels. The system was successfully applied: a) to detect Kv1-specific peptide blockers in animal venoms; b) to determine affinity and specificity of peptide blockers to the target Kv1 channels; c) in combination with transcriptomics, to perform comprehensive study on the variability of Kv1.1 blockers in the venom of the scorpion *Mesobuthus eupeus*; d) in combination with molecular modeling, to study molecular basis of interactions of peptide toxins with the pore domain of Kv1.X channels; e) to obtain a selective peptide blocker of the target channel by means of screening mutant peptides. To facilitate our studies of channel–ligand interactions, a new bioengineering approach was worked out to high-yield production of disulfide-rich peptide toxins with retained native N-terminal amino acid residues. Also, genetically encoded fluorescent peptides (GEFP) were produced that can be used as fluorescent probes of Kv channels and for screening purposes. Further perspectives of development and applications of bioengineered analytical system are discussed. This work was supported by the Program for Fundamental Research of the Presidium of Russian Academy of Sciences No. 1.

P.28-017-Tue
New tools in the *Leishmania* gene expression toolbox

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Leishmania is an obligate, intracellular parasite that multiplies in macrophage phagolysosomes of its vertebrate host causing leishmaniasis. This severe disease affects several hundred million people mainly in the tropical and subtropical countries. In order to successfully infect macrophages *Leishmania* must handle the oxidative stress within macrophages. Many other parasites utilize catalase for this purpose. Conspicuously, the gene, encoding this clearly advantageous in the oxygen environment enzyme, was secondarily lost from genomes of all *Leishmania* spp. investigated thus far. Previously, we overexpressed catalase of the phylogenetically related insect trypanosomatid *Leptomonas pyrrhocoris* in *Leishmania mexicana* and demonstrated that transcription of this gene was significantly reduced in infective metacyclic and intracellular amastigote stages. The developmentally regulated untranslated regions (UTRs) used in the T7 polymerase-driven Tet-inducible protein expression system likely determined such an expression profile. In this study we replaced one allele of the *L. mexicana* β-tubulin gene (*LmxM.32.0792*) by Hygromycin antibiotic resistance ORF and confirmed its stable expression during *Leishmania* life cycle. To bypass the need of additional UTRs we constructed bicistronic vectors in which the catalase (or GFP as a control) and the selectable marker gene (Nourseothricin) were linked by a self-cleaving 2A peptide derived from the *Porcine teschovirus-1* maintaining a single ORF. The polypeptides were successfully processed in *L. mexicana* and yielded active catalase enzyme (or GFP). Western Blotting analysis confirmed high expression level of the reporter genes and efficient 2A cleavage. Considering post-transcriptional regulation of gene expression and unknown regulatory mechanisms of this process in *Leishmania* parasites, 2A-based self-processing polyproteins provide an opportunity to stably express multiple exogenous proteins.

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P.28-018-Wed
Proton-independent activation of acid-sensing ion channel 3 by isoquinoline alkaloids

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Acid-sensing ion channels type 3 (ASIC3) are expressed mainly in peripheral sensory neurons and play an important role in pain perception and inflammation development, but their therapeutic potential is limited by the lack of ligands activating them at physiological pH. We found that compounds, such as a bisbenzylisoquinoline alkaloid, lindoldhamine (LIN) from laurel leaves, as well as endogenous isoquinoline alkaloids (EIAs), have a fundamentally new effect on ASIC3 channels. At pH 7.4 or higher, LIN and EIAs activated a sustained, proton-independent, current through rat and human ASIC3 channels, but not rat ASIC1a or ASIC2a channels. LIN and EIAs also potentiated proton-induced transient currents and promoted recovery from desensitization in human, but not rat, ASIC3 channels. The decrease of EIA levels could be suggested as a novel therapeutic strategy for treatment of pain and inflammation. These unique, species-selective, ligands of ASIC3 channels, open new avenues in studies of ASIC structure and function, as well as providing new approaches to drug design.

P.28-019-Mon
TCRbeta CDR3 motif is detected in synovial fluid of patients with different spondyloarthropathies

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Spondyloarthropathies (SpA) are a group of autoimmune diseases with similar clinical and genetic features, which includes ankylosing spondylitis (AS), psoriatic arthritis (PsA), enteropathic arthritis, undifferentiated SpA and reactive arthritis (ReA). Recently a characteristic TCRbeta motif represented by highly homologous $CD8^+$ $TRBV9^+$ clonotypes was revealed in peripheral blood of patients with AS and detected in a few samples of synovial fluid of the patients. Also clonotypes from this motif were described in peripheral blood and synovial fluid of patients with reactive arthritis. Here we expanded our cohort of donors, including samples of synovial fluid from AS and PsA patients, and investigated presence of the motif. Using quantitative molecular-barcoded 5′-RACE, we performed TCRbeta repertoire profiling of synovial fluid samples from HLA-B*27-positive and HLA-B*27-negative patients with AS and PsA, including magnetically sorted TRBV9+ T-cells. The TCRbeta motif was detected in synovial fluid samples from both AS and PsA patients. The most frequently detected clonotype from the motif was CASSVGLYSTDTQYF. No additional similar to the motif clonotypes was detected in the repertoires. Consistent with previous observation, the motif was found only in HLA-B*27-positive but not in HLA-B*27-negative patients, that supports HLA-B*27 restriction of the clonotypes. Presence of the motif in synovial fluid of patients with at least 3 (AS, PsA and ReA) diseases, belonging to the family of spondyloarthropathies, suggests similar mechanism of initiation and progression of different types of the SpAs. Funding: RSF grant No 17-75-10220.

P.28-020-Tue
Chlamydia trachomatis biodiversity detected in clinical urogenital samples in the Southeastern European Region of Russia

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Chlamydia trachomatis (CT) is one of the most successful pathogens responsible for ocular trachoma and sexually transmitted diseases worldwide. Chlamydial infection has a high epidemic potential that is possibly depends on the strains polymorphism. Thus, CT clonal diversity is now under intensive investigation worldwide. Recently, we have analyzed the sequence of 1156 bp-fragment of the CT *ompA* gene, comprising of four variable (VD) and five constant (CD) domains. Classification of CT into biovars is based on polymorphism in the VD regions. We found both synonymous and non-synonymous substitutions as single nucleotide polymorphisms (SNP): 5 of 17 (29.4%) were located in VDI, VDII, VDIV, while 12 of 17 (70.6%) positioned in CDI, CDIII and CDIV. In overall, we revealed the presence of 13 variants of the *ompA* gene. Here we investigated polymorphisms of the seven CT housekeeping genes (*gatA*/*oppA*/*hfiX*/*gitA*,/*enoA*/*hemN*/*fumC*) (7HKG) using multilocus sequence typing (MLST) method. For this purpose, we determined sequences of 7HKG genes analyzing DNA derived from clinical samples (cervical or urethral swabs) of heterosexual patients (n = 856). All patients were from Saratov Region located in Southeast Russia bordered Kazakhstan. We found five sequences types (ST), such as ST4 (56%, 95% CI, CI, 70.0 to 41.3), ST6 (10%, 95% CI 21.8 to 3.3), ST9 (22%, 95% CI 35.9 to 11.5), ST10 (2%, 95% CI 10.7 to 0.05) and ST38 (10%, 95% CI 21.8 to 3.3). According to the classification based on both SNPs in the *ompA* gene and 7HKG, the Saratov CT strains were associated with non-invasive urogenital disease of the Haplotype 2 CT group. There were no strains found that belong either to the Haplotype 1 (invasive urogenital disease caused by LGV), or Haplotype 3 (trachoma caused by typical A-C genovars). This work represents a pilot study focused on the unraveling micro-evolution of CT aimed to understand better the global epidemiology of this pathogen. This work was supported by the RSF #17-16-01099

P.28-021-Wed
Ontogenetic variation in trypsin, chymotrypsin and metallo-peptidase activity in *Litopenaeus vannamei* shrimp

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During shrimp larval development, changes occur at ecological, physiological and biochemical level. Proteolytic activity was assayed in larval stages of the shrimp *Litopenaeus vannamei*

including 5 nauplius stages, 3 protozoeal stages, 3 mysis stages, and numerous postlarval stages. To assess the larval variations in the activity of trypsin, chymotrypsin and metalloproteinases along larval development, we measured the proteolytic activity using zymograms and specific fluorogenic substrates. Trypsin reached its maximum activity at the beginning of postlarval stage, while chymotrypsin and metallopeptidase activity increased sharply at the third protozoea stage; in general, protease activity decreased significantly on subsequent stages. Using class-specific inhibitors and mass spectrometry followed by bioinformatics analysis allowed us to distinguish and identify protease bands on zymograms. It was clear that each type of showed a particular pattern of appearance and disappearance during larval development, indicating that such peptidases fulfill a role in larval development yet to be investigated.

P.28-022-Mon
Anti-inflammatory effect of palmitoleic acid on effector mechanisms of neutrophils

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Neutrophils play a pivotal role orchestrating the inflammatory response through migration to the inflammatory focus, phagocytosis of microorganisms and/or cellular debris, and release of cytokines, reactive oxygen species and other inflammatory mediators. Palmitoleic acid, an n-7 monounsaturated fatty acid that occurs in plant oils from macadamia nuts and sea-buckthorn, is found in the sebum cutaneum of young humans and decreases with age. Recent studies have reported that palmitoleic acid is a useful tool in treating disorders related to skin inflammation, but the mechanisms of action are unclear. Herein, an investigation was made into the effects of palmitoleic acid in neutrophil death (apoptosis and necrosis), migration to the inflammatory focus and cytokine release. In the concentration range studied (0–200 μM), palmitoleic acid is not toxic to neutrophils as observed by the maintenance of membranes and DNA integrity in palmitoleic acid treated neutrophils. In in vivo assays for neutrophils, including migration and the formation of exudate in a sterile inflammatory air pouch, it was observed that palmitoleic acid has potent anti-inflammatory activity, inhibiting LPS-induced TNF-α (73%, $P \leq 0.05$), IL-1β (66%, $P \leq 0.001$), IL-6 (75%, $P \leq 0.001$), MIP-3α (70%, $P \leq 0.05$) and l-selectin (16%, $P \leq 0.05$) release. Palmitoleic acid also inhibited LPS-stimulated neutrophil migration. In vitro, palmitoleic acid inhibited LPS-stimulated cytokine production by cultured human neutrophils. We concluded that palmitoleic acid has anti-inflammatory effects, decreasing the neutrophil inflammatory response. Further studies are needed to elucidate the mechanisms involved in these effects. Financial Support: FAPESP (Process 14/03947-1 and 11/15360-7) and CNPq (Process 307769/2014-3).

P.28-023-Tue
Serum amyloid a induces cytokine and growth factor production in HaCaT keratinocytes

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Serum amyloid A (SAA), an acute phase marker with potent proinflammatory activity, can modulate regenerating tissue, influencing cutaneous healing. Poor wound healing is associated with chronic inflammation and involvement of the migration and proliferation of keratinocytes, among other factors such as infection, poor angiogenesis and increased oxidative stress. Keratinocytes play a central role in healing, maintaining skin integrity, and participating in tissue repair through the release of cytokines, chemokines and growth factors. Although SAA is involved in skin disorders, the mechanisms of action of this protein are unclear. Our objective was to study the effect of SAA on the release of TNF-α, IL-1β, IL-6, IL-8, VEGF and FGF in cultured HaCaT keratinocytes. To define the toxic dose of SAA in keratinocytes, we analysed the number of viable cells by MTT assay. Cytokines and growth factors were quantified by ELISA in the supernatant of keratinocytes treated with SAA. After 24 h, the supernatants were collected, and cytokines were determined by ELISA. In the concentration range studied, SAA (0, 5, 10, 20 and 40 μg/ml) was not toxic to keratinocytes. A dose-dependent increase in TNF-α ($r = 0.99$, $P < 0.05$), IL-1β ($r = 0.99$, $P < 0.05$), IL-6 ($R = 0.99$, $P < 0.05$), IL-8 ($r = 0.99$, $P < 0.05$) and VEGF-α ($r = 0.97$, $P < 0.05$) by keratinocytes incubated in the presence of SAA was observed. In the studied conditions, SAA did not alter the production of FGF-1. Our results point to SAA as an important protein involved in signalling for healing, scarring, angiogenesis and fibrosis. Added SAA could contribute to psoriasis pathogenesis via keratinocyte activation, maintaining a cutaneous inflammatory and proliferative environment. Knowing the mechanisms by which SAA interferes with signalling for cutaneous healing is essential for the development of new therapeutic agents. Financial Support: FAPESP (Process 16/24758-8) and CNPq (Process 307769/2014-3).

P.28-024-Wed
Detection and *Chlamydia trachomatis* genovar distribution in blood and genital samples of Chlamydia patients

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Zoonotic Chlamydia could be routinely detected in blood of infected animals as well as in both their genital and extra-genital sites. In contrast, *C. trachomatis* (CT) is typically found in the urogenital, rectal or pharyngeal sites of Chlamydia patients. The main goal of this study was to investigate a possibility of detecting CT DNA in the blood samples of Chlamydia patients who have demonstrated a positive response for the presence of CT DNA in their genital samples. In total, clinical specimens from genital sites (urethra and cervix) and blood from 22 individual Chlamydia patients (males and females), mean age 26 years, and 22 healthy subjects (males and females), mean age 25 years, were enrolled in this study. DNA samples from both cohorts of Chlamydia patients and healthy controls were amplified by PCR with commercial duplex TaqMan PCRs followed by sequencing of the *ompA* gene for genovar typing. Our results demonstrated the presence of CT DNA in the blood samples of 21/22 (95,4%) and in genital specimens of 19/22 (86,3%) of Chlamydia patients, respectively. There were CT genovars E (%), F (%) and G (%) in the blood and CT genovars D (%), E (%) and G (%) in genital samples. Based on the CT *ompA* single nucleotide polymorphisms (SNP), identical SNPs at positions 995 bp and 997 bp of the genovar E were found in the samples from both blood and genital sites. The data obtained from DNA testing were confirmed when each CT-positive blood and genital specimen was

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examined by slide technique and direct immune-fluorescent test with CT A-L serovar-specific monoclonal antibodies. No positive responses were seen in any specimen from healthy controls. This is the first study that examined the impact and efficiency of detection of CT DNA in blood samples of Chlamydia patients compared with their genital sites. This work was supported by the Russian Science Foundation (Project No. 17-16-01099).

P.28-025-Mon
Saliva extract of *Dermacentor marginatus* (Acari: Ixodidae) leads to apoptotic cell death

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Physiologically, biologically and morphologically distinct, about 900 different tick species have been reported worldwide. Some properties such as antioxidant, anticoagulant and anti-angiogenetic activities of tick-saliva have been shown in the studies conducted on some tick species. In this study, it was aimed to determine the effects of salivary gland extract of *Dermacentor marginatus,* tick species which has not been studied for such a purpose, on the cell culture in some aspects. For this purpose, adult male and female ticks were fed on the rabbits using ear bag method, and at the eighth day of feeding/in the fast engorgement phase, ticks were detached. Of those, the female ticks were weighed, washed with distilled water, and salivary glands were dissected and washed three times with phosphate-buffered saline. Total protein extraction was done in PBS via mechanical disruption and protein content was determined by Bradford's assay. Different doses of the extracts were added to MEFs and cell viability tested by MTT assay as well as microscopic examination. Apoptosis-related proteins (such as c-PARP, caspases) were determined by immunoblotting. As MTT assay is a marker of mitochondrial activity, oxygen consumption rates were determined by using Clark-type electrode in digitonin-permeabilized cells. As a result, cell viability was decreased in a dose and time-dependent manner. A significant PARP cleavage via caspase pathway induction was also found. Oxygen consumption rate of the cells treated with saliva extracts were found to be lower than the control group. After these initial findings, effects of tick saliva on different cancer cell lines examined. Our preliminary data evidenced that saliva of *D. marginatus* induces apoptotic cell death.

P.28-026-Tue
Optimization of aptamer-based impedimetric biosensor for detection of sarcosine

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Sarcosine, a natural amino acid, is an intermediate of glycine metabolism. Recent studies revealed the role of sarcosine in activation of prostate cancer. Therefore, sarcosine has been identified as a potential biomarker for monitoring the prostate cancer progression to metastasis. In this study, the interaction between sarcosine-specific DNA aptamer and sarcosine was monitored by electrochemical impedance spectroscopy (EIS). Specific DNA aptamer was selected using graphene oxide-assisted systemic evolution of ligands by exponential enrichment (GO-SELEX). Then, the aptamer was obtained in 5′-TTTTTT-thiol modified form. Before each measurement, the thiolated aptamer was treated with 10 mM Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) for 1 h at room temperature. The reduced aptamer was then dropped on a gold electrode. After incubation for certain time, the electrode was incubated with 6-mercapto-1-hexanol (6-MCH) for complete blocking of the electrode surface. For determination of optimum measurement conditions, different incubation times with different concentrations of aptamer and 6-MCH were tested. In addition, the effects of buffer type and pH were investigated. 17 h of aptamer incubation, 1 h of 6-MCH incubation, 1 μM of aptamer, and 1 mM of 6-MCH were identified as optimum conditions. The EIS measurements were recorded in 5 mL of 5 mM $[Fe(CN)6]^{3-/4-}$ in 0.01 M phosphate buffer (pH 7.5) under the following conditions: a potential of 0.223 V, the frequency range from 10 kHz to 100 mHz, and an amplitude of 0.01 V. A linear detection range between 5 pM and 500 nM of sarcosine was achieved. Preliminary results show that aptamer-based biosensor is promising an alternative method for simple, reliable, sensitive and specific detection of sarcosine in urine. The forthcoming experiments will focus on further characterization of biosensor and measurements in artificial urine samples. We thank the financial support of TUBITAK (grant number 215Z182).

P.28-027-Wed
The effect of vitamin d on ovarian inflammation in experimental polycystic ovarian syndrome in rats

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Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age. In addition to diagnostic criteria such as chronic anovulation, infertility, hyperandrogenism, hirsutism; it is associated with diseases such as insulin resistance, obesity, endothelial dysfunction and metabolic syndrome. In recent years, the relationship between PCOS-associated diseases and PCOS has been thought to be chronic inflammation and vitamin D deficiency. However, the results of studies investigating the association of vitamin D with PCOS are contradictory, and immunohistochemical studies have not been found to explain how the cytokines IL-1 beta IL-6 and TNF-alpha, which are thought to increase in the case of inflammation, affect the ovarian tissue. In this study, short-time and mid-term effects of vitamin D on IL-1β, IL-6 and TNF-α in the ovary were investigated in dehydroepiandrosterone-induced PCOS rat model. We investigated whether pre-administration (daily from 2 h before PCOS induction) and post-administration (DHEA injection daily for 20 consecutive days and 20 days after the first injection of DHEA, daily from 2 h before DHEA injection) of vitamin D could have effect dehydroepiandrosterone-induced PCOS rat model. Vitamin D has not been shown to have a significant effect on the IL-1 IL-6 and TNF-α markers in the short-term in the ovary, but it has been shown to reduce the number of cystic follicles. However, increased expression of IL-6, IL-1β and TNF-α was observed in the longer exposure group to DHEA.

P.28-028-Mon
The effect of sodium pentadecylsulfonate on the electrical conductivity of bilayer lipid membranes

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The effect of an anionic surfactant sodium pentadecylsulfonate (SPDS) on the conductivity of bilayer lipid membranes (BLM) was investigated. It's shown that in the presence of SPDS in a solution of NaCl and with the increase of its concentration the electrical conductivity of the BLM increases. In the life activity process, the continuity of the bilayer membrane can be violated with the formation of structural defects such as through hydrophilic pores. It is quite natural to expect changes in all functions of the cell membrane, including permeability and stability. Among the wide spectrum of factors, leading to loss of membrane stability chemically induced instability of membranes is important. The importance of this factor is associated with the fact that a cell membrane operates surrounded by large number of various low-molecular substances whose adsorption onto the membrane can lead to loss of its stability and change of its permeability. For this reason, studies of the influence of different chemical agents are important. SPDS is widely used as detergents, cleaning agents, emulsifier and wetting agent. The electric capacitance of BLM was measured by applying symmetric triangle voltage. From the analysis of CVC the following values of the specific capacity and specific conductivity of BLM were obtained in the absence of SPDS: $g_{sp} = (0.42 \pm 0.03)\cdot 10^{-7}\mathrm{Om}^{-1}\ \mathrm{cm}^{-2}$, $\mathrm{Csp} = 0.34 \pm 0.02\mu\mathrm{Fcm}^{-2}$. In the presence of SPDS at concentrations of 10^{-5} M the results were accordingly such: $g_{sp} = (0.86 \pm 0.02)\cdot 10^{-7}\mathrm{Om}^{-1}\ \mathrm{cm}^{-2}$; $\mathrm{Csp} = 0.45 \pm 0.04\ \mu\mathrm{Fcm}^{-2}$. Because of positive spontaneous curvature of the SPDS molecule the presence of SPDS leads to the loss of stability of the BLM and increase in conductivity, which is associated with the decrease in the value of the linear tension of pore edge in BLM. This changes in conductivity correlates well with the result that with increasing SPDS concentration the linear tension decreases.

P.28-029-Tue
Age-dependent expression of MSTN paralogs in muscles of brown trout (*Salmo trutta* L.) and Atlantic salmon (*Salmo salar* L.)

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Myostatin (MSTN), a member of TGF-β family, is a negative regulator of muscle growth and inhibits proliferation and differentiation of muscle cells in mammals. The mechanism of MSTN action on growth in fish probably differs from mammals. In salmonids, MSTN is presented by genes *MSTN1a, MSTN1b*, *MSTN2a* and *MSTN2b* (which is a pseudogene). It is known, that paralogs expression is tissue-and species-specific. The study was conducted to evaluate the age-dependent features of MSTN paralogs genes expression levels in parr of brown tout *Salmo trutta* L. and salmon *Salmo salar* L. The gene expression levels of *MSTN1a, MSTN1b* and *MSTN2a* along with myogenic regulatory factors (*MyoD1* paralogs, *myogenin*, *Myf5*) and myosin heavy chain (*MyHC*) were evaluated in white muscles of parr at ages 0+ (under-yearling), 1+ (yearling) and 2+ (two year old). According to analysis, in brown trout *MSTN-1a* and *MSTN-1b* mRNA level was higher in parr 1+ and 2+ in comparison with 0+. In salmon *MSTN-1a* and *MSTN-1b* mRNA level was peaked at 1+ parr. In contrast, expression level *MSTN-2a* was the highest at 0+ both in salmon and brown trout. The *MSTN-1a* and *MSTN-1b* expression level was high at 1+ along with *MyHC* and Myf5 expression level in brown trout. The high level of *MSTN-1a* and *MSTN-1b* expression in yearling salmon accompanied by *MyHC*, *MyoD1a/b* and *myogenin* expression. The high expression of *MSTN2a* and its decrease in 1+ group corresponded to increased expression *MyoD1c* mRNA levels both in trout and salmon. Our findings suggests that in the result of duplication MSTN gene there are functional differences between *MSTN*-1 and *MSTN*-2, that is shown by different expression pattern between age-groups and its correlation with MRFs. But no differences in functioning were found between *MSTN*-1a and *MSTN*-1b. The study was supported by the grant of the Russian Science Foundation № 14-24-00102.

P.28-030-Wed
Expression of myogenic regulatory factors in muscle of Atlantic salmon (*Salmo Salar* L.) fry reared under different photoperiod regimes

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Postnatal muscle growth in fish occurs by both hyperplasia and hypertrophy. The main role in myogenesis regulation belong to myogenic regulatory factors (MRF), the transcription factors of bHLH family: MyoD, Myf5, myogenin and MRF4. MRFs expression depends on various factors affected fish growth. Photoperiod, classified as directive factor, controls fish growth through its influence on endogenous rhythms and circulating levels of growth hormone. Expose to extended light photoperiod regimes has been shown to lead increased growth rate in different fish species. In spite of numerous studies there is not enough information about precise mechanisms of myogenesis regulation during light stimulated growth. The effects of two photoperiod regimes LD16:8 (16 h light:8 h dark, group (1) and LD24:0 (24 h light:0 h dark, group (2) on growth and MRFs expression in muscles of Atlantic salmon fry (0+) were investigated. The experiment was conducted in conditions of fish farm. The fishes were subjected to different photoperiods for 3 months (August–October). Salmon reared under light regime of fish farm were used as a control group. The gene expression of MRFs (*MyoD1b* and *myogenin*) and myosin heavy chain (*MyHC*) in white muscles were studied. Results revealed that the fry reared under long light periods (LD 24:0) grew faster and were bigger at the end of the experiment. There was no significant photoperiod effects on the survival rate. The significant differences in MRFs gene expression were revealed in October. Myogenin expression were higher in LD24:0 group. Moreover *MyHC* expression tended to be higher in group 2 in comparison to control group in October. It was suggested that long light period positively affected on duration and intensity of muscle growth processes in autumn, the season when fish growth rate generally decreased. The study was supported by the grant of the Russian Science Foundation № 14-24-00102.

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P.28-031-Mon
Neurotrophin 3 as a new bidirectional signaling molecule between lung inflammation and the diffuse neuroendocrine system

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Neurotrophin 3 (NT-3) was investigated and understood in its functionality in the central nervous system (CNS), but it is still insufficiently studied in the periphery. Neurotrophins can be active outside the CNS, on different cellular types. They participate in the genomic (slow) regulation and non-genomic (fast) regulation of cellular growth and survival. Immunohistochemistry tests performed on lung epithelium samples have proven the local expression of nerve growth factor, brain derived growth factor and NT-3. Inflammatory cytokines TNF-α and IL-1β stimulate the epithelial synthesis of neurotrophins, but the way in which these participate in the regulation of cell functions is still unknown. The aim of this work was to study the effects of lung inflammation on the whole organism. We worked on blood samples taken from patients with chronic obstructive pulmonary disease (COPD), using ELISA. The serum levels of IL-1β, TNF-α, IL-6, IL-8, IL-10 and NT-3 were determined. Our results demonstrated increased serum levels of all parameters, but only NT-3, IL-1β, TNF-α and IL-8 had statistically significant elevations. The presence of NT-3 in a greater concentration has a strong functional and informational correlation with the lung inflammation (it was demonstrated that TNF-α stimulates NT-3 synthesis as a result of the nerve endings aggression in the lungs and airways). Thus, NT-3 is responsible for stimulating cell differentiation and survival during hypoxia and also for the leukocyte activity amplification which increases inflammation and the immune response. This higher serum level of NT-3 facilitates the transduction of intero- and nociceptive information from the pulmonary tissue to the CNS and also the transduction of efferent signals to the lungs. Elevated NT-3 serum levels in COPD do not necessarily have regenerative effects on the lung parenchyma, as these effects depend on the cytokine and metabolic context of the NT-3 secretory cells.

P.28-032-Tue
A serpin from the red king crab *Paralithodes camtschaticus* inhibits R/K-specific peptidases in hosts hemocytes and plasma

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The red king crab, *P. camtschaticus*, is a large commercially important crustacean in Russia, Norway, and the US, with annual landings estimated in the range of tens of millions of kgs. Previously, from the *P. camtschaticus* we isolated a serpin (further serpin PC), which had an anticoagulant and an anticomplement effects on the human blood. Based on its action we hypothesized its role in host's immunity since these proteins were reported to be engaged in defense systems of other crustaceans, are highly homologous, and there is still a lack of valuable biochemical data on their exact role, partner peptidases, and specificity. Recombinant serpin PC's reactivity was previously tested towards various serine and cysteine proteases, and it was found that it inhibits a bovine cationic trypsin, the reaction proceeds through the characteristic serpin mechanism, with the unusual reaction site. After we proved its inhibitory function on the model partner peptidase, we decided to find whether it performs as the inhibitor in a host organism and clarify its function. Earlier we found that its highest level of transcription is in the hemocytes, and, due to the presence of the secretion signal, it can be secreted to the hemolymph. Thus, we performed a peptidase activity profiling of hemocytes and plasma with the recombinant serpin PC and other peptidase inhibitors, and found that it inhibits at least two R/K-specific activities. In arthropods, hemocyte serpins are known to regulate the melanization cascade, that involves a number of peptidases of aforementioned specificity, therefore we further assessed serpin PC's effect on its activation. We showed that it twice inhibits the induction of phenoloxidase activity, through which the melanization manifests. As a result, we approached further to the identification of serpin PC's native targets and function. The research was carried out within the framework of RSCF project No. 16-14-00191.

P.28-033-Wed
Inflammatory cytokines as prognosis factors in chronic lung inflammation

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Chronic obstructive pulmonary disease (COPD) is characterized by the progressive restriction of air flow through the lungs, determined by an excessive inflammatory response to the chronic inhalation of certain particles. The pathophysiology of COPD was the subject of numerous studies up until this point, but although we know the stages of inflammation in the small airways, lung tissue and pulmonary vascular walls during COPD, the mechanisms of the particular inflammatory response are not completely elucidated. The present study aims to evaluate the various inflammatory cytokines involved in the complex signaling pathways and systems that develop in COPD, both inside the lungs and between the lungs and the rest of the organism. Blood samples were obtained from patients with clinically confirmed COPD. ELISA was used to determine serum levels of interleukin 1 β (IL-1β), tumoral necrosis factor α (TNF-α), interleukin 6 (IL-6), interleukin 8 (IL-8) (as inflammatory markers) and interleukin 10 (IL-10), as the prototypical anti-inflammatory cytokine and marker of the adaptive response of the organism. The patients included in the study did not have any other active inflammatory processes in the organism when the blood samples were taken. We obtained significant increases in the serum levels of TNF-α, IL-1β and IL-8. Chronic evolution of inflammation with acute periods was demonstrated by increased serum levels of TNF-α. This cytokine also participates in neoplastic lung transformation and in muscle atrophy development. Thus, monitoring of TNF-α serum levels in patients with COPD may be a prevention method in lung cancer and cachexia. Increased serum levels of IL-1β and IL-8 may be considered markers for the aggravation of inflammation and for the permanent neutrophil lung infiltration and for the morphological reshaping by necrosis and apoptosis.

P.28-034-Mon
Development an amperometric microbial-enzyme hybrid cholesterol biosensor based on ionic liquid MWCNT carbon paste electrode

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In this study, an amperometric microbial-enzyme hybrid biosensor were constructed for cholesterol determination. Cholesterol metabolism induced and organic phase tolerant *Pseudomonas sp*. and catalase enzyme were used as a biocomponent of biosensor. *Pseudomonas sp*. and the catalase enzyme were immobilized into multi-wall carbon nanotube (MWCNT)/ionic liquid (IL)/carbon paste electrode (CPE). Immobilization steps of the biosensor were characterized by CV and impedance spectroscopy. Hydroquinone was used as the mediator system in the measurements. The amperometric working potential was found to be −0.2 V. Optimization and characterization studies were also carried out by amperometric method. The optimization of working conditions of microbial biosensor were carried out and optimum temperature and pH were found to be 35°C and 7.0 (Phosphate buffer, 50 mM). The effect of amounts of bacteria on the biosensor responses was investigated. The linear range of this microbial biosensor that developed for the determination of cholesterol was found to be 5–600 μM and the detection limit was found as 1.52 μM. The effect of steroid compounds such as cortisone, cortisol, testosterone, β-sitosterol and estradiol, and the presence of endogenous analytes such as glucose and ascorbic acid on the biosensor response was investigated in substrate specificity and interference effect experiments. After optimization and characterization studies of the microbial biosensor, the biosensor response to cholesterol in saturated cyclohexane was investigated and also responses obtained were compared with response obtained by water phase. Cholesterol determination was carried out both in cyclohexane and in water phase by developed microbial biosensor.

P.28-035-Tue
Cardiolipin containing membranes are more resistant to permeabilizing effect of surfactin

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Surfactin is a lipopeptidic antibiotic produced by *Bacillus subtilis*. Due to its amphiphilic nature, surfactin is able to enter lipidic membranes and interfere with their barrier function by forming conductive pores or even disrupting their integrity in detergent-like manner. Exact details of membrane disruption mechanism and producer's mechanism of self-resistance which it employs to avoid the effect of its own toxic product remain unknown. None of the notoriously used resistance proteins, such as ABC efflux pumps, was linked to surfactin so far. Only minor increase of yerP product was detected, however its deficiency had no effect on sensitivity to surfactin. Thus, the most feasible mechanism is target modification, which in this case is the cytoplasmic membrane. We have previously shown that *B. s.* significantly alters the proportions of its membrane lipids during surfactin production, main difference being the increased content of cardiolipin. Using carboxyfluorescein loaded liposomes resembling the membrane composition of *B. s.* adapted and not adapted to surfactin (ternary systems consisting of synthetic phosphatidylglycerol, phosphatidylethanolamine and cardiolipin in different ratios) we now show that the adapted membrane is less prone to surfactin-induced permeabilization. We further tested the contribution of individual phospholipid components of *B. s.* membrane on its stability towards surfactin action. We found that the presence of cardiolipin in liposome membrane profoundly decreases surfactin induced permeabilization. Membrane permeabilization assay with propidium iodide confirmed that cardiolipin deficient strain of *B. s.* is significantly more susceptible to antimicrobial effect of surfactin compared to wild type. We can thus conclude that cardiolipin is major determinant of membrane resistance against surfactin both in vitro and in vivo and that increased level of cardiolipin can play a key role in *B.s.* resistance against surfactin.

P.28-036-Wed
Larvicidal activity of secondary metabolites of medicinal plants against *Aedes aegypti* and determination of the mode of action based on DNA, acetylcholinesterase and mitochondrial respiratory chain

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Aedes aegypti is the principal vector of dengue, zika and chikungunya diseases. Due to the absence of a specific treatment or an effective vaccine, the control of this illnesses focusses on the elimination of the mosquito. In addition, many colonies have developed resistance to the most common insecticides such as organophosphorus and pyrethroids. Therefore, there is a need to discover new pesticides and many studies point at plants as source of new structures with insecticidal effect. In this study, we used bioinformatic tools to do a virtual screening and select seven secondary metabolites (of 33 reported in a previous study), using physical-chemical properties and the prediction of biological activity as selection criteria. Larvicidal activity on *Ae. aegypti* of these metabolites was assessed, calculating the lethal concentrations 50% (LC_{50}): 44, 41, 48, 84, 87, 98 and 183 ppm, for geranyl acetate, α-humulene, β-caryophyllene, geraniol, nerol, n-octanol and 1,8-cineol, respectively. The six molecules with LC_{50}< 100 ppm were tested on DNA of *Ae. aegypti* showing no-degradation or fragmentation effect. Additionally, these compounds were assessed on acetylcholinesterase and complexes of Mitochondrial Respiratory Chain (MRC). Four secondary metabolites showed striking inhibition on several targets. Geranyl acetate inhibited the acetylcholinesterase activity in 38%, α-humulene inhibited complexes II, III and IV of MRC (38, 35 and 30%, respectively), β-caryophyllene inhibited complexes II and III (30 and 36%, respectively) and geraniol inhibited complexes I and II (27 and 29%, respectively). In contrast, nerol and n-octanol did not show marked inhibition on the different targets tested. The larvicidal activity of geranyl acetate, geraniol, α-humulene and β-caryophyllene was greater than nerol and n-octanol, and it is related to the effect on the assessed targets. These compounds are potential active ingredients to the vector control.

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Immobilization stress affects the circadian rhythm of leydig cell endocrine function

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It is well known that immobilization stress (IMO) has ability to decrease Leydig cells steroidogenesis and serum testosterone level, but the effects of IMO on circadian rhythm of Leydig cells endocrine function are not completely clear. This study was designed to evaluate the effect of acute (3 h daily) and repeated (3 h daily for 10 consecutive days) IMO, applied at different times during the 24 h on circadian rhythm of testosterone secretion and expression of clock and steroidogenic genes in Leydig cells. The result showed that acute IMO changed transcription of some genes involved in steroidogenesis (decreased and canceled rhythm of *Cyp11a1* and *Cyp17a1* and decreased circadian pattern of *Star*) and some core clock genes (increased rhythm robustness and mesor of *Per1* and *Reverbα*) which is associated with decreased level and canceled circadian pattern of testosterone secretion. Ten time repeated IMO also decreased and flattened oscillatory pattern of testosterone secretion and changed rhythm of steroidogenic genes transcription (decreased rhythm of *Cyp17a1,* increased and initiated cyclic pattern of *Hsd3β1/2*). The transcription of clock genes were also deregulated (*Bmal1, Per1*, *Cry1* and *Cry2* decreased while *Reverbα* increased). However, comparing the IMO effects at different periods during the 24 h, it was found more pronounced IMO effect on the clock genes in the morning. Accordingly, presented data suggest severe effect of IMO on Leydig cell endocrine function.

P.28-038-Tue
Effect of the gold nanoparticles on the catalytic properties of the porcine pancreatic lipase

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Interactions of the enzymes with nanoparticles (NPs) are the subjects of intensive study. The formation of such complexes is promising for application in biomedical areas, including genomics, biosensorics, immunoassay, clinical diagnostics, etc. In our previous work we found the influence of silver nanoparticles on the catalytic activity of porcine pancreatic lipase (PPL). Our ongoing research is focused on the PPL interaction with colloidal gold. The PPL particles in salt solutions have sizes of about 4–5 nm that was measured by dynamic light scattering. Lipase is more prone to aggregation in water than in saline that is proved by an increase in the particles dimensions up to 18–30 nm. Gold nanoparticles (AuNP) were obtained from the gold (III) chloride hydrate and sodium borohydride at 0°C. The obtained sol was stable and the particle sizes were at about 12 nm. The adsorptive and diffuse layer of such particles consist of anions. The effect of the gold nanoparticles on the catalytic activity of the enzyme in solution was revealed. The activity of the lipase was measured by the potentiometric titration method: a) 10 mM NaOH was used as the titrant; b) as substrate a 50 mM triglycerol solution was used (2,182 ml triacetin dissolved in 100 ml 50 mM of NaCl solution and 100 ml 50 mM of $CaCl_2$ solution); c) PLL was dissolved in the same solution. The average PPL activity in the presence of AuNPs was lower by 9% (after 30 min) as compared to the pure lipase. In contrast, PPL-AuNP system retained 74% activity, while pure PPL – only 49% (after 7 days of storage solutions at +4°C). Thus, AuNPs stabilized PPL and prolongs the enzyme activity as compared to the pure lipase. The work was supported by the Russian Foundation for Basic Research (grant 15-29-01193).

P.28-039-Wed
The effect of anti- and prooxidants on methemoglobin formation in neurodegenerative diseases

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Methemoglobin formation was examined in erythrocytes of 25 patients with Parkinson's disease (PD) (stage 3–4 by the Hoehn and Yahr scale). The patients receiving levodopa-containing drugs (madopar, nakom) were also treated with intramuscular injections of mexidol (daily dose 100 mg/day) for 14 days. Control group included 12 clinically healthy persons. The erythrocyte methemoglobin content was determined by spectrophotometric method (λ = 630 nm) and electron paramagnetic resonance (EPR) using the EPR signal intensity with the g-factor 6.0. The methemoglobin content was significantly higher in erythrocytes of PD patients than in healthy donors. The complex therapy with mexidol normalized the methemoglobin content in erythrocytes of PD patients. Using electron paramagnetic resonance, the dose-dependence effect of dopamine on methemoglobin formation in erythrocytes of patients with Parkinson's disease under the activation of oxidative stress induced by acrolein and the possibilities for the correction of this pathological process using antioxidant in vitro experiments have been examined. It was shown that incubation of erythrocytes with 1.5 mM dopamine did not change the methemoglobin content, while incubation with 15 mM dopamine caused a two-fold increase in the methemoglobin content compared to its initial level; 10 μM acrolein increased methemoglobin formation threefold. Administration of 15 mM dopamine and, after 1 h, 10 μM acrolein to the incubation system increased methemoglobin formation tenfold compared to its initial level. Preincubation of erythrocytes with carnosine followed by acrolein addition prevented the increase in the methemoglobin content, while carnosine had no effect on methemoglobin formation induced by dopamine. Preincubation of erythrocytes with polyamines – spermine and spermidine followed by acrolein and dopamine addition prevented the increase in the methemoglobin content.

P.28-040-Mon
Effects of mutations mimicking phosphorylation of myosin essential light chain on the actin-myosin interaction in optical trap assay

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It is known that myosin essential light chain (ELC) can be phosphorylated at two sites, Thr68 and Ser194, in skeletal and cardiac muscles. However, till now it is unknown how the ELC phosphorylation can affect the molecular mechanism of muscle contraction. In the present work, we tried to elucidate how the ELC phosphorylation affect the mechanical properties of the myosin motor, i.e., the isolated myosin head (myosin subfragment 1, S1). For this purpose, we produced, using site-directed mutagenesis, three recombinant ELC constructs with mutations T65D, S193D, and S193D/T65D, which mimic the naturally occurring ELC phosphorylation, and these ELC mutant constructs were then introduced into the S1 molecule. We applied an optical trap technique to investigate the effects of these ELC mutations on the mechanical characteristics of the S1 interaction with actin filament. This method makes it possible to measure both step size of a single S1 molecule during its interaction with actin filament and duration of the step. It has been shown that all these ELC mutations have no appreciable influence on the step size of S1 (from 8 to 11 nm). Mutations T65D and S193D/T65D significantly decreased the duration of the S1 step, by 30%, from 26–28 ms to 19 ms, whereas the ELC mutation S193D had no effect on the step duration. This implies that mutation T65D, but not S193D, can affect the lifetime of the S1-actin complex during the ATPase cycle, thus making faster the ATP-dependent interaction of the myosin head with actin filament. These results indicate for the first time that ELC phosphorylation at Thr65 may affect the kinetic characteristics of the interaction of the myosin heads with actin filament in the absence of load and may play an important role in actin-myosin interaction during muscle contraction. This work was supported by the Russian Science Foundation (grant 16-14-10044).

Biotechnology

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Novel cucumovirus as a candidate for plant biotechnology applications

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The Baltic isolate of Gayfeather mild mottle virus (GfMMV), belonging to the genus *Cucumovirus*, family *Bromoviridae*, was isolated from *Liatris spicata* plant and characterized earlier by one of the NGS methods (454 pyrosequencing of total RNA, Adams *et al*., 2009). This approach is intended for rapid sequence determination without purification of virions, especially in cases of mixed infections. Unfortunately, it does not allow sequencing of satellite RNAs that are characteristic for this viral group and significantly affect the development of infection; analysis of encapsulated subgenomic RNA(s) is not possible as well. In our laboratory three genomic RNA sequences of a new Moscow isolate (GfMMV-Mo) discovered in *Arctium lappa* were defined by conventional Sanger method. Sequences of all terminal genome segments had noticeable discrepancies with the data published in the GenBank. Exact determination of the 5′-proximal and 3′-proximal parts of the genome is necessary for cloning cDNA copies of genomic RNAs. Comparison of the gene sequences responsible for replication revealed the high level of homology (99.1% to 99.5%). Phylogenetic analysis demonstrated an early divergence of GfMMV from other *Cucumovirus* group members. Moscow isolate was capable of establishing systemic infections in *Nicotiana clevelandii*, *N. tabacum* and *N. benthamiana*; the last two species are widely used in "green" biotechnology. Infection of Baltic isolate in *N. tabacum* was limited to inoculated leaves (Adams *et al*., 2009). GfMMV-Mo is easily transmissible by mechanical inoculation and accumulation level of coat protein in *Nicotiana spp*. was about 3–4 g/kg of fresh upper leaves. Thus, GfMMV-Mo is a promising object for plant biotechnological applications such as constructing a viral vector(s) designed for expression of target protein in plants as well as assembly of genetically or chemically modified icosahedral nanoparticles or nanocontainers.

[Correction added on 14 September 2018, after online publication: abstract P.07-063-Wed has been added in this version.]

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