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O.P.1.
Structural biology and Bioinformatics

Structure and functioning of copper chaperones

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Copper chaperones compose a specific class of proteins assuring safe handling and specific delivery of potentially harmful copper ions to variety of essential copper proteins like Cu-ATP-ases, Cu,Zn-superoxide dismutase and cytochrome c oxidase. Metallation of Cu-ATP-ases is performed with copper efflux chaperone Atx1 (yeast) or Hah1 (human). Cu,Zn-SOD is metallated with copper chaperone for SOD; Ccs (1). Metallation of cytochrome c oxidase (CCO) is apparently the most complicated task of copper delivery as it requires highest number of assisting proteins, such as Cox11, Cox17, Sco1, Sco2, Cox19 and Cox23. Copper chaperones compose structurally heterogeneous class of proteins, which can exist in multiple metal-loaded as well as oligomeric forms. By using NMR we have resolved 3D structures of Cox17 and Sco1 protein, which are folded into coiled-coil helix coiled-coil helix and thioredoxine-like folds respectively. Moreover, many copper chaperones (Ccs, Cox17, Sco1) exist in various oxidative states and participate in redox catalysis, connected with their functioning. It is demonstrated that Cox 17 can reduce oxidized Sco1 protein as well as Sco1 can reduce oxidized CuA site in CCO (2). Analysis of the metal-binding properties of copper chaperones and their partners allowed us to establish affinity gradients determining copper transport in the cell (3).

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O.P.2.
Biotechnology

Application possibilities of yeast-expressed virus-like particles derived from polyomaviruses

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Virus-like particles (VLPs) are complex molecules comprised of multiple copies of one or more viral structural proteins. They mimick infectious viruses in their structural and immunological features but are non-infectious and highly safe because of the lack of a viral genome. Polyomaviruses (PyVs) are small, non-enveloped icosahedral viruses. Major capsid protein VP1 of PyVs assembles into VLPs which are used in diagnostics, show great potential in vaccine development and other biomedical purposes. Hamster polyomavirus (HaPyV) VP1-derived VLPs were used not only for basic research applications, i.e. in structural and assembly studies, but VP1-based VLPs showed great potential as epitope display system for the presentation of foreign antigens. It was demonstrated that yeast-expressed chimeric HaPyV VP1-derived VLPs harbouring inserts of different origin from 9 to 120 amino acids long induced a strong insert-specific humoral response without any adjuvant and represented a useful tool for generating monoclonal antibodies with desired specificity. HaPyV VP1-based VLPs were able to trigger the maturation of human dendritic cells, IL-12 production and stimulation of CD8+ T cells. In addition, using model GP33 CTL epitope derived from murine Lymphocytic choriomeningitis virus inserted into HaPyV VP1 protein it was shown that chimeric VLPs were efficiently processed in antigen presenting cells and were capable to induce insert-specific CD8+ T cell proliferation and CTL response in mice. Moreover, HaPyV-derived VLPs were used as a carrier for functionally active complex molecules such as single-chain antibody fragment (scFv-Fc). It was shown that scFv-Fc fused with VP2 protein and presented on pseudotype VP1/VP2 VLPs was functionally active: it neutralized its target vaginolysin by assay as efficiently as the parental monoclonal antibody. The exploitation of VP2 protein fused with large (370-472 aa long) protein as a subunit of VLPs represents the new way to obtain correctly folded and functionally active complex proteins expressed on the surface of pseudotype VP1/VP2 VLPs.

O.P.3.
Biotechnology

Biotechnologically relevant prokaryotes: search and identification using molecular techniques

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An important focus of our current research is the search for biotechnologically relevant prokaryotes in the unique environments - in the caves. Caves are considered to be extreme environments for life. The increased interest in microorganisms of the caves is associated with the potential of these new, unique microorganisms to produce novel biotechnologically relevant products. Enrichment-based and cultural studies have identified 34 novel species of the phylum *Actinobacteria* in the caves from 2000 to 2009. Culture-independent, molecular techniques have also shown that many novel organisms can be found in the caves. We used barcoded pyrosequencing of 16S rRNA genes for analysis of bacterial diversity in the KarabiJaila caves, the Crimea. Two different caves were investigated – the frequently visited cave Venta and the rarely visited one Radzievsky. We have found that 13.82 % of 16S rRNA gene

sequences from the cave Venta belonged to novel previously unclassified bacteria. The portion of 16S rRNA gene sequences of unclassified bacteria was considerably higher in the unexplored branches of the caves - even 46.34 % of all sequences.

The other focus of our research work is the development of identification and genotyping methods for the biotechnologically relevant prokaryotes. Geobacilli constitute an excellent resource of biotechnologically important products. These bacteria are also associated with heat-treated foods, they can cause food spoilage. Identification of these species by traditional biochemical techniques is imprecise and time consuming. We have successfully applied restriction analysis of 16S rRNA gene, sequence and restriction analysis of 16S-23S rRNA internal transcribed spacers, sequence analysis of *spo0A* gene as well as repetitive DNA of these biotechnologically important bacteria for the development of identification and genotyping methods. It should be noted that our methods have allowed us to reveal the previous misidentifications of geobacilli.

O.P.4.

Knowledge-based design of enzyme functional properties

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Understanding how changes in protein structure affect biological function is of great importance for both fundamental enzymology and biocatalysis. During evolution of proteins from a common ancestor one functional property can be preserved while others can change leading to functional diversity. Homologous enzymes within a family or superfamily can be divided into subfamilies with different functional properties. New method of bioinformatic analysis has been developed [1-3] to identify subfamily-specific positions – conserved only within protein subfamilies, but different between subfamilies – that seem to play important role in discrimination of substrate specificity, enantioselectivity, catalytic activity, stability, etc. The developed method was used to study evolution of structure-function relationship in several enzyme families: Ntn-hydrolases, penicillin-binding proteins, α/β-hydrolases. Subfamily-specific positions of corresponding enzyme families were identified and used as hotspots for mutations to improve functional properties of wild type enzymes: increase stability of penicillin acylase from *Escherichia coli* in alkaline medium, expand substrate specificity of D-aminopeptidase from *Ochrobactrum anthropi* and introduce amidase activity into *Candida antarctica* lipase B. Molecular modeling was applied to evaluate influence of subfamily-specific substitutions on stability as well as catalytic properties of *in silico* constructed mutants and to select the most promising variants. Experimentally produced mutants of penicillin acylase, D-aminopeptidase and lipase B demonstrated significantly improved functional properties [4,5]. Bioinformatic analysis of subfamily-specific positions can be used as a systematic tool to study structure-function relationship in enzymes, characterize and rank their binding sites [6]. Selection of subfamily-specific positions as hotspots for mutation can be recommended to rationalize different protein engineering approaches and design enzymes with requested functional properties.

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O.P.5.
Biotechnology

Novel mechanisms of site-specific endonucleases

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Since their discovery in the 1970s, the type II restriction endonucleases (REases) became the 'work horses' of modern molecular biology. The practical value of REases for DNA manipulations has prompted extensive searches for new enzymes of this type, and over 4000 REases have been identified. For a long time it was believed that most restriction enzymes are homodimeric, Mg^{2+}-dependent enzymes that symmetrically interact and cleave short (4-8 bp) palindromic DNA target sites. However, the research conducted during the past decade revealed that such 'orthodox' REases comprise only a minor fraction of all Type II enzymes. Indeed, REases vary greatly in their oligomeric structure and domain organisation, and employ at least five evolutionary unrelated domains to accomplish DNA cleavage. Restriction endonuclease BfiI from *Bacillus firmus* is 'unorthodox' in every possible way: it recognises and cleaves a non-palindromic DNA sequence 5'-ACTGGG-3', binds two copies of cognate DNA, and, most strikingly, cleaves DNA in the absence of Mg^{2+} ions. Biochemical and structural studies of BfiI revealed that it forms a dimer with a single catalytic site at the subunit interface, which resembles enzymes of the phospholipase D (PLD) superfamily. Uniquely among site-

specific endonucleases, BfiI employs a two step reaction mechanism: it first makes a covalent enzyme–DNA intermediate, and then cleaves it by a nucleophilic attack of water. Surprisingly, the covalent BfiI-DNA intermediate can also be resolved by other nucleophiles, such as alcohols, nucleosides and 3'-termini of other DNA molecules, yielding various DNA transesterification products. To cleave double-stranded DNA, BfiI must use its single catalytic center sequentially, first on the bottom, and then on the top DNA strand. This novel DNA cleavage mechanism requires a single active site to not only move between strands, but also to switch its orientation on the DNA.

O.P.6.

CRISPR/CAS9 system application for mammalian genome editing

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CRISPR/Cas system, part of anti-viral defense mechanism used by bacteria, has generated a surge of interest over the past couple of years due to its unprecedented flexibility and versatility in targeted genome modification already proven to work within a wide variety of cell types and multicellular organisms. Majority of studies so far have investigated Cas9 activity by delivering protein and guide RNA encoded in DNA vectors. Here we present an alternative approach and transfect mammalian cells with *in vitro* pre-assembled Cas9-crRNA-tracrRNA complexes. We demonstrate Cas9-RNA complex activity on integrated dual reporter (GFP-RFP) gene as well as endogenous genes PPIB, PVALB, DNMT3B and state the potential that pre-assembled complexes can be successfully used for genome modulation in mammalian cells.

O.P.7.
Biotechnology

Immunogenic and antigenic properties of recombinant virus-like particles

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Protein engineering provides opportunities to generate new antigens with desired features. Virus-like particles (VLPs) generated by the heterologous expression of viral structural proteins mimic native viruses and therefore can be exploited as potential vaccines or diagnostic tools. Our studies demonstrate that yeast-expressed VLPs of hamster polyomavirus-derived VP1 protein are highly immunogenic and induce in mice strong B- and T-cell responses. Chimeric VLPs harboring foreign sequences at certain surface-exposed positions have been successfully used for generation of monoclonal antibodies against selected sequences of non-immunogenic viral proteins. The immunogenicity of inserted peptides is enhanced due to the repetitive multimeric structure of chimeric VLPs. Yeast-expressed recombinant proteins also represent safe and easily produced alternative antigens for use in routine serologic diagnosis of viral infections. We have successfully used the recombinant antigens and virus-specific monoclonal antibodies as components of immunodiagnostic assays for paramyxovirus and hantavirus infections. The reactivity of virus-specific serum antibodies with recombinant viral antigens confirms their proper antigenic structure and allows development of VLP-based serologic assays.

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O.P.8.
Biochemistry of Prokaryotes

Is Gardnerella vaginalis the pathogen responsible for the initiation of bacterial vaginosis?

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Gardnerella vaginalis was recovered in vaginal fluid in close to 100% of women with clinically diagnosed bacterial vaginosis (BV). BV is the leading vaginal disorder globally characterized by the displacement of commensal vaginal lactobacilli and the over-growth of mixed anaerobes. Despite clinical manifestations (abundant malodorous vaginal discharge) BV is linked with infertility, adverse pregnancy outcomes, postsurgery infections and may increase the risk of acquiring sexually transmitted diseases. The debating questions concern *G. vaginalis* role favoring increased growth of host-anaerobes along with suppression of lactobacilli and whether *G. vaginalis* is a part of normal vaginal flora. The association of *G.vaginalis* with different clinical phenotypes could be explained by different cytotoxicity of the strains, presumably based on disparate gene content. The contribution of horizontal gene transfer to shaping the genomes of *G. vaginalis* is acknowledged. We have isolated 17 *G. vaginalis* strains from the BV patients. A half of the analyzed *G. vaginalis* isolates contained CRISPR/Cas system. The origin of the spacers in the CRISPR locus raises the hypothesis that the transfer of the genetic material among *G. vaginalis* strains could be regulated by the CRISPR/Cas mechanism. *G. vaginalis* is equipped with a number of virulence properties (toxin vaginolysin, sialidase; capacity to adhere to vaginal cells and to form biofilms) promoting adherence to host epithelium and further invading of the host. We determined VLY production level by different *G. vaginalis* clinical isolates cultivated *in vitro*. A great diversity of the level of VLY may be related to a

different cytotoxicity of the strains. The disparate production level of sialidase and different thickness of *G. vaginalis* biofilms varied among isolates. In conclusion, *G. vaginalis* strains isolated from BV patients revealed genetic and biochemical diversity presumably reflecting the severity of manifestations of the disease.

O.P.9.

Toxin antitoxin systems in bacterial pathogens: battle ready weapons

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Toxin-antitoxin systems are small genetic modules vastly spread throughout bacterial genomes. TA systems are composed of a toxic protein and its antidote that prevents the toxicity. System can be activated upon antitoxin degradation, which leads to bacterial growth inhibition or death. Due to their addictive nature TA systems stabilize mobile genetic elements and ensure their spread. When settled in chromosome TAs seem to gain new functions important to bacterial stress response and survival. Important bacterial pathogens are remarkably armed with TA systems and it rises the question of their importance in pathogenicity. We have shown the importance of TA systems that accompany antibiotic resistance genes in mobile genetic elements of an important nosocomial pathogen *Acinetobacte rbaumannii*. Moreover we have found novel TA systems that effectively inhibit bacterial growth. We expect that studying novel toxicities will reveal the weak spots of bacteria and will be used in creating new antibacterial strategies.

O.P.10.
Biochemistry of Prokaryotes

Biosynthesis of wyosine derivatives in tRNAPhe of Archaea: role of a remarkable bifunctional tRNAPhe:m1G/imG2 methyltransferase

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The presence of tricyclic wyosine derivatives 3'-adjacent to anticodon is a hallmark of $tRNA^{Phe}$ in eukaryotes and archaea. In yeast *Saccharomyces cerevisiae*, formation of wybutosine (yW) results from five enzymes acting in a strict sequential order. In archaea, the intermediate compound imG-14 (4-demethylwyosine) is a target of three different enzymes, leading to the formation of distinct wyosine derivatives (yW-86, imG, and imG2). Based on our previous experimental data (de Crecy-Lagard et.al.Mol.Biol.Evol., 2010) and present comparative genomics analysis, we predicted the existence of a peculiar methyltransferase displaying a dual-specificity (aTrm5a) in several archaeal species. Combining a TLC and HPLC/MS analysis, we confirmed that Trm5a enzyme of *Pyrococcusa byssi* catalyzes two distinct reactions: N^1-methylation of guanosine and C^7-methylation of imG-14, whose function is to allow the production of isowyosine (imG2), an intermediate of the 7-methylwyosine (mimG) biosynthetic pathway. Based on the formation of mesomeric forms of imG-14, a rationale for such dual enzymatic activities is proposed. This bifunctional tRNA: m^1G/imG2 methyltransferase, acting on two chemically distinct guanosine derivatives located at the same position of $tRNA^{Phe}$, is unique to certain archaea and has no homologues in eukaryotes. This enzyme here referred to as Taw22, probably played an important role in the emergence of the multistep biosynthetic pathway of wyosine derivatives in archaea and eukaryotes.

O.P.11.
Molecular biology and Functional genomics

Catalytic plasticity of DNA methyltransferases

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Methyltransferases (MTases) catalyze highly specific methyl group transfers from the ubiquitous cofactor S-adenosyl-L-methionine (SAM) to a multitude of biological targets in the cell. Despite the importance of the transmethylation reactions in biology, the naturally transferred methyl group has limited utility for practical applications. We have redesigned the methyltransferase reaction for targeted transfer of larger chemical groups [1] by rational engineering of the catalytic center or a target recognition domain [2,3] and employing synthetic SAM analogs. In these analogs, the sulfonium-bound methyl group is replaced by an extended side chain that typically carries a transferable propargyllic moiety, a linear linker and a terminal reporter/functional group [3,4]. The new approach, named mTAG (methyltransferase-directed Transfer of Activated Groups), provides a new enabling tool for precise, targeted functionalization and labeling of large natural DNAs and RNAs [5-7]. More recently, we discovered novel atypical reactions of the cytosine-5 MTases with non-cofactor-like compounds (aliphatic aldehydes, thiols, selenols) leading to enzymatic exchange or modification of 5-hydromethyl or 5-carboxyl groups on target cytosines in DNA [8-10]. 5-hydroxymethylcytosine and 5-carboxycytosine have recently been identified in genomic DNA from the human brain and mice ES cells and is implicated in new yet unknown epigenetic regulatory mechanisms [11]. Our studies provide new insights into the catalytic versatility of MTase enzymes

and offer new molecular tools for a variety of *in vitro*, *in situ* and single molecule applications that require targeted covalent deposition of functional and reporter groups on DNA or RNA.

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O.P.12.
Emerging techniques and new methods in biochemistry

Optical mapping and transmural microelectrode recordings for investigation of excitation propagation and arrhythmogenesis in the heart

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Background: Coincidence between optical and electrical action potential (AP) is moderate, when the near-infrared (NIR) dye is used, because of optical signal blurring in the tissue. **Objective:** Combination of optical and electrical methods enables: (1) more precisely evaluate the shape of optical AP (OAP) upstroke, (2) uncover disturbances in the pathological situations.
Methods: Simultaneous recordings of optical (with di-4-ANBDQBS dye) and of electrical (with microelectrode) signals were assessed at a various pacing and frequency or during ischemia on Langendorff-perfused rabbit heart.
Results: We present a new experimental approach, which allows under the real experimental conditions, but not with a mathematical modeling, firstly, to split optical signal in two directions (transmurally and parallel to epicardium). Accordingly, calculate both components that form OAP upstroke, i.e. depth-weighting and lateral-scattering. Transmural-AP recordings with microelectrode have enabled to disjoin and to evaluate separately impacts of electrical and optical (of dye/tissue) components on OAP upstroke shape formation. Secondly, we demonstrate that only transmural-APs allows to evaluate clearly the ongoing processes during the pathological events (i.e. ischemia, arrhythmias, etc.), when the amplitude and/or duration of APs at different depths in LV wall can become irregular. While, OAP sums up the signals of unequal amplitude/duration achieved from various depths of the LV wall. Accordingly, some information could be lost, when OAP signals analyzed alone.
Conclusions: (1) In order to evaluate the influence of scattering, induced with NIR dye, on optical upstroke formation, primarily, the electrical impact on OAP upstroke must be detected. (2) Development of unequal AP amplitude/duration during ischemia progressed dissimilarly in LV wall, and in the deep layers a conduction block has been recorded.
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O.P.13.

Electrochemical monitoring of enzymatic reactions

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Brief review of the application of electrochemical methods in the investigation of enzyme action in heterogeneous systems will be presented. Electrochemical monitoring of the product of enzymatic reaction will be described as well as application of soluble and insoluble mediators. Direct electron transport between active center of enzyme and surface of the electrode will be analyzed. Action of biosensors and bioreactors based on oxidases, PQQ-dehydrogenases and some hydrolases will be presented.

O.P.14.
Cancer Cell Biology and Biomedicine

Electrical and metabolic communication between laryngeal carcinoma cells through tunneling nanotubes

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Adjacent cells communicate with each other via gap junctions and synaptic junctions. In contrast, communication between remote cells is usually mediated by secreted signaling molecules or exosomes. Tunneling nanotubes and epithelial bridges are recently discovered new forms of intercellular communication between remote cells allowing their electrical synchronization, transfer of second messengers and even membrane vesicles and organelles. In the present study, we demonstrate for the first time in primary cell cultures prepared from human laryngeal squamous cell carcinoma (LSCC) samples that these cells communicate with each other over long distances (up to 1 mm) through membranous tunneling tubes (TTs), which can be open-ended or contain functional gap junctions. In LSCC tissue and cell culture, we identified three types of connexins, Cx43, Cx26 and Cx30, among which only Cx43 formed functional gap junctions between adjacent and connected through TTs cells. We found two types of TTs, containing F-actin alone or F-actin and α-tubulin. In the LSCC cell culture, we identified 5 modes of TT formation and performed quantitative assessment of their electrical properties and permeability to fluorescent dyes of different molecular weight and charge, Alexa Fluor-350 (326; -1), Lucifer Yellow (443; -2), DAPI (279; +2) and Alexa Fluor-488

3000 dextrane (3000; -1). We show that TTs, containing F-actin and α-tubulin, transport mitochondria and accommodate small DAPI-positive vesicles suggesting possible transfer of genetic material through TTs. We advocated this hypothesis by demonstrating that even TTs, containing gap junctions, were capable of transmitting double-stranded small interfering RNA. To support the idea that the phenomenon of TTs is not only typical of cell cultures, we have examined microsections of samples obtained from human LSCC tissues and identified intercellular structures similar to those found in the primary LSCC cell culture.

O.P.15.
Emerging techniques and new methods in biochemistry

Silicon-rhodamine - a far-red fluorophore for cellular structures imaging

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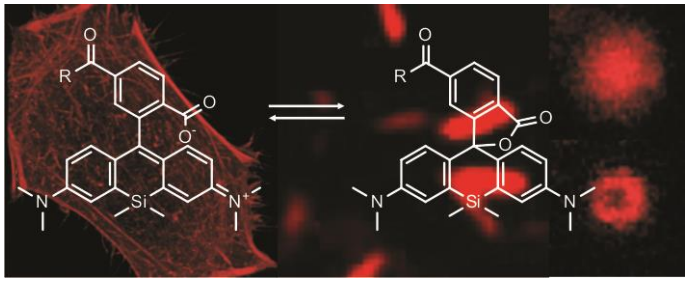
The ideal fluorescent probe for bioimaging is bright, absorbs at long wavelengths and can be flexibly implemented in living cells and in vivo. However, the design of synthetic fluorophores that combine all of these properties has proven to be extremely difficult. I will introduce a biocompatible near-infrared silicon-rhodamine probe that can be specifically coupled to proteins using different labeling techniques. Importantly, its high permeability and fluorogenic character permit imaging of proteins in living cells and tissues, while its brightness and photostability make it ideally suited for live-cell superresolution microscopy. The excellent spectroscopic properties of the probe combined with its ease of use in live-cell applications make it a powerful new tool for bioimaging[1-3].



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O.P.16.
Emerging techniques and new methods in biochemistry

How to make a blood vessel: insight from zebrafish

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Blood vessel development is associated with multiple vascular diseases in humans such as vascular malformations, diabetic retinopathy and cancer. However, molecular mechanisms that regulate vascular development are poorly understood. Zebrafish has recently emerged as an advantageous model system to study blood vessel formation. Transparent embryos are easily accessible for observations and experimental manipulations. We have previously demonstrated that an evolutionarily conserved transcription factor Etv2/Etsrp functions as a master regulator of early endothelial differentiation, vasculogenesis in zebrafish embryos. However, its later role in vascular development is not known. Here we show that a related ETS factor Fli1b functions partially redundantly with Etv2 and has a critical function in vasculogenesis and angiogenesis during later developmental stages in zebrafish embryos. While *etv2* mutants exhibit significant recovery of vasculogenesis and angiogenesis during later stages, *etv2* and *fli1b* double knockdown embryos fail to form angiogenic sprouts. This combined effect is associated with a greatly reduced number of vascular endothelial cells that fail to differentiate and an expanded zone of apoptosis throughout the axial vasculature. Overexpression analyses indicate that both *etv2* and *fli1b* induce the expression of multiple vascular markers and of each other. Temporal inhibition of Etv2 function using photoactivatable morpholinos suggests that the later function of Etv2 during angiogenesis is independent from its early requirement during vasculogenesis stages. These findings argue that *fli1b* and *etv2* function together as critical regulators of endothelial differentiation during both vasculogenesis and angiogenesis. We further demonstrate that Etv2 function is critical during lymphatic development since lymphatic vasculature fails to form upon conditional inhibition of Etv2 function. Furthermore, Etv2 expression is associated with tumor angiogenesis in a zebrafish tumor xenograft model. These results suggest that inhibition of Etv2 may present a novel approach to inhibit pathological angiogenesis including tumor angiogenesis.

O.P.17.

Surface-immobilized phospholipid bilayers for studies of protein interactions with membranes

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Immobilized (tethered) phospholipid bilayers allow localization of membrane associated proteins and peptides and application of surface sensitive techniques such as surface plasmon resonance, electrochemical impedance spectroscopies, neutron reflectometry for structural and functional studies. In addition to providing experimental

basis for the study of cellular/membrane functions (e.g. membrane fusion, ion transport, signal transduction), immobilized bilayers are promising platforms for biosensor applications. Developed in our laboratory bilayer systems are simple, yet versatile: comprised only of three components - a synthetic molecular tether, that provides the membrane anchoring to the substrate, a molecular backfiller ensuring enough submembrane space for the functional reconstitution of transmembrane proteins, and a phospholipid/sterol mixture. The formation of the surface immobilized system occurs spontaneously via self-assembly. Neutron reflectometry attests for the structural integrity and presence of water-filled reservoir between bilayer and solid support. Even though a simple premixing of lipids may provide quite wide variety of membrane compositions, several techniques allowing post-formation modifications were developed. In particular, the vesicle fusion, and methyl-β-cyclodextrin complexes of sterols can be used to vary composition of bilayers, as well as creating compositional asymmetry of the phospholipid sheets.

An electrochemical impedance spectroscopy proved to be one of the most versatile techniques for qualitative functional characterization of immobilized bilayers. However, lack of analytical solutions for spectral response precluded utilization of this method for quantitative analysis of the bilayers. We developed mathematical solution for the radially symmetrical defects, which allows estimation of the defect density, cluster formation as well as other structural parameters of the membranes. Such mathematical framework can be used in practice for detection of membrane damaging proteins such as pore-forming and other toxins.

O.P.18.

Targeted Genome Editing using CompoZr ZFN and CRISPR Technology

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The development of engineered nucleases for targeted genome editing have had a dramatic influence on Life Science research. Especially the recent discovery of the type II prokaryotic CRISPR/Cas immune system, that allows for RNA guided genome editing, have gained particular interest due to its simplicity and low cost profile. Sigma Aldrich have developed a number of options for researchers interested in the CRISPR/Cas technology, including a single vector system with a Cas9-GFP and a U6-guideRNA expression cassette for CRISPR/Cas9 delivery and expression, as well as a paired Nickases approach. The Sigma CRISPR/Cas systems along with our CompoZr ZFN technology provide alternative solutions for targeted genome editing in a wide variety of species and cell types. This presentation highlights how both technologies can be utilised to generate precisely targeted genome edits.

O.P.19.

Transcriptomics, lipidomics and fluxomics, reveal unexpected relations between cancer and lipids metabolism.

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De novo synthesis of lipids has been known for a long time, to be necessary for cancer proliferation. Many tumors show up-regulation of FAS I (cytosolic fatty-acid synthase). By using a genome-scale human metabolic model and gene expression data from the NCI-60 panel of cancer cell lines, we have identified that cytosolic synthesis of fatty acids, coupled to their transport into the mitochondria by the carnitine shuttle and mitochondrial β-oxidation, forms a cycle that shuttles cytosolic reductive power (in form of NADPH) into the mitochondria. The redox equivalents transferred into the mitochondria by this mechanism seem to play a key role in energy generation and cell proliferation. It was also found that the activity of this mechanism correlates negatively with the survival of colon cancer patients. Measurements of the lipid composition of lung tumors are also consistent with this mechanism.

Lipid transport and degradation could constitute a new potential therapeutic window for the treatment of cancer.

O.P.20.
Cancer Cell Biology and Biomedicine

Epigenetic and Genetic Profile of Stemness and Cancer

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Stem cells are characterized by increased expression of pluripotency factors *OCT4*, *SOX2,* and *NANOG*. Cancer cells also possess some features of stemness. Activation of stem cell-like transcriptional program usually relates with more aggressive forms of cancer and resistance to chemotherapy. For identification of common features of stem cell and cancer, gene and miRNA expression was profiled in human mesenchymal stem cells (MSCs) of different origin and compared to expression profile of cancer cells. DNA methylation and hydroxymethylation marks of stemness genes were also analysed.

Genome-wide DNA methylation, gene and miRNA expressionprofiling was performed on Agilent microarrays. Expression of 399 genes and 12 miRNAs were significantly down-regulated during induced stem cell differentiation. A set of these stemness-related genes was over-expressed in primary tumours. Expression levels of the main pluripotency

factors *OCT4*, *SOX2,* and *NANOG* were similar in MSCs and cancer cell lines. Identical DNA methylation marks were detected in promoters of *OCT4* and *NANOG* in both cell types, while the promoter of *SOX2* was unmethylated in MSCs but methylated in a part of tumours. Analysis of 5-hydroxymethylcytozine (5hmC) in parallel with 5-methylcytosine (5mC) in the pluripotency-associated loci revealed an accumulation of 5hmC in regulatory region of the stemness genes in MSCs, with the most abundant levels detected in regulatory regions of *OCT4* and *NANOG.* In primary tumours the levels of 5hmC were similar to that observed in MSCs, while in cultured cancer cell lines 5hmC mark was less abundant. In summary, the data of our study shows that active expression of stemness genes in adult stem cells and cancer cells might result from similar epigenetic profiles of these cells.

O.P.21.

Molecular radiobiology for the strategies of more efficient anticancer radiotherapy: aims and challenges

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Anticancer radiotherapy is one of most frequently applied treatment strategy in oncology, nevertheless the current efficacy of the treatment is not satisfactory. Obviously better understanding of cellular response to the exposure of ionising radiation would promote the development of efficient radiotherapy. To achieve this aim, however, different challenges are met. Advantages and disadvantages of different research strategies as well as results of recent research will be discussed.

O.P.22.

Towards the role of 5-hydroxymethylcytosine in neuronal DNA

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Methylation of cytosine modulates the affinity of DNA binding proteins, typically resulting in transcriptional silencing, which translates into biological outcomes such as genomic imprinting, suppression of retrotransposition and X-chromosome inactivation. Until recently DNA methylation referred to a single modified base - 5-methylcytosine (5mC). The repertoire of DNA modifications was expanded with discoveries of 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxycytosine (5caC). These 5mC derivatives have so far being implicated in DNA demethylation, especially in the setting of whole genome reprogramming in zygotes and imprinting. In neuronal cells however, high density of 5hmC is found on the gene bodies of actively transcribed genes. Our most recent results help to interpret possible roles of 5hmC in neuronal cells by studying how this modification impacts transcription.

O.P.23.

Modulation of cell developmental potency - a tool to tackle tumorigenesis

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Any cell of a multicellular organism can be characterized through its developmental potency, which describes the ability of cells to develop into more differentiated cells. In one end of this scale are totipotent zygote (able to develop into all types of adult cells as well as into extraembryonal cells) and pluripotent embryonic stem cells (ES cells, which are able to develop into all cell types but extraembryonal ones). In addition, pluripotent state is characterized by the ability of cells to self-renew, i.e. to form, during proliferation, similar pluripotent cells. In the other end of the "potency scale" are cells in fully differentiated states (e.g. adult fibroblasts or, more extremely, human erythrocytes). In the middle of this scale there are numerous more or less committed progenitor cells, exhibiting different levels of developmental potential depending on the status of their state on "maturation". After it was demonstrated by Gurdon and Yamanaka (Nobel Prize 2012), that differentiated mature cells can be reverted into pluripotent state, our understanding of cell developmental flexibility and its plasticity had to be considerably revised.

The prevailing view of the cell pluripotency is that there are specific proteins (usually transcription factors like Oct4, Nanog, Sox2 and others), which, when expressed, switch on their downstream genes and actively keep the cell in pluripotent status [1,2]. The differentiation of a cell is accompanied by gradual decrease of expression of such "pluripotency factors" and generally they are not expressed in mature differentiated cells.

Tumor cells can also be described by different extent of their developmental potency. They often express classical pluripotency markers like Oct4, Nanog and Sox2. Higher expression of these proteins has generally been found in poorly differentiated tumors and often associated with poor clinical outcome [3,4]. Certain types of tumors, first of all embryonic carcinoma (EC) cells are considered to be very close to pluripotent state, although this can differ between different cell lines. There are other types of more benign tumor cells, which have lost much of their developmental potency and rather resemble terminally differentiated cells. A special class of cells is tumor stem cells, which have been found recently in many different tumors and which are believed to carry on the tumor phenotype and form metastases. They form just a small sub-population among

the main tumor mass, which is mostly consisted of relatively harmless differentiated (albeit incorrectly) cells.

We have characterized the molecular mechanisms determining the status of cell pluripotency and their role in tumorigenesis. Our ultimate aim is to be able to modulate these molecular control pathways to drive cells into more differentiated status. If we know how to push semi-potent tumor cells into more terminally differentiated status, we can use this to enhance the prognosis of the disease.

We have been studying the ability of several small molecular compounds to reduce the pluripotency of cells. Some years ago we were the first to demonstrate that activation of tumor suppression protein p53 in hES cells by nutlin leads to cell cycle block in G1 phase due to activation of cdk inhibitor p21, and rapid differentiation of the cells [5]. Treatment of hES cells with nocodazole, an agent which similarly to taxanes is used in cancer chemotherapy and causes cell cycle arrest in the G2/M phase, resulted in a decrease of the number of cells simultaneously expressing Oct4 and Nanog. After removal of nocodazole the cells returned into normal cycle, but the expression of pluripotency markers Nanog and Oct4 was not restored. At the same time, expression of Sox2 was not affectedbynocodazole [6]. We also recently showed that Sox2 is regulated differently from Nanog and Oct4 in hES cells during early differentiation initiated with sodium butyrate [7]. Cdk inhibitor NU1640 arrests hES and hEC cells in G2 phase and inhibits the entry into M phase of cell cycle, whereas hES cells are more sensitive to NU1640 than hES cells (submitted).

In addition to the effects of small molecular compounds to the state of cell developmental potency we also are studying the effects of micro-RNAs, which are more specific compared to small chemicals. Their delivery into cells is, however, more complicated and we will discuss the technical aspects of this process.

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O.P.24.

Proteinaceous Infectivity

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Infectivity refers to the ability of a pathogen to spread among hosts. Since the discovery of genetic code, infectivity was exclusively associated with nucleic acids. However, at the end of last century, prof. S. Prusier raised protein-only hypothesis, which stated that some proteins can be infective even in absence of nucleic acids. These proteins were named prions (proteinaceous infecious particles).

The mechanism of infecitivity is based on the ability of pathogenic prions (PrP^{Sc}) to self-replicate by conformational conversion of normal, cellular form of prion protein (PrP^{C}). While PrP^{C} has been well characterized, the structure of the infectious PrP^{Sc} conformer is still debated. Our work suggested PrP^{Sc} structure to be similar to amyloid fibrils.

Amyloid deposits are associated with a number of disorders, including Alzheimer‘s and Parkinson‘s diseases and type II diabetes. If prion structure is similar to amyloids, does it mean infective nature of all amyloid-related disorders? Some of the most recent findings show a possibility of such scenario.

Elongation is the main driving force for self-replication of amyloid fibrils, thus it may be the key process in understanding proteinaceous infectivity. We concentrate on understanding mechanisms and driving forces of amyloid-like fibril elongation *in vitro*.

O.P.25.
Molecular mechanisms in Cell Biologyand Virology

Molecular signals for synaptic pruning by microglia

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Brain immune cells called microglia are known to play an important part in the removal of dead neurons, their debris or unwanted cellular material in injured or diseased brain. In contrast, their role in healthy brain remained concealed for many years. Recent evidence suggests that these motile cells continuously monitoring brain environment are in fact required for postnatal brain development as well as adult plasticity. The development of a complex nervous system is accompanied by a generation of superfluous neuronal connections that are removed when neural circuits mature. Permanent elimination of immature synapses is an activity-dependent process that leads to selective maintenance and strengthening of a subset of synapses. The maturation of synaptic circuits is tightly related to the presence of microglia cells that actively contact and engulf unnecessary synaptic terminals. Aberrant or impaired microglial function leads to abnormal synaptic densities and dysfunctional connectivity that causes morphological, functional and behavioral deficits. The elimination of immature synapses is dependent on microglial phagocytic pathways, implicating microglial phagocytosis as a mechanism of synaptic pruning. A couple of pathways have been suggested to mediate the removal of synapses, including immune complement cascade proteins, apoptosis-related secreted opsonins and corresponding microglial receptors. The question remains which neuronal

surface signals label weak synapses for elimination or mark strong synapses for strengthening thus ensuring proper brain development and maintenance.

O.P.26.

Mitochondrial dysfunction in experimental acute pancreatitis

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Acute pancreatitis (AP) is an inflammatory disease of pancreas [1]. It is known, that systemic inflammatory response syndrome in acute severe pancreatitis leads to multiple organ failure, which is the main cause of death [2, 3]. Mitochondrial disturbances in different organs during severe acute pancreatitis are poorly described. In this study the effects of experimental severe acute pancreatitis on mitochondria from rat pancreas, kidney, lungs and liver were investigated.

Acute pancreatitis was induced by intraductal application of sodium taurocholate (5%, 1,75 mL/kg). Animals were divided into six groups (1, 3, 6, 12, 24 and 48 hours) reflecting the time from induction of the AP. Pancreas, liver, lungs and kidney were removed and mitochondrial function was measured oxygraphically. Mitochondrial Complex I activity was measured spectrophotometrically.

Our results revealed, that mitochondria from pancreas were affected early - within the first 6 hours after onset of AP (State 3 respiration rate with glutamate/malate as substrate decreased by 80%, and the respiratory control index by 59%, $p<0.05$). Succinate-dependent State 3 respiration rate was inhibited later - 12 h from onset of AP (decreased by 51%, $p<0.05$). Mitochondria from kidney were impaired 24 hours after onset of AP (State 3 respiration rate (substrate glutamate/malate) decreased by 68%, $p<0.05$). Mitochondria from lungs were affected 48 hours after induction of AP (State 3 respiration rate was diminished by 47%, $p<0.05$). Liver mitochondria remained unaffected during AP within 48 hours. Complex I–depended glutamate/malate oxidation was more affected during AP than Complex II-depended succinate oxidation. Mitochondrial Complex I activity in pancreas, kidney and lungs during AP was also diminished.

In conclusion, our data revealed that the impairment of mitochondria in pancreas, kidney and lungs may play an important role in the development and progression of acute pancreatitis as a systemic disease.

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O.P.27.

miRNA-mediated regulation of membrane trafficking

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MicroRNAs (miRNAs) are short non-coding RNAs (18 to 24 nt in length) regulating gene expression transcriptionally and post-transcriptionally. miRNAs bind to target mRNAs in a complementary or partially complementary way, resulting in degradation and/or translational repression of mRNAs. Roles of miRNAs in the regulation of cell cycle progression, senescence, development and tumour biology are well established, with numerous miRNAs identified as key regulators by now. In contrast, regulatory roles of miRNAs in membrane trafficking, one of the most fundamental cellular processes, that is necessary for the entry, delivery and distribution of diverse molecules is only starting to emerge. In order to facilitate the discovery of miRNAs regulating membrane trafficking, we have performed anunbiased fluorescence microscopy-based screen and assigned potential regulatory roles for 44 miRNAs. Detailed analysis of the selected miRNA, miR-30b, confirmed its profound role in regulation of membrane trafficking and revealed the underlying novel molecular mechanisms.

O.P.28.
Enzymology and Metabolism

Yeast vacuoles as a model system for studying polyphosphate metabolism in acidocalcisomes

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Virtually all organisms contain inorganic polyphospahate (polyP). The polyP functions in eukaryotes range from osmoregulation and virulence in parasitic protozoa to modulating blood coagulation, bone mineralization and glia signaling in mammals. A hallmark of polyP metabolism in eukaryotic cells is its enclosure within membrane-bound compartments, often in the evolutionary conserved organelles acidocalcisomes. How and why polyphosphates are sequestered in dedicated organelles has been unknown.

We studied this question with yeast vacuoles, which show all essential characteristics of an acidocalcisome. Whereas yeast can synthesize and accumulate large amounts of polyP inside the vacuole, we showed that polyphosphates produced by an ectopically expressed cytosolic polyphosphate polymerase are not translocated into vacuoles and that the accumulating cytosolic pool of polyphosphate becomes toxic to the cells.

In yeast polyP is synthesized by a large multi-subunit protein complex, vacuolar transporter chaperone (VTC).Large cytoplasmic domains of VTC proteins were shown to contain the active and regulatory sites, whereas transmembrane domains were proposed to integrate the polyP synthesis with

its translocation through the membrane into the vacuole lumen. We demonstrated that isolated yeast vacuoles can efficiently synthesize polyP *in vitro* and translocate it into the lumen, thereby providing a good model system for detailed studies of eukaryotic polyP metabolism and paving the way to deciphering molecular details of acidocalcisome functions. We showed that polyP synthesis import requires the vacuolar electrochemical potential, probably as a driving force for polyP translocation. This suggests that synthesis of polyP is obligatorily coupled to its import in order to avoid toxic intermediates in the cytosol. This may explain why acidocalcisomes exist as a universal class of organelles in all organisms storing polyphosphates.

O.P.29.

Conditional gene trap mutants for regeneration studies in zebrafish

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Genetic and pharmacological tools enabled tremendous progress in descriptive analysis of regeneration, including demonstration of the importance of classical developmental pathways - Wnt, Fgf, Bmp and retinoic acid. Conditional mutants are needed for more mechanistic studies of genetic control of regeneration. However, conditional mutants can only be readily generated in the mouse, which has very limited regenerative capacity. Conversely, in vertebrate model systems with extensive regenerative capacity, such as the zebrafish, very few conditional mutants exist. To address this chiasmus in regeneration biology, we have constructed a fully conditional gene trap vector for use in zebrafish. In our pilot screen we recovered an insertional mutant of *tbx5a*. Heterozygous *tbx5a* gene trap mutants display haploinsufficient pectoral fin phenotypes remarkably similar to forelimb phenotypes of human patients with Holt-Oram syndrome, while homozygous mutants display a fully penetrant linear heart phenotype and complete absence of pectoral fins. Notably, fish heterozygous for the *tbx5a* gene trap allele also fail to regenerate their hearts. Inversion of the gene trap cassette using Fl precombinase completely reverts all mutant phenotypes. We then used a *tnnt2:CreERT2* line to specifically re-mutate *tbx5a* in the cardiomyocytes. We observed a mosaicism-dependent regeneration defect: topical failure to regenerate where a large percentage of cells immediately adjacent to the injury site carried *tbx5a* mutation. Thus, *tbx5a* plays a critical role in cardiac regeneration in zebrafish.

O.P.30.

Microscaffolds for Cell Arrays and Tissue Models

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Modern cell analytics and cell manipulation-based tissue repair strategies are hardly possible without advanced fabrication techniques. Originally developed as the basis of semiconductor manufacturing, the concept of integrated circuitry is now entering different areas of life science and medicine. The broad arsenal of fabrication tools that are employed for arraying, stacking and interfacing of living cells ranges from inkjet printing, direct laser writing to photolithography and electron beam. Our laboratory is focusing on a particular class of tools that are based on contact printing of biomaterials, including proteins, lipids, polymers, peptides and other compounds. We have been using this approach to fabricate architectures consisting of cell specific vs. cell repellent components on a variety of substrates for different applications. This lecture will provide a brief overview of recent activities, with a particular focus on hydrogel engineering for in vitro tissue models and regenerative medicine.

O.P.31.

Stromal guides for T cell development in the Thymus

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Thymic T cell development requires a specialized microenvironment that is largely composed of cortical and medullary thymic epithelial cells (TECs).
The TEC master gene transcription factor Foxn1 was identified 20 years ago as a causative mutation in nude mice lacking hair and thymus. However, the molecular mechanisms of Foxn1 action in TECs remain elusive. Using ChIP-seq approach we have mapped Foxn1 DNA binding sites and discovered the binding motif. By combining Foxn1 DNA binding information with transcriptome analysis of novel mouse models of Foxn1 insufficiency in TECs we have identified Foxn1 target genes critical for TEC function to support T cell development.

O.P.32.
Enzymology and Metabolism

Correction of Brain Ischemic and Reperfusion Injuries by Metabolic Substances

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Nowadays many medicines with direct influence at specific injury mechanisms are used to achieve neuroprotection in the acutely ischemic brain. Unfortunately, most of them act in clinical practice less effective than in experimental conditions and have pronounced side actions. Application of the metabolic substances that help to maintain energy metabolism and redox status of glutathione system may be useful for remedying damages to the brain after ischemia-reperfusion because they have no toxic effects and may be used safely for prolonged period.

It is believed that the physiological function of CoA system is participation information of redox potential of glutathione and proteins, redox signaling and maintenance of biological membrane stability, especially in brain tissue. We showed high protector efficacy of pantothenic acid derivatives – CoA precursors, for example, D-panthenolin respect to changes of energic metabolism and glutathione system activity during brain ischemia-reperfusion. Succinic acid derivatives are effective modern antioxidants in the brain because succinate regulates activity of SDH in the Krebs' cycle and restores activity of respiratory mitochondrial chain not only, but increases microcirculation in tissues.

We showed that injections of panthenol and succinate following brain ishemia-reperfusion stabilize levels of lipoperoxidation in blood and in brain hemispheres, stabilize levels of protein SH-groups in blood, lead to significant decrease of the GSSG level and normalization of glutathione enzyme activities, as well as glutamate and glutamine metabolism in the brain to control values.

Addition of a selenium source to panthenol and succinate strengthened protective potential of the substances with respect to changes for enzyme activities of glutamate and glutamine which play an important role in maintaining of energy supply and detoxication in ischemic brain tissue and confirms the antiischemic effect of the composition.

O.P.33.
Structural biology and Bioinformatics

Viral DNA replication: new insights and discoveries from large scale computational analysis

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Ability to replicate is essential for all living entities. Duplication of genetic information is carried out by replication proteins. DNA replication is well studied in T7, T4 phages and herpes viruses, however information about replication apparatus from other groups of viruses is „gappy" or missing. Double-stranded (ds) DNA viruses infect cells from all domains of life, evolve fast and are very diverse. Their genome size varies from 5 to 2500 kbp. To better understand viral DNA replication we identified replication proteins in dsDNA viruses using current state-of-art homology detection methods. Over 150000 proteins from 1574 genomes were analyzed. We found out that the composition of replication machinery depends on virus genome size. Small viruses (<40 kbp) use protein-primed DNA replication or rely on proteins from the host. Large viruses (>140 kbp) have their own RNA-primed replication apparatus often supplemented with processivity factors and DNA topoisomerases to increase replication speed and efficiency. Latter insight led us to a search for a „missing" replication components in a large genomes. This has resulted in a discovery of single-stranded DNA binding (SSB) proteins in largest eukaryotic viruses. Surprisingly these proteins were homologs of SSB proteins previously thought to be specific for T7-like phages. Other surprise came from the analysis of a herpesviral helicase-primase. We found that its component (UL8) is inactivated and highly diverged B-family DNA polymerase.

Symposium "Young biochemistry": 17th June.

O.P.34.
Molecular mechanisms in Cell Biologyand Virology

MK-801 protective effect against Aβ1-42 oligomer-induced neuronal death

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Soluble beta amyloid (Aβ) oligomers are critical factors in the pathogenesis of Alzheimer's disease (AD), particularly $A\beta_{1-42}$ form. However, the molecular mechanism how $A\beta_{1-42}$ oligomers trigger neurotoxic cascades are not fully elucidated.

The aim of this study was to investigate the toxic effects of small $A\beta_{1-42}$ oligomers on neurons and glia cells in primary neuronal/glial cerebellar granule cell cultures and pure microglia cultures, evaluating whether these neurotoxic effects caused by $A\beta_{1-42}$ oligomer are sensitive to NMDA receptor antagonist MK801.

We found that the plasma membrane of neurons and microglia was rapidly depolarized by $A\beta_{1-42}$ oligomers but by different mechanisms. During 0.5-1h incubation small $A\beta_{1-42}$ oligomers caused depolarization of neurons and microglia membranes and MK-801 protected only the microglia cells from $A\beta_{1-42}$-induced membrane depolarization. The effect of MK-801 was also seen in pure microglia cultures, indicating that microglia plasma membrane depolarization may be associated with activation of NMDA receptors. Measuring glutamate level in CGC culture medium we found that during 0.5-4h incubation $A\beta_{1-42}$oligomers increased glutamate level and this was prevented by MK-801. In pure microglial culture, $A\beta_{1-42}$ oligomers did not cause release of glutamate

into medium. We found that MK-801 preserved neuronal viability and protected CGC from $A\beta_{1-42}$-induced neurotoxicity during 24 h incubation. In conclusion, $A\beta_{1-42}$ oligomers induced rapid NMDA receptor–independent neuronal depolarization and NMDA receptor–dependent release of glutamate in CGC cultures leading to excitotoxic neuronal death. $A\beta_{1-42}$ oligomers also induced rapid and NMDA receptor –dependent microglial plasma membrane depolarization.

O.P.35.
Cancer Cell Biology and Biomedicine

Potential immunosuppression, drug resistance and stemness biomarkers for ovarian cancer

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Ovarian cancer is the most lethal gynecologic malignancy. Due to challenges in early detection and clinical monitoring of ovarian cancer, different biomarker combinations have recently been investigated. The aim of this study is to evaluate relationships between potential drug resistance, stemness and immunosuppressive markers in ovarian cancer patients. For this study, 20 pathologically verified tumor specimens and peripheral blood samples were obtained from women with serous epithelial ovarian cancer. Flow cytometry was used to assess immunosuppressive T lymphocyte subsets in peripheral blood, to determine tumor leukocyte infiltration and to evaluate the expression of a panel of stemness-associated markers in tumor cells. Immunosuppressive enzymes in serum and tumor lysate were quantified by an ELISA method. The relative expression of a panel of platinum resistance-associated genes was quantified using real-time qPCR. The expression of all markers differs between patients. Several statistically significant ($p<0,05$) relationships were elucidated after applying correlation analysis. It was shown that the greater the quantity of immunosuppressive enzyme IDO in tumor lysate, the higher the expression of multidrug resistance proteins ABCC1 (R=0,91) and ABCG2 (R=0,74) in tumor cells. Also, expression of stemness-associated marker CD44 in tumor cells correlates with the relative expression of *FN1*, *ATP7B*, *ATP11B* gene set (R=0,69), tumor leukocyte infiltration (R=0,66) and serum IDO level (R=0,76). Additionally, serum IDO level is associated with *FN1*, *PCNA*, *ATP11B* gene set expression (R=0,74). Moreover, the size of the immunosuppressive T cell subset CD8+/CD57+/FoxP3+ correlates with the expression of *ASS*, *PCNA*, *ATP11B* gene set (R=0,89). More thorough investigation of immunosuppressive, stemness and resistance properties of tumors and their relationships with clinical data could support the establishment of novel prognostic and predictive marker set for ovarian cancer.

O.P.36.
Structural biology and Bioinformatics

Mapping the functional domains of plant small RNA methyltransferase HEN1

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Small, 21-33-nucleotide RNA molecules are essential for post-transcriptional gene regulation in eukaryotic organisms including humans. All types of small interfering RNAs (siRNAs) and microRNAs (miRNAs) in plants, piwi-interacting RNAs (piRNAs) in animals require 2’-O-methylation on the 3’-terminal nucleotide for their stabilization, preventing them from a 3'-end uridylation and degradation. This specific modification is carried out by the S-adenosyl-L-methionine–dependent small RNA 2'-O-methyltransferases, which are also found in metazoan, fungal, and bacterial proteomes. The best studied representative of them is *Arabidopsis thaliana* HEN1.

Analysis of tertiary protein structure revealed that *Arabidopsis* small RNAs methyltransferase consists of five domains. To elucidate experimentally the function of each domain, miRNA/miRNA* and siRNA/siRNA* binding analysis, steady-state and pre-steady-state kinetic studies of truncated variants of methyltransferase and HEN1 mutants with point mutations were done. The obtained data indicate that: the methyltransferase domain of HEN1 is important for methyl group transfer; the first double-stranded RNA-binding domain is required for substrate recognition and its tight binding; the second dsRNA-binding domain as well as the extra loop between beta sheets β1 and β2 in R1 (residues 31 to 49), which communicates with methyltransferase domain, are the essential factors decelerating the decay of ternary complexes after methylation reaction. Similar binding and methylation parameters observed with siRNA and miRNA substrates suggest that the HEN1 does not encompass any domain necessary for distinguishing two types of small non-coding RNAs *in vitro.* As the central part of HEN1 is not responsible for the interaction with substrates, it was supposed that this part can be important for binding others biogenesis proteins in plants.This hypothesis was confirmed by data obtained using electrophoretic mobility shift assay, yeast two-hybrid system and pull-down method. The obtained results provide valuable insights into the enzymatic mechanism and biological role of an abundant class of small RNA 2’-O-methyltransferases, which share similar catalytic domains, and are widely distributed in all biological kingdoms except archea.

O.P.37.
Cancer Cell Biology and Biomedicine

Novel inhibitory monoclonal antibodies against tumour-associated carbonic anhydrase XII

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Human carbonic anhydrase XII (CA XII) is a single-pass transmembrane protein with an extracellular catalytic domain. This enzyme is being recognized as a potential biomarker for different tumours. It is involved in tumour progression by acidification of the extracellular milieu and regulation of intracellular pH. CA XII is aberrantly overexpressed in breast, cervix, brain cancers, renal carcinomas. The current study was aimed to generate monoclonal antibodies (MAbs) neutralizing the enzymatic activity of CA XII. Bioinformatics analysis of CA XII structure revealed surface-exposed sequences located in a proximity of its catalytic centre. Two MAbs against the selected antigenic peptide spanning 167; 180 aa sequence of CA XII were generated. The MAbs were reactive with recombinant catalytic domain of CA XII expressed either in *E. coli* or mammalian cells. Inhibitory activity of the MAbs was demonstrated by a stopped flow CO2 hydration assay. The study provides new data on the surface-exposed linear CA XII epitope that may serve as a target for inhibitory antibodies with a potential immunotherapeutic application.

O.P.38.
Biochemistry of Prokaryotes

Cascade complex – identifying the clue to PAM recognition

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Cascade complex from *Streptococcus themophilus* is a Type I CRISPR/Cas surveillance complex that uses crRNA to target invading DNA species [1]. *S. thermophilus* Cascade is composed of five cas proteins (CasA, CasB, CasC, CasD, CasE) that bind crRNA to form a large ribonucleoprotein complex [2]. Cascade guided by the crRNA binds to the complementary target DNA if the correct proto-spacer adjacent motif (PAM) is present. Subsequently, the DNA structure named R-loop is formed and it serves as a binding platform for the Cas3 nuclease/helicase that degrades the invading DNA sequence [3].
The correct PAM sequence is critical for the target recognition in the invading DNA. In *S. thermophilus* Type I CRISPR-Cas system PAM is limited to single nucleotide A or T [1]. In this project we aimed to understand the mechanism of PAM recognition in the *S. thermophilus* Type I CRISPR-Cas system.

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O.P.39.
Biochemistry of Prokaryotes

Archaeal protein aFib and Nop5p heterodimer methylates 16S rRNA site-specifically, independently of C/D guide RNA

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Archaeal fibrillarin (aFib) is a well-characterized RNA 2'-O-methyltransferase that acts in C/D RNP complexes. It has been established that the methylation target is selected via base-pairing of the guide RNA of the complex and aFib finds it only by interactions with the other proteins of the complex: L7Ae and, especially, Nop5p (1). Nevertheless, we investigated functions of C/D RNP components and showed that *Pyrococcus abyssi* aFib in a heterodimer with Nop5p, and without other components of a C/D RNP, effectively methylates 16S rRNA site-specifically *in vitro*. We further identified methylated positions using tritium-methyl incorporation into RNA assay, mass spectrometry, reverse transcription stop analysis, alkaline hydrolysis. This aFib-Nop5p heterodimer activity could be an example of proteins that evolved from stand-alone enzymes to C/D RNP components acting in RNA guided manner.

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O.P.40.
Biochemistry of Prokaryotes

Vaginolysin from Gardnerella vaginalis: a candidate for a new group of cholesterol-dependent cytolysins

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Vaginolysin (VLY) – a cholesterol-dependent cytolysin (CDC) from *Gardnerella vaginalis* has been suggested to be an important factor in the development of bacterial vaginosis by causing lysis of vaginal epithelium cells. While the majority of CDCs oligomerize through interaction with the cholesterol in

eukaryotic cell membranes only; a couple of CDCs, namely intermedilysin, lectinolysin and vaginolysin need as well to bind to a human cellular marker CD59 (hCD59). Vaginolysin and other hCD59 binding CDCs also oligomerize differently from other CDCs: they do not possess π-stacking between β4 and β1 chains in addition to hydrogen bonding in monomer-monomer interaction. The exact role of hCD59 in VLY induced cytolysis, however, is not clear, as well as the way in which VLY monomer-monomer interaction is established. Using site-directed mutagenesis we mutated VLY by disrupting particular oligomerization sites. We then examined if these mutations influenced lytic properties of VLY by quantifying the level of lysis of erythrocyte suspension or cell culture. Further we investigated discrete steps in the mechanism of the VLY action. By using cholesterol-rich 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC) liposomes we observed VLY oligomerization which was consistant with its hemolytic manner. Dot blot assay was used to confirm ongoing interaction between VLY mutants and cholesterol. In order to study VLY binding to hCD59 we derived hCD59 expressing Chinese hamster ovary (CHO) cell line. We showed that VLY binding to hCD59 is important but not critical for cytolytic activity as opposed to ILY binding. Complete VLY-induced cytolysis of non-human cells lacking hCD59 was achieved only with higher amount of the toxin. Cholesterol, but not hCD59, was shown to be an indispensible factor for VLY activity proven by the impairment of the insulating properties of VLY-treated artificial tethered bilayer lipid membrane.

O.P.41.

Development and Characterization of Monoclonal Antibodies against Human Parvovirus 4 Major Capsid Protein VP2

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The recently discovered human parvovirus 4 (PARV4) has been associated with human immunodeficiency virus, hepatitis B virus and hepatitis C virus infections. Intravenous drug users have been described as virus carriers. Moreover, the virus has been detected in blood products and in multiple transfused patients. Epidemiology and transmission routes of the PARV4 are poorly investigated. Virus detection is usually performed by polymerase chain reaction or enzyme-linked immunosorbent assay (ELISA). However, there is no virus identification system approved worldwide. Due to lack of convenient and reliable methods, more research is needed.
This study was aimed at generation and characterization of monoclonal antibodies (MAbs) against yeast-expressed PARV4 major capsid VP2 protein with respect to use them in diagnostic assays. The purified PARV4 VP2 protein was self assembled to virus-like particles. Previously, it was demonstrated that yeast-expressed PARV4 VP2 protein shares antigenic properties with virus-derived VP2 protein. The recombinant PARV4 VP2 protein was used as an immunogen for the production of MAbs. Twenty-eight hybridomas producing MAbs of IgG isotype were generated. The specificity of the MAbs was analyzed by indirect ELISA and Western blot. Their cross-reactivity with other yeast-expressed VP2 proteins of the members of *Parvovirinae* subfamily – human bocavirus and human parvovirus B19 – was investigated. All MAbs reacted specifically with recombinant PARV4 VP2 protein and did not react with the related proteins. Competitive ELISA was used to identify the immunodominant sites of PARV4 VP2 protein. Three major antibody-binding sites were identified. Further research is directed to the potential use of the MAbs in diagnostics, in particular to the development of IgM/IgG capture ELISAs for PARV4 infection.
This study was supported by the Lithuanian Science Council Grant No. MIP-060/2011.

O.P.42.

Membranes and bioenergetics

The Comparative Analysis of Multidrug Resistance Pumps in Jurkat and MX1 Cells

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Multidrug resistance pumps are possessed by the majority of cells. These pumps protect us from toxic compounds and harmful refuse but also extrude anticancer drugs and cause problems in cancer chemotherapy. In this study the efflux pumps in Jurkat and MX1 cells were explored. DNA intercalating agent ethidium was used as indicator to assess the efficiency of multidrug resistance pumps. One of the tasks was to optimize the conditions for permeabilization of these cells. Digitonin was chosen as the most effective agent to destroy cell permeability barrier. In addition, we determined the effects of tetraphenylphosphonium cations (TPP^+) and ionophoric antibiotics nigericin and monensin on the efficiency of these pumps in Jurkat and MX1 cells. The results of our study suggest that TPP^+ ions upregulate the efficiency of multidrug resistance pumps. The effects of nigericin and monensin on these pumps depend on the duration of exposure: short (15 min) cell exposure to antibiotics enhances the efficiency of the pumps whereas 24 hour cell exposure to these ionophores has the opposite effect.

O.P.43.
Cancer Cell Biology and Biomedicine

Characterization of A 5-fluorouracil-resistant Human Colon Cancer Cell Line

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5-Fluorouracil (5-FU) is antimetabolite drug which is still widely used in threatment of a range of cancers. Nonetheless, response rates for 5-FU-based chemotherapy as a first-line treatment for advanced colorectal cancer are only 10–15%. These low response rates are due to intrinsic or acquired drug resistance. In this study, we investigated 5-fluorouracil-resistant cell line which was developed from human colon cancer HCT116 cells. The resulted derivative HCT116/100FU cells were ~100-fold more resistant to 5-FU. These cells had a prolonged doubling time, a lower adhesive ability, and increased resistance to doxorubicin and irinotecan, but not mTHPC-PDT. Some molecular changes between parental and 5-FU-resistant cell lines were identified using high throughput proteome analysis. HCT116/100FU cells had decreased expression of proteins which are involved in endocytosis, apoptosis; increased expression of enzymes essential in nucleobase, nucleoside and nucleotide biosynthesis and DNA repair.

O.P.44.
Structural biology and Bioinformatics

The PPI3D web server for searching, analyzing and modeling protein-protein interactions in the context of 3D structures

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Easy-to-use computational tools for structural analysis of protein-protein interactions are valuable for studies of molecular mechanisms of biological processes. We have recently developed the PPI3D web server, which provides a possibility to query experimentally determined 3D structures of protein complexes, to analyze the identified protein-protein interactions and to generate homology models of protein complexes. Structural data for experimentally determined protein-protein interactions are represented by biological assemblies available from the Protein Data Bank. All the protein-protein interactions accessible through PPI3D are clustered according to both sequence and interaction interface similarity. This removes the redundancy of structural data while preserving alternative protein binding modes. The server enables users to explore interactions for individual proteins or interactions between a pair of proteins (protein groups). In both modes, structural data on protein-protein interactions are detected using sequence search with either BLAST or PSI-BLAST depending on the desired level of similarity. The PPI3D output enables users to interactively explore both the overall results and every detected interaction. The server reports the total protein-protein interface area, the sortable list of interface residues and their individual contribution to the interface area. The interface residues can be inspected in the sequence alignment or in the context of 3D structure. In addition, the server provides a possibility to construct a homology model for the protein complex. The server is freely accessible at http://www.ibt.lt/bioinformatics/ppi3d/.

O.P.45.
Biotechnology

Heat shock at the late-log growth phase improves protein translocation and invertase secretion in Saccharomyces cerevisiae

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The yeast *Saccharomyces cerevisiae* is a widely used cell factory for the production of heterologous proteins. However, secretory expression of heterologous proteins in yeast is often subject to several bottlenecks that limit yield. The translocation of newly synthesized proteins into the endoplasmic reticulum (ER) is the first stage of the secretion pathway. It was shown earlier that synthesis of measles virus hemagglutinin (MeH) is inefficient mostly due to the bottleneck in the translocation of viral protein precursors into the ER of yeast cells. The aim of this study was to improve the translocation of MeH in *S. cerevisiae* by manipulating cell culture conditions. We found that heat shock with subsequent induction of MeH expression at 37ºC improved translocation of MeH precursors when applied at higher cell densities. The amount of MeH glycoprotein increased about three-fold after heat shock in the late-log phases of both glucose and ethanol growth. Heat shock at the late-log glucose growth phase also improved endogenous invertase yield by approximately 2.7-fold. We propose that increased temperature enhances protein secretion by facilitating translocation across the ER membrane after heat shock at higher culture densities, when yeast cells enter the late logarithmic growth phase. Our results demonstrate that heat shock may be employed for the improvement of heterologous protein expression in *S. cerevisiae* secretory pathway.

O.P.46.

Studies on the prevalence of oncogenic HPV types among Lithuanian women with cervical pathology

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Human papillomavirus (HPV) is the main cause of cervical cancer. Therefore, the detection of oncogenic HPV types is important in predicting the risk of cervical cancer. The aim of the current study was to estimate the prevalence of 16 carcinogenic and potentially carcinogenic HPV types in the study group of Lithuanian women with various grades of cervical pathology in comparison to healthy women. A total of 824 cervical specimens were investigated for HPV DNA: 547 specimens of women with abnormal cytology and 277 specimens of healthy women. Cytological diagnosis was confirmed by histology. For the detection of HPV infection, HPV DNA was amplified by PCR using three different primer systems. HPV DNA-positive samples were investigated for the presence of 16 HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 66, 73 and 82) by multiplex PCR. HPV DNA was detected in 67.6% of specimens collected from women with abnormal cytology and 24.2% of specimens collected from healthy women. The frequency of HPV-positive specimens correlated with the severity of cervical pathology: it ranged from 50.0% in ASC to 80.6% in cervical cancer. In cases confirmed by histology the frequency of HPV-positive specimens ranged from 68.6% in CIN I to 89.2% in CIN III/CIS. The most common HPV type was HPV16 (detected in 42.3% of HPV-positive specimens) followed by HPV31 (10.1%), HPV33 (8.2%) and HPV56 (5.7%). The prevalence of HPV16 among HPV-positive specimens ranged from 27.6% in ASC to 60.0% in cervical cancer as diagnosed by cytology and from 37.5% in CIN I to 61.3% in cervical cancer as diagnosed by histology. In contrast, the frequency of HPV18 was lower as compared to other countries. HPV18 was identified in 4.1% of HPV-positive women with various grades of cervical abnormalities and in 11.3% of those with cervical cancer.

O.P.47.
Synthetic Biology

Edinburgh iGEM 2013 Project: Remediation and Valorisation of Industrial Waste

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The Edinburgh iGEM 2013 team, WastED, focused on remediation and valorisation of industrial waste streams, with a particular focus on Scottish leather, textile, and whisky industry waste waters, which contain toxic heavy metal ions as well as fermentable organic components. Using *Bacillus subtilis* as a chassis, we attempted to engineer organisms to capture metal ions using chelators and metal binding proteins, and to ferment organic components to produce biofuels. We also tested a new assembly procedure, GenBrick, which is based on the Genabler assembly system. GenBrick allows assembly of multiple RFC10-compatible BioBricks in a single reaction, and is also well suited for the preparation of fusion proteins and addition of terminal tags. Enzyme fusions may enhance metabolic pathways through substrate channelling. We are testing the effect of protein fusions on fermentation efficiency for biofuel production. In addition, we are examining the implications of possible Scottish independence, following the 2014 referendum, for Synthetic Biology in Scotland.

O.P.48.
Emerging techniques and new methods in biochemistry

Response of perennial plants to seed treatment by low temperature plasma and electromagnetic field: reactive oxygen species are involved in changes of seed germination and early plant development

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Low-pressure radiofrequency plasma (cold plasma, CP) and radiofrequency electromagnetic field (EM) treatment of non-stratified seeds of perennial plants *Morusnigra (MN)*, *Rhododendron smirnowii (RS), Pinussylvestris(PS) and Picea abies (PA)* was studied: the impact on seed germination rate, seed surface structure and early seedling development was determined using seed treatment by vacuum (15 min) as an additional control. Scanning electron microscopy analysis revealed that CP treatment had strong etching effect on the seed surface. Vacuum exerted no effect on fresh *MN* seeds, but activated germination of fresh (but not after-ripened) *RS* seeds by 70%. Plasma treatment (2 min) increased germination yield of *MN* seeds by 50% and stimulated further seedling development, but negatively affected *RS* and *PA* seed germination. EM treatment (5-15 min) resulted in significant improvement of seed germination for *MN, RS* and *PA* species, but had obvious negative effect on *PS*. EPR spectra in *MN* seeds was registered immediately after treatment and registration repeated in few following days. The results indicated strong burst of ROS production (up to 90%) on the first day after the seed treatment. EPR signal remained lower but stable above the control level in several following days. The effects of treatment by stressors on early *MN* seedling development and the correlation of the

registered EPR signal and the observed effects will be presented and discussed. The treatments efficiently reduced fungal contamination of plant seeds, but this effect was strongly pronounced only for *MN* seeds that were the most contaminated (colonies were obtained on 94% control seeds). We suggest the hypothesis that seed response to treatment by physical stressors starts from the induced burst of ROS in plant seeds that is followed by a very complex and species specific changes in signal transduction possibly involving hormonal response and finally resulting in a long-term changes in protein expression.

O.P.49.
Cancer Cell Biology and Biomedicine

IDH1 mutation promotes migration and invasion and acts synergistically with p53 and PTEN loss in murine tumour cells

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INTRODUCTION: Over 70% of low- or high grade gliomas carry *IDH1* gene mutations. The understanding of the role of *IDH* mutations in human glioma cells is limited by concomitant genetic and epigenetic modifications in glioma cell lines, making it difficult to precisely dissect the role of *IDH* in proliferation, migration and invasion.
METHOD: Neural stem/progenitor cells (NSC) were isolated from the subventricular zone of mice carrying combinations of conditional gene mutations: IDH1(R132H), p53lox/lox, Ptenlox/lox, or Rblox/lox. The *IDH1* mouse model carries a cre-inducible human R132H allele. Neurospheres were recombined *in vitro*, and transferred to adherent conditions to form monolayers, for quantification of growth, invasion and migration. Further, we compared murine to human glioma initiating cells (hGIC) with wild-type or mutant IDH1.
RESULTS: *IDH* mutations in mNSCs increased proliferation, migration and invasion but required inactivation of at least one additional tumour suppressor. In contrast, *IDH* mutant hGICs showed lower proliferation and wound healing rates than IDH wild type counterparts.
CONCLUSION: Our study demonstrates an important role of mutant IDH in promoting growth, migration and invasion in murine primary NSC. The striking difference to human glioma cells may be explained by a different spectrum of mutations that have accrued in *IDH* mutant human tumours. We hypothesise that long-standing *IDH* mutations modify the epigenome differently from that in the short-term murine culture model. We are currently characterising the genome and methylome of murine IDH mutant cells during long-term *in vitro* culture and in our allografting paradigm to better characterise the role of IDH in tumourigenesis from NSC.

O.P.50.

The immunogenicity of amyloid beta oligomers and their role in macrophage-mediated inflammation

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The central molecule in the pathogenesis of Alzheimer's disease (AD) is believed to be a small-sized polypeptide – beta amyloid (Aβ) which has an ability to assemble spontaneously into oligomers. Studies concerning therapeutic and prophylactic approaches for AD are based on the immunotherapy using antibodies against Aβ which in some cases of clinical trials led to neuroinflammation. However, knowledge on the mechanisms of Aβ-induced immune responses is rather limited. Research on Aβ oligomeric antigens in complex with antibodies showed that neurotoxic effects on primary neurons are increased by Fc-dependent microglia activation. In the current study, we evaluated the dependence of immunogenicity of Aβ on the size of oligomeric particles and investigated how Aβ oligomers alone or in complexes with antibodies influence macrophage phenotype. **Results:** The analysis of serum antibodies in immunized mice revealed that 1-2 nm Aβ oligomers are highly immunogenic while larger Aβ oligomers and monomers induced a weak IgG response. Epitope mapping of Aβ-specific antibodies demonstrated that the main immunodominant region of the Aβ oligomers is located at its N-terminus, between amino acids 1 and 19. Cytokine profiles of cell growth supernatants from mice spleen macrophage and J774 cell line cultures treated with Aβ or their complexes with antibodies suggest that macrophages are directed towards M1 phenotype. **Conclusions:** Small Aβ oligomers induce the strongest immune response in mice. The N-terminus of Aβ oligomers represents an immunodominant epitope which indicates its surface localization. Aβ oligomers in complex with antibodies form inflammatory conditions in macrophage cultures. The results of the current study may be important for further development of Aβ-based vaccination and immunotherapy strategies.
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O.P.51.
Biochemistry of Prokaryotes

Characterization of Streptococcus thermophilus Csm complex mediated CRISPR interference in vitro

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CRISPR-Cas systems are an adaptive prokaryotic defence mechanism set against viral infection and horizontal gene transfer. The sequence-specific degradation of invasive nucleic acids (NA) is carried out by ribonucleoprotein effector complexes. While the cleavage is executed by protein subunits, the recognition of specific target nucleic acid is performed mostly by an RNA molecule. The effector complexes of Type I (Cascade) and II (Cas9-dual RNA) cleave foreign DNA while RNA is degraded by Type III-B Cmr complexes. Although genetic evidence suggests that Type III-A Csm complex targets DNA, there is no biochemical data on the interference mechanism. Moreover, while the active sites have been identified for the Type I and II effector complexes, no such data has been reported for the Type III systems in general. Here we aimed to establish the complex assembly and silencing mechanism of the Type III-A system Csm-machinery using *Streptococcus thermophilus* as a model system. We expressed the Type III-A CRISPR-Cas locus in *Escherichia coli*, isolated the Csm effector complex and characterized its activity *in vitro*. Further, we demonstrated that Csm complex may be reprogrammed to cleave the NA substrate of interest.

O.P.52.

Analysis of atopy-associated genetic markers in the Lithuanian birth cohort

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Over the last decades, the prevalence of allergic diseases has been increasing in Europe and currently approximately 2 % of the population suffers from food allergy and 4 % – from asthma. As few effective techniques to cure allergic diseases are in use, research into predictive atopy-associated markers could provide ways to prevent the start of the atopic march. This study is the first analysis of atopy-associated genetic markers in the Lithuanian birth cohort.

Seven single nucleotide polymorphisms (SNPs) were investigated – *CD14* rs2569190, *FLG* rs11584340 and rs2184953, *FCER1A* rs2427837 and rs2251746,*IL13* rs20541 and rs1800925 by either allele-specific PCR or PCR restriction fragment length polymorphism (PCR-RFLP).

A total of 133 children (mean age 5.2 years; 57 % male) were genotyped and separated into cases and controls according to the result of a skin prick test (SPT). In the combined cohort, only *FLG* rs2184953 A/G genotype was linked to increased odds of producing a positive SPT. An association between *IL13* rs20541 T alele and at least one positive SPT was observed in the male subgroup. Within the female subgroup, positive SPT was associated with *FCER1A*rs2251746 C alele.

In conclusion, 3 SNPs were linked to allergy (positive SPT) in the analysed cohort. A significant difference in the prevalence of the atopy-associated SNPs among the genders was also observed.

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O.P.53.
Biotechnology

Immobilization of Enzymes on Silica Nanoparticles

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Oil-based polyols are important starting materials for the manufacture of polyurethanes. Currently, they are produced by a two-step chemo-enzymatic reaction involving epoxidation and hydrolysis [1]. For the first step commercial immobilized lipase (Novozym 435) is applied and for the second step commercial ion exchange resin based catalyst Amberlyst 15 is used [2]. Application of other enzymes with epoxidizing and hydrolyzing activities can achieve more economical and environmental benefits for biopolyol production. The enzyme reusability, operational activity and stability, recovery and self-life can be enhanced by immobilization. In general, three methods of immobilization are used: i) binding to a support (carrier), ii) entrapment (encapsulation) and iii) cross-linking [3].

In this research, enzymes with epoxidizing and hydrolyzing activities were immobilized on solid surface. Immobilization procedure consisted of two steps: sorption of enzyme to amino-modified silica nanoparticles and the subsequent covalent cross-linking using two linkers: glutaraldehyde and triacryloylhexahydrotriazine. Seven objects were choosen for immobilization: four bacterial strains (*B. pumilus*, *P. pertucinogena*, *E10* and *E12* (from metagenomic library) and three commercial yeast strains (*Y. lipolytica* DSMZ 1345, *Y. lipolytica* DSMZ 3286, *C. cylindracea* DSMZ 2031). Optimal conditions for immobilization of samples from bacteria and yeasts origin were determined. Influences of carrier, pH, sorption time, concentration of linker, cross-linking time for immobilization were estimated. Further, the stability of immobilized derivatives under optimal conditions was investigated.

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O.P.54.
Cancer Cell Biology and Biomedicine

Analysis of oncogene GLi1 protein expression levels between differring grades of astrocytoma

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Background: Aberrant expression of oncogene GLI1 has been linked to malignancies in many types of tissues including brain, pancreas, skin and breast [1-4]. It has been found that GLI1 expression is important for tumor progression and has been linked to tumor grade [1,5-7]. In this study, GLI1 expression has been analyzed in different malignancy grade astrocytomas.

Materials and Methods: GLI1 protein expression has been evaluated in 87 clinical tumor samples by using Western blot analysis. Associations between GLI1 expression and tumor grade were analyzed by applying the Mann-Whitney or Kruskal-Wallis tests. Kaplan-Meier survival analysis was performed to evaluate the prognosis of patients.

Results: Western blot analysis did not show statistically significant correlation between GLI1 protein expression and astrocytoma tumor grade (P = 0.294). GLI1 has been detected only in 45 gliomas (52% of total samples), whereas no signal has been detected in 42 gliomas (48% of total samples). Kaplan-Meier survival also did not show correlation between survival rates and GLI1 expression (P = 0.081).

Conclusion: Our results show that GLI1 protein expression levels in astrocytomas have higher variability than previously shown. Furthermore, our data indicates that GLI1 protein expression is not an absolute requirement for the process of gliomagenesis. Yet, the relatively high (52%) occurrence of GLI1 expression in astrocytomas suggest the need for further analysis on the involvement of GLI1-mediated Hedgehog signaling pathway in gliomatumorigenesis.

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O.P.55.
Biotechnology

Better Biocatalysis Through Immobilization of Enzymes

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Enzymes are Natures sustainable catalysts and their biocatalytic processes are more cost-effective, more environmentally friendly and, eventually, more sustainable. Notwithstanding all these advantages, application of enzymes is often hampered by difficult recovery and re-use of the enzymes and a lack of long-term operational stability. [1] These drawbacks can generally be overcome by immobilization of the enzyme. Historically, methods for immobilization are divided into three categories: adsorption on a carrier (support), encapsulation in a carrrier and cross-linking (carrier-free).

In this presentation, a brief overview of the why, what and how of enzyme immobilization for use in biocatalysis will be given. Our latest research results on immobilization of enzymes will be presented. The studies on immobilization of wild type lipase from *Serratia sp*. and its prototypes by CLEA method revealed that it can be successfully applied for the biocatalytic synthesis of 2-ethyl-1-hexyl oleate (biolubricant). Another immobilization method, adsorption on a carrier, will be discussed. Recent results on immobilization of enzymes with lypolytic activity from bacteria and yeast strains on silica nanoparticles will be explored. Additionally, immobilization of other types of enzymes such as laccases [2] and alcohol oxidases briefly will be described.

Financial support by the BIOLUBRICANT project (MITA) and B. Tvaska for implementation of this project are gratefully acknowledged. We thank Boris Kolvenbach for implementation of Sciex Junior Researchers' Fellowship project (IMMOZYME).

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O.P.56.
Molecular biology and Functional genomics

Citokine TGF-β1 - a potential diagnostic biomarker for Cardiovascular patients

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Thoracic aortic aneurysms (TAA) develop asymptomatically and cause aortic ruptures which are associated with high mortality rate. Our laboratory of Molecular Cardiology investigates mechanisms associated with TAA in order to find genetic and biochemical markers that could be implemented in hospitals for early diagnosis of TAA.

A pilot study on patients with TAA (without Marfan syndrome features) focuses on interactions between *Fibrillin1* (*FBN1*) genetic polymorphisms and levels of transforming growth factor beta 1 (TGF-β1) in patients blood plasma. Previous studies have shown that certain *FBN1* polymorphisms increase the risk of developing sporadic TAA but the exact mechanism remains unclear. *FBN1* and TGF-β1 interplay could be one of the major factors causing pathological defects in the thoracic aorta's wall. We

investigated *FBN1* polymorphisms and TGF-β1 concentration in blood of 39 patients undergoing aortic reconstruction due to TAA and compared it to 60 healthy individuals from random population in Kaunas.

TGF-β1 levels were significantly higher ($p < 0.05$) in patients with TAA (mean 7.4) compared to healthy population (mean 4.3). TAA patients were further divided into 3 groups according to their diagnostic categories: aneurysms (21), dissections (5), and post stenotic dilatation (13). Highest levels of TGF-β1 were detected in dissections group (mean 14.5), however due to small patient number the results are not statistically reliable. Aneurysms group showed reduced levels of TGF-β1 (mean 8.3); lowest TGF-β1 concentration was observed in post stenotic dilatation group (mean 6.4).

Our results show that *FBN1* polymorphisms have no significant impact on TGF-β1 blood levels, thus the genetic defects in *FBN1* cause TAA through a different biochemical pathway. We found increased TGF-β1 concentration in TAA patients of dissections group which could be further developed as a biochemical marker in diagnosis of TAA at an early stage.

O.P.57.

Cultivation of Microalgae Chlorella sp. and Scenedesmus sp. as a Potential Triacylglycerol Sources

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Algae are characterised as multifunctional sources used as additive source of food and feed and others high-value derivatives because of their ability to produce and accumulate a variety of useful materials.Two robust algae strains *Chlorella sp.* and *Scenedesmus sp.* growing in Lithuanian lakes were of interest with the aim to involve algae as potential triacylglycerol (TAG) sources.

The growth of algae species and lipids accumulation in mixotrophic conditions in the respect of autotrophic conditions was investigated. The highest concentration of *biomass* was 1.58 and 1.67 g/Lin autotrophic medium for *Chlorella sp*. and *Scenedesmus sp.,* respectively.The investigation of the biomass growth of both algaestrains in the mixotrophic conditions showed that the algae biomass grow well in media prepared with glycerol. The highest concentration obtained in the media with 5 g/L of glycerol for *Chlorella sp., Scenedesmus sp.* was 1.2 and 1.3, times larger than during the autotrophic growth of these species. Mixotrophic conditions improve not only growth of microalgae, but also accumulation of TAG. The content of TAG of 3.79 and 1.39% was reached in autotrophic medium (medium BG11) for *Chlorella sp.* and *Scenedesmus sp.*, respectively. Increases in TAG of up to 10 times was observed for *Scenedesmus sp.* under mixotrophic conditions (with 10 g/L of glycerol), whereas an increase of 4 times was noticed for *Chlorella sp.* (2 g/L of glycerol). The amount of 50% of saturated acids for both species was characteristic for samples grown in autotrophic BG11 medium. The content of saturated fatty acids of *Chlorella sp.* (2 g/L of glycerol), and S*cenedesmus sp.* (10 g/L of glycerol), was 67.11 and 34.63%, and the amount of unsaturated fatty acids was 32.9 and 65.06%, respectively.

Investigated cultures could be declared as a promising resource for lipids source regarding the possibility to regulate lipid content and fatty acid composition of oil with additives in growing medium.

Posters presentations:

P.P.1.
Molecular mechanisms in Cell Biologyand Virology

Regulation of calcium-induced opening of MPTP by cyclosporin A and rotenone in mitochondria isolated from rat cerebral cortex and cerebellum

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Mitochondrial damage, particularly opening of mitochondrial permeability transition pore (MPTP), is thought to be critical in ischemic insults and neurodegeneration. The opening of MPTP can induce mitochondrial depolarization and inhibition of ATP synthesis leading to cell death. Therefore, pharmacological inhibition of MPTP may be powerful strategy to improve brain tissue functions in pathological situations. Mitochondria isolated from various brain regions were shown to have different sensitivity to calcium induced MPTP and this may account for different vulnerability to ischemic stress. In this study we investigated mitochondria isolated from different brain areas- cerebral cortex and cerebellum andtested effects of selective inhibitor of MPTPcyclosporin A (CsA) and respiratory chain complex I inhibitor rotenone to calcium induced MPTP measured as calcium retention capacity (CRC). Respiration and CRC were similar in both types of mitochondria. In the presence of 0.5 μM CsA70% increase of CRC of cortical mitochondria was observed, however, 5 μM CsAconcentration was necessary to increase CRC of cerebellum mitochondriaby 70%. We also found that rotenone (1 μM) was more potent than CsA and increased CRC by 100% and 115% in cerebral cortex and cerebellum mitochondria, respectively. Importantly, CsA and rotenone acted synergistically and significantly increased CRC of both, cortical and cerebellum mitochondria (up to 170% and 180%, respectively). These results demonstrated that inhibition of complex I may regulate MPTP and that CsA and rotenone can act in synergistic protective manner against

calcium induced opening of MPTP in isolated rat cerebral cortex and cerebellum mitochondria.

P.P.2

Molecular mechanisms in Cell Biologyand Virology

The role of MAP kinases in endothelial progenitor cells exposed to oxidative

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Endothelial progenitor cells (EPCs) play an integral role in the cellular repair mechanisms for endothelial regeneration, maintenance, and postnatal vasculogenesis. However, inflammation-associated oxidative stress diminishes the number and bioactivity of EPCs [1].

The aim of this work was to elucidate the role of mitogen activated proteinkinases (MAPKs) in blood vessel-associated endothelial progenitor cells exposed to oxidative stress.

Blood vessel-associated EPC were established from rat pulmonary trunk. Hydrogen peroxide was used to mimic oxidative stress *in vitro*. The effect of oxidative stress on EPC migration and regenerative functions was assessed using live cell imaging technology – Cell-IQ(CM Technologies).AO/EB staining was applied to determine the mode of cell death. Expression and phosphorylation of signalling molecules was evaluated via Western blot analysis method. In order to determine the role of ERK, JNK, and p38 MAPK in EPC during oxidative stress, specific inhibitors were used: PB098059, SP600125, and SB203580, respectively. Transcription factor c-Jun generated biological response to oxidative stress was assessed using genetically engineered EPC: with up-regulated and down-regulated c-Jun expression.

The data obtained proved the apoptotic mode of cell death. A decreased migration rate of cells exposed to the oxidative stress was observed. The studies of the role of MAPKs in oxidative stress-induced cell death revealed the proapoptotic action of ERK, JNK, and p38. Transcription factor c-Jun played distinct, prosurvival action in blood vessel-associated EPC in response to oxidative stress. Moreover, ERK, JNK, p38 MAPKs are implicated in the phosphorylation/activation of c-Jun.

Acknowledgments. This work was financially supported by the European Social Fund under National Integrated Programme grant No VP1-3.1-SMM- 08-K01-001.

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P.P.3.
Molecular mechanisms in Cell Biologyand Virology

SNX482 inhibits Semaphorin 3A induced DRG growth cone collapses and Ca2+ elevation

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During development and regeneration of the nervous system axon pathfinding is controlled by various guidance molecules. One of the guidance molecules repelling axons during development and inhibiting regeneration after injuries of the nervous system isSemaphorin 3A (Sema3A). Although extensive researches are being carried to understand Sema3A signaling pathways, they still remain elusive.

The aim of this study was to investigate if E15 mouse embryo dorsal root ganglion (DRG) axon growth cone collapse in response to Sema3A is Ca^{2+} dependent process. In order to reach the goal intracellular Ca^{2+} imaging by Ca^{2+} sensitive dye Fura2 as well as growth cone collapse assays were employed. Ca^{2+} dynamic in different compartments of neuron, namely bodies, axon parts and growth cones were investigated. Moreover different Ca^{2+} channel and pump blockers were used to evaluate influence of Ca^{2+} channels/pumps in response to Sema3A.

Results showed that Sema3A induces sustained and prolonged Ca^{2+} elevation in growth cones but not in neuron bodies or axons. Moreover growth cone collapse assay, showed that non-selective voltage gated Ca^{2+} channel blockers (Cd^{2+} and Ni^{2+}) do inhibit Sema3A induced growth cone collapses. Finally by using selective R-type Ca^{2+} channel blocker SNX482 we showed, that Sema3A induced increase in intracellular Ca^{2+} is significantly reduced in presence of SNX482. This result was further supported by growth cone collapse assay showing that inhibition of R-type Ca^{2+} activity significantly decreases Sema3A induced growth cone collapse rate. Although this finding is primarily fundamental, results of the study can also be employed for developing novel strategies for the treatment of the injuries of nervous

P.P.4.
Molecular mechanisms in Cell Biologyand Virology

The role of U2AF in hypoxia-dependant pre-mRNA splicing regulation

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Oxygen (O_2) is essential for the life of all aerobic organisms. In mammals, at the whole body level, oxygen supply is optimized by tight regulation of ventilation, arterial blood hemoglobin saturation and systemic oxygen transport. The oxygen tension has been demonstrated to be a key regulator to optimize specific organ functions. Hypoxia-inducible factors (HIF-1; HIF-2; HIF-3) play a central role in oxygen-regulated gene expression. HIFs DNA binding complex

consists of a heterodimer of HIF-α and HIF-β subunits. All these proteins belong to the family of basic helix-loop-helix (bHLH) proteins that contain a PAS domain. HIFs bind to hypoxia responsive elements (HRE) and activate the transcription of a variety of genes involved in the regulation of erythropoiesis, angiogenesis, vasomotor control and energy metabolism. In mice a dominant negative regulator of hypoxia-inducible gene expression (IPAS) is generated in hypoxic cells by alternative splicing from HIF-3α pre-mRNA.

One of the essential splicing factors which play an important role in early splicesome complex formation is the U2AF, which comprises of two subunits – the 65 kDa and the 35 kDa. U2AF is responsible for recruiting the U2 snRNP to the branching point sequence.

Our research tries to identify the mechanism by which cells, exposed to hypoxia, regulate gene splicing and produce alternative isoforms products of which are important for cell survival under hypoxic conditions. It has been observed that in the U2AF heterodimer formation the 65 kDa subunit interacts with the 35 kDa subunit differently in cells under hypoxia compared to normoxic cells. This feature is currently under investigation.

P.P.5.
Molecular mechanisms in Cell Biologyand Virology

New Insights Into Investigation Of Chemical Gating Of Connexin 36 By Altering Cysteine Residues

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Proper regulation of connexin 36 (Cx36) gap junctions (GJ) is critically important under normal and pathological conditions of central nervous and secretion systems. Alcohols are an attractive model to study the mechanisms of anesthesia in CNS. We examined the weightiness of cysteines in chemical gating of Cx36 GJs using short carbon chain alcohols (SCCAs: pentanol, hexanol and heptanol) which stimulate and long carbon chain alcohols (LCCAs: octanol, nonanol, and decanol) which inhibit Cx36 GJ conductance (g_j). In our previous study, the cutoff for Cx36 *wt* g_j stimulation by SCCAs was determined to be heptanol.

Based on the resolved crystal structure of Cx26, 3-D structure of Cx36 was generated, and the cysteines of transmembrane domains 2 and 4 (TM2 and TM4), and cytoplasmic loop (CL) were suggested as possible targets involved in chemical gating by alcohols. To examine experimentally this assumption, we constructed a set Cx36 mutants having following mutations: C87S (TM2), C92V (TM2), C168A (CL) and C264S (TM4). In addition, mutants having the deletions of C87 and CL loop were constructed.The properties of Cx36 mutants were examined in HeLa transfectants using dual cell patch-clamp set-up.

Cysteine substitutions caused significant changes in response of Cx36 GJ channels to alcohols and voltage. C264S and C87S mutations increased the sensitivity to voltage gating, while C92V and C168A decreased it. C87S and C92V reduced the cutoff for stimulation of g_j by SCCAs to hexanol and to pentanol, respectively, while C168A did not modify the stimulatory effect of SCCAs on g_j. C264S reversed the effect of SCCAs on Cx36 g_j from stimulation to inhibition. C92V, C168A and the deletion of CL loop decreased the g_j blocking efficacy of nonanol.

Obtained data demonstrate for the first time that cysteines residing in the TM2 and TM4 domains play an important role in binding of SCCAs and regulation of Cx36 GJ gating. Structural modeling and experimental data suggest that TM2 domain and CL loop may be involved in forming the binding cavity/ies for LCCAs.

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P.P.6.
Molecular mechanisms in Cell Biologyand Virology

Immune complexes of antibodies with Aβ oligomers cause neuronal death in primary neuronal-glial culture by activating microglia

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Alzheimer disease (AD) is the most common dementia disorder in the elderly. Recent research suggests that amyloid-beta (A) oligomers may contribute to the pathogenesis of AD, thereby leading to A as a target for treatment. A immunotherapy, either, immunization with A or administration of A antibodies, was successful in preclinical studies, however, clinical trials were halted due to brain inflammation, the mechanisms of which are poorly understood. In the present study we aimed to investigate the effects of antigen-antibody complexes formed by Aand specific monoclonal antibodies in primary neuronal-glial cultures. We found that complexes formed by A oligomers or by oligomeric virus proteins and their specific antibodies can cause death and loss of neurons. Antibodies bound to monomeric A or virus protein fragments were non-toxic to cultured neurons. The neurotoxicity of antibody-oligomeric antigen complexes was abolished by removal of the Fc region from the antibodies or by removal of microglia from cultures, and was accompanied by inflammatory activation and proliferation of the microglia in culture. In conclusion, immune complexes formed by A oligomers or other oligomeric antigens and their specific antibodies exert strong toxic effects on neuronal cells via Fc-dependent microglial

activation. Therefore, if endogenous antibodies to oligomeric proteins cross the blood-brain barrier, they may cause neuroinflammation and neurotoxicity. The results suggest that therapies resulting in antibodies to oligomeric A or oligomeric brain virus proteins should be used with caution or with suppression of microglial activation.

This work was supported by the Research Council of Lithuania (grant LIG-04/2012 MALPAMA).

P.P.7.
Molecular mechanisms in Cell Biologyand Virology

Model system for research of the influence of oligomeric proteins and their immune complexes on the phenotype of macrophages

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BACKGROUND: Macrophages, cells of the immune system, play a key role in defending organism from intracellular and extracellular pathogens. In certain pathologies, chronic inflammation can be a result of the immune system disorders. As a consequence, various tissues can be damaged, for example the neurodegenerative process can occur in the central nervous system. One of these pathologies is Alzheimer's disease (AD). This disease is caused by β-amyloids (Aβ), especially $A\beta_{1-42}$ oligomers, which are detected in the brain tissue of the AD patients. Immunotherapy was appliedin order to decrease the effects ofAβ. However, the clinical research was discontinued due to the side effects such as meningoencephalitis. The aim of this work is to investigate the influence of $A\beta_{1-42}$ oligomers and their immune complexes on macrophage phenotype.

METHODS: The study model is macrophage cell line J774, originated from a BALB/c line mouse. The research model of various macrophage activation states was based on the classically activated M1 and alternatively activated M2 macrophage population markers. The variations in the levels of cell markers - M1 marker CD86; M2 marker CD206; phagocytosis marker CD68; macrophage and cell activation marker F4/80 - were investigated by flow cytometry.

RESULTS: After treatment of J774 cells with $A\beta_{1-42}$ oligomers and their immune complexes an increase in expression of cell activation markers CD68, CD86 and F4/80 was detected. However, there was no change in the expression of CD206 marker.

CONCLUSION: Based on our research, activated mouse macrophages J774 show changes in CD68, CD86 and F4/80 expression. This suggests that activation of macrophages with $A\beta_{1-42}$ oligomers and their immune complexes can lead to the inflammatory phenotype M1.

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P.P.8.
Molecular mechanisms in Cell Biologyand Virology

Role of the beta-glucans for the action of Saccharomyces cerevisiae K2 toxin

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Budding yeast produces four types of dsRNA virus-encoded killer toxins (K1, K2, K28, and Klus) capable of killing non-killer yeast as well as killer yeast of the different types. Despite some similarities in their mode of production, killer toxins have different biochemical properties and profoundly distinct modes of action. While K28 toxin primarily acts in the nucleus of the host cell interfering with gene expression, K1 and K2 are active at the cell wall where they ultimately disrupt the structural and/or functional integrity of the plasma membrane. Mode of action of the Klus toxin is not completely understood.

In order to explore the killing mode of K2 toxin, we investigated the dynamics of K2 protein binding to the cell wall-localized receptors, evaluated the dependence of K2 binding and killing activities on the presence of different glucans *in vitro* and *in vivo*,and demonstrated the essential role of beta-1,6-glucan in the action of the killer protein. It was experimentally confirmed that increased level of beta-1,6-glucan is directly correlated with the number of toxin molecules bound, the sensitivity to K2 and changes in cell morphology, whereas decreased level of such glucan augments the yeast survival. We established that cytotoxic effect of the K2 toxin on the*S. cerevisiae* cells assert very quickly, when fast decline of living cell population occurs due to the toxin-induced damages.

P.P.9.
Biotechnology

Muscle-derived stem cell and blood vessel-associated endothelial progenitor cell interactions with 3D printed PLA scaffolds

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Materials with optimal mechanical properties, biodegradability, biocompatibility and biological activity are critical to the success of tissue engineering. Moreover, cheap and efficient technologies allowing the formation of scaffolds with desired architecture and pore size are required. Such

structures should mediate the crosstalk between cells and biomaterial surface. PLA (polylactic acid) – an *in vivo* biodegradable polyester – fulfills all of these requirements.
The aim of this study was to investigate muscle-derived stem cell and blood vessel-associated endothelial progenitor cell differentiation as well as to study the effect of Akt kinase on adhesion and proliferation of the cells grown on different PLA scaffolds.
3D printing (fused filament fabrication) was employed to create scaffolds for stem cell investigation [1]. The viability and morphology of the cells grown on the scaffolds was evaluated by light, fluorescent and scanning electron microscopes. Myogenic and osteogenic cell differentiation was induced and evaluated according to Kalvelyte et al [2]. The role of Akt kinase for cell adhesion and proliferation on the tested scaffolds was evaluated in cells overexpressing Akt kinase. Cellular adhesion and proliferation rates were determined by shaking and MTT test, respectively.
We have found that the scaffold properties such as porosity, pore distribution, hydrophilicity and surface topography influence many vital cellular responses – from the rate of scaffold infiltration up to the entire tissue engineering. The results of this work are encouraging for future research employing 3D printing and using PLA as a scaffold material and progenitor cells in creating tissue engineering products.
This work was supported by the European Social Fund under National Integrated Programme Biotechnology & Biopharmacy, grant VP1-3.1-SMM-08-K01-005.
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P.P.10.
Biotechnology

Properties and biocontrol potential of endophytic bacteria from apple (Malus × domestica Borkh.) buds

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Inner tissues of plants are inhabited by various microorganisms. At least parts of them are presumed to have beneficial effect on plant health. Endophytic microorganisms are attractive object for biotechnological studies due to their potential in plant growth improving and biocontrol. They are also in focus of research interest as a promising source of applicable compounds.
In this study we estimated properties of bacterial isolates from apple tree buds which are potentially involved in interaction with host plants and other plant inhabiting microorganisms, such as IAR, siderophore, HCN production, and chitinase activity. We also established that some bacteria of apple endophytic origin were able suppress growth of other endophytic isolates as well as laboratory strains of *Micrococcus luteus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium* and pathogen fungus of domestic apple *Venturia inaequalis.*
Analysis of 16S rRNA sequence showed that among obtained isolates pseudomonads were most abundant, also members of genera *Pantoea, Curtobacterium, Bacillus, Stenotrophomonas*were present.

P.P.11.
Biotechnology

ProMer™ technology – extended plasma half life of biopharmaceuticals

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Biological drugs became significant players in the pharmaceutical market. Nonetheless, biological drugs based on recombinant proteins (or so called First Generation Biopharmaceuticals) may have some disadvantages such as their instability in the plasma, limited solubility, fast clearance from plasma, immunogenicity or toxicity. Due to these reasons a lot of efforts are focused to developing biological drugs with improved pharmacokinetic pK and (or) pharmacodinamic pD.
The current research was aimed to develop an innovative technology for biologicals with improved pK/pD properties. ProMer™ technology involves genetic fusion of two proteins through the specific linkers. We adapted this approach to granulocyte-colony stimulating factor (G-CSF). Three different types of polypeptide linker of fused proteins were analyzed; non-helical glycine-rich, containing 11 or 34 amino acids, and helical, containing 54 amino acids. All homodimers showed improved pK or/and pD properties in comparison with monomeric protein. The best characteristics have been obtained in the case of protein with alfa-helical linker. Within current study a procedure for the purification of the latter protein was optimized. The purity of rhG-CSFLα reached 98% and mass recovery of refolded and purified dimeric rhG-CSFLα was 15%. The homodimeric protein was characterized using a set of analytical methods and compared with G-CSF monomer. In conclusion, the successful production and advantageous properties of this new recombinant fusion protein represent a promising approach to a clinical therapy.
ProMer™ technology is currently expanded for the development of heterodimer constructs containing synergetically acting partners of various growth factors of therapeutical value.

P.P.12.
Biotechnology

Better Living Through Biocatalysis: Synthesis of Biopolyols from Natural Oils

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Petrochemical based polyols, which are currently used for polyurethane production [1], depend directly on the petroleum and its price. In addition, the petrochemical based products increase CO_2 production which is claimed to cause global warming. Therefore, bio-based polyol is an excellent alternative which is inherently sustainable, renewable and biodegradable. The aim of our current research is to develop eco-friendly polyols form natural oil – rapeseed oil, which is a cheap and abundant raw material in Lithuania [2].

We perform the synthesis of polyols in a two stage process: chemo-enzymatic epoxidation of the double bonds and latter nucleophilic cleavage of the epoxide ring to form polyols, in this case - hydrolysis of the epoxide ring. By this current research we investigated temperature and reaction time influence for the oxirane oxygen content formation during enzymatic epoxidation process [3]. Further, the hydrolysis of epoxidized rapeseed oil was investigated to determine optimum reaction parameters.

The obtained results conclusively demonstrated a sustainable chemo-enzymatic process for the synthesis of polyol from rapeseed oil. The results will be presented in more detail during the conference at the poster session.

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P.P.13.
Biotechnology

<u>Accumulation of dehydrin-like proteins in micro-shoots of plants of Rosaceae family during cold acclimation in vitro</u>

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For Lithuania and related northern regions, cold damage to plants is a major problem in agriculture. Plants of *Rosaceae* family, such as strawberry, apple, sweet and sour cherry, are often damaged in winter due to the lack of acclimation and cold hardiness. Therefore improvement of winter hardiness traits and development of biological markers directly and indirectly associated to cold acclimation traits are among the main goals of modern horticultural breeding practices. During cold acclimation, proteins of dehydrin family are accumulated in plant cells. There is only limited data on dehydrin proteins of *Rosaceae* family plants that presents only fragmented view on the role of the protein family in cold hardiness of the *Rosaceae* plants. Therefore, the aim of this research is to establish diversity of the dehydrin-like proteins characteristic for *in vitro* cold-acclimated microshoots of pear, apple, cherry and strawberry. It was demonstrated that incubation of microshoots under low temperature (4 ^{o}C) conditions induces acclimation and leads to decrease in damage to microshoot tissues in all genotypes included in the study. Maximum reduction of the KT_{50} values was from 0.7 to 2.1 ^{o}C. Dehydrin-like protein immunoblot analysis using polyclonal antibodies against the K-segment [Close, 1993] revealed species and genotype specific accumulation pattern of dehydrin proteins during the acclimation process. Presented data on analysis of dehydrin-like proteins for the selected pear genotypes demonstrated that up to eight dehydrin-like protein bands were differentially expressed in microshoots during cold acclimation. The results of effect of different medium composition indicated that cytokinin plant growth regulator or mannitol had limited effect on cold-induced stress response under *in vitro* conditions.

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P.P.14.
Biotechnology

<u>Oxidative stress injury in apple shoot culture in vitro</u>

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Vegetative propagation methods are commonly used for horticultural plants of *Rosaceae* family, such as apple, pear, cherry, peach and strawberry. Development of *in vitro* micropropagation methods is important for plant biotechnology research and agricultural applications. *In vitro* environment, such as cultivation media supplemented with sugars and growth regulators, low irradiance, low CO_2 concentration during light period and high air humidity, imposes unfavorable conditions that lead to imbalance of plant physiological equilibrium and induction of stress. Production of reactive oxygen species (ROS), such as superoxide and hydroxyl radicals, hydrogen peroxide, singlet oxygen, is important manifestation of plant environmental stress. ROS cause lipid peroxidation leading to membrane injury, enzyme inactivation and DNA damage. The aim of this study was to characterize oxidative stress and injury in apple (*Malus* × *domestica* Borkh.) microshoots grown under *in vitro* conditions. Microshoots of apple cv. Gala, Golden Delicious, Orlovim and hybrid Noris × Paprastasis Antaninis were cultivated on MurashigeandSkoog medium supplemented with 0,75 mg/l 6-benzylaminopurine and 3% sucrose. Production of ROS and oxidative stress injury was assessed during replanting, growth and senescence stage of the microshoot culture. ROS production in microshoot tissues was investigated by histochemical staining using superoxide

and H_2O_2 specific dyes NBT and DAB. Superoxide production was mostly detectable in leaf and injured tissues. A more uniform staining with higher intensity at upper parts of stems of microshoots was characteristic to the DAB staining. An analysis of lipid peroxidation using quantitative TBARS assay was applied to estimate level of oxidative stress injury. The results demonstrated that damage of membrane lipids of *in vitro* apple microshoots is more prominent during first week after replanting and during onset of senescence of the culture.

P.P.15.
Biotechnology

DNA fragmentation with modified Mu transposons for the next-generation sequencing

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Phage Mu is a mobile DNA element encoding MuA transposase, the critical catalytic component of the integration machinery. Minimal Mu transposition reaction composed of MuA transposase, 50 bp Mu transposon end segments, and target DNA as the only macromolecular components is used in a variety of applications, including sample preparation for the next-generation sequencing. Transposons containing modified sequences, either in an attacking strand or in both DNA strands, can be used for transposon/transposase complex formation and subsequent fragmentation of DNA of interest. As a result, transposition reaction products contain uniform terminal sequences which length and structure are determined by a modified transposon end sequence position in primary transposons. In addition, the target sequence specificity of the substituted transposon/MuA complexes was assessed and was shown to differ substantially from that characteristic to the non-modified complexes.

P.P.16.
Biotechnology

METAGENOMICS – A MODERN METHOD FOR SEARCHING OF POTENTIAL BIOCATALYSTS

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The feature of enzymes (biocatalysts) to operate effectivelly not only inside the cell, but also *in vitro* is essential for biocatalytic application of enzymes for various scientific and industrial needs. Therefore, the demand for enzymes with new or specific characteristics is constantly increasing. Currently, in the laboratoryis possibleto cultivateless than 1% of themicroorganismsfound inthe nature, which is why only a smallpart of theirgenetic diversity can be covered by standardmicrobiologicalmethods? Metagenomics is one of the modernmethodsfor searching andinvestigation of new enzymes, thus, reducing thecultivationproblem [1]. This method is based on the direct extraction and analysis of total DNA in the environmental samples.

The aim of our work was to detect potential biocatalysts in the constructed metagenomic DNA library. DNA extracted from sludge was fractionated, ligated with multicopy pUC19 vector and transformed into *E. coli* DH10B cells. Construction of metagenomic DNA library resulted in more than 3400 recombinant clones. Functional analysis of the recombinants on the selective growing media revealed several clones with potential enzymatic activity. The partial sequence of the inserts revealed the presence of peroxidase, sulfatase, acylesterase, arylsulfatase genes. The results of functional analysis, protein electrophoresis and performed DNA sequencing will be presented in more details during poster session.

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P.P.17.
Biotechnology

Inhibition resistance of mutant reverse transcriptases

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Reverse transcriptases (RT) are often used in variety of assays to make cDNA from RNA. The activity of these enzymes can be compromised by various inhibitors coming from RNA source, RNA purification or preservation components, or the reaction itself. Therefore a lot of effort is put to improve RT inhibition resistance by mutagenesis. This work is focused on the M-MuLV RT mutant (mut7-10) with seven mutations that cumulatively result in higher inhibitor tolerance. Seven M-MuLV RT point mutants with individual mutations found in the mut7-10 protein were constructed and analyzed seeking to identify specific mutations that contribute to inhibitor resistance and understand the mechanism of resistance. Mut7-10 shows higher resistance to sodium dodecyl sulfate (SDS), formamide, xylan and heparin than wild type M-MuLV RT. Our results indicate that different residues in mut7-10 are responsible for resistance to formamide, SDS and xylan or heparin, thereby suggesting different molecular mechanisms determining inhibitor tolerance in RT enzyme.

P.P.18.
Biotechnology

In vitro evolution of phi29 DNA polymerase using isothermal compartmentalized self-replication (iCSR) technique

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Compartmentalized self replication (CSR) is widely used for in vitro evolution of thermostable DNA polymerases able to perform PCR in emulsion. We have modified and adapted CSR technique for isothermal DNA amplification using mezophilic phi29 DNA polymerase and whole genome amplification (WGA) reaction. Library of phi29 DNA polymerase mutants was subjected for seven rounds of isothermal CSR (iCSR) selection. After the selection polymerase variant containing most frequent mutations was constructed and characterized. Mutant phi29 DNA polymerase can perform WGA at elevated temperatures (37^{o}C – 45^{o}C), generate more (2-5 folds) of DNA amplification products, and has significantly increased half-life at 30^{o}C, 37^{o}C both in the presence or absence of DNA substrate. Moreover WGA of *E.coli* genomic DNA performed with mutant polymerase at 42^{o}C has generated less DNA amplification bias comparing to the same WGA reaction performed at 30^{o}C with wild-type enzyme.

P.P.19.
Biotechnology

Production and Charakterization of Membrane-Bound Carbonic Anhydrase XIV

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Carbonic anhydrase (CA; E.C.4.2.1.1) are zinc metalloenzymes that occur in human tissues as fifteen (CA I-XIV) genetically distinct isozymes, twelve of which catalyze the reversible hydration of carbon dioxide. The CAs arebeing extensively studied because of their broad physiological importance in all kingdoms of life and clinical relevance as drug targets. CA XIV is a membrane-bound enzyme that is expressed in numerous metabolically active tissues such as the brain and retina, kidney, liver, heart and skeletal muscle, where its activity figures prominently in the regulation of extracellular *pH* [1]. CA XIV is the major membrane associated enzyme in human retina and plays an important part in producing a normal retinal light response [2]. Furthermore, the finding of such intense CA XIV activity in retinal pigment epithelium suggests that CA XIV is the retinal target of CA inhibitors, when used to treat cystoid retinal edema [3]. Therefore, the development of more selective CA inhibitors shall improve the understanding of this CA function and treatment of macula edema with lower side effects.
In our research, various physical properties such as stability profile, protein-inhibitor interaction thermodynamics and enzyme kinetics were determined for human CA XIV. We worked out the expression conditions for the production of soluble and catalytically active recombinant CA XIV in bacterial cells. The catalytic domain of CA isoform was purified using metal chelation and affinity chromatography. Thermodynamics of binding was determined by isothermal titration calorimetry and the fluorescent thermal shift assay. Investigated stability properties can be exploited for finding optimal purification and storage conditions. The intrinsic enthalpies, entropies, and the Gibbs free energies provide the direction for the improvement of the compounds binding affinity and selectivity.

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P.P.20.
Biotechnology

Development and characterization of monoclonal antibodies against recombinant metapneumovirus nucleocapsid protein

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Human metapneumovirus (hMPV) is a member of the *Pneumovirinea* subfamily within the *Paramyxoviridea* family. Since its discovery in 2001, hMPV has been isolated in several continents, which suggests its worldwide prevalence. hMPV resembles human respiratory syncytial virus with regard to disease symptoms and its ability to infect and cause disease in young infants as well as individuals of all ages. The aim of the current study was to develop hMPV N protein-specific monoclonal antibodies (MAbs) and evaluate their diagnostic potential. The major nucleocapsid gene of hMPV was cloned and expressed in *S. cerevisiae* yeast cells. Expression of recombinant protein in yeast cells led to the formation of nucleocapsid-like nanoparticles. A panel of MAbs was generated after immunization of mice with recombinant hMPV N protein. The specificity of the newly generated MAbs was confirmed by immunofluorescence analysis of hMPV-infected cells. Epitope mapping using truncated variants of hMPV N revealed the localization of linear MAb epitopes at the N-terminus of hMPV N protein, between amino acid residues 1 and 90. The MAbs directed against conformational epitopes did not recognize hMPV N protein variants containing either N- or C-terminal truncations. The reactivity of recombinant hMPV N protein with hMPV-positive serum specimens and the ability of MAbs to recognize virus-infected cells confirm the antigenic similarity between yeast-expressed hMPV N protein and native viral nucleocapsids. In conclusion, recombinant hMPV N protein and hMPV-specific MAbs provide new diagnostic reagents for hMPV infection.

P.P.21.
Biochemistry of Prokaryotes

The Change of Amount of Surfactin in Suspension of Bacillus subtilis

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There are various surfactants widely used in domestic abstergents and cleaners, detergents, foam forming agents, emulsifiers, etc. Unfortunately, most of surfactants used in industry and daily needs are synthetic and toxic to the environment. Biosurfactants are less harmful, surface-active, better foaming substances that works selectively in extreme temperatures, pH and salinity conditions [1, 2]. The extraction of these substances in big amounts by using microorganisms is a difficult and expensive process. On purpose to increase the synthesis and to optimize the extraction of biosurfactants, cheaper growing conditions and effects of various agents are being investigated. The gram-positive bacteria *Bacillus subtilis* which produces a high surface-active anionic biosurfactant surfactin was chosen for the research. Surfactin is also valuable for its antibacterial and antiviral activities [3, 4]. The aim of this research was to examine the influence of synthetic surfactants on growth and production of surfactin by *Bacillus subtilis*. Fluorescence spectroscopy method was used for the estimation of produced surfactin in the suspension of *B. subtilis* [5]. The bacteria were grown at various temperatures in LB medium (Lennox) with the addition of iron salt, glucose, anionic or nonionic synthetic surfactant. It was estimated that the production of surfactin depends on various growing temperatures, ferrous salt and glucose solutions, and the presence of synthetic surfactants in the suspension of *B. subtilis.* The results showed that anionic sodium dodecyl sulphate as a competitive surfactant did not increase the production of surfactin compared to the values of surfactin, produced in medium without this supplement, independently of chosen temperatures, but the growth of bacteria was not suppressed. Suppressed growth of bacteria and surfactin production by addition of cocamidopropyl betaine may be explained by the antibacterial activity of quaternary nitrogen atom.

P.P.22.
Biochemistry of Prokaryotes

The impact of AcrAB-TolC efflux pumps activity on to Anthraquinone dye sorption into gram-negative bacteria

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AcrAB-TolC efflux pumps can transport a large variety of substrates, such as antibiotics, dyes, detergents from the periplasm to the extra-cellular spaces [1]. A tripartite AcrAB-TolC efflux pump is composed of AcrA, AcrB and TolC components, and is the most important multidrug efflux pump system responsible for gram-negative bacteria resistance to lipophilic and amphiphilic drugs [2].

The aim of this work is to evaluate the impact of activity of the AcrAB-TolCefflux pumps on the Anthraquinone dye sorption into gram-negative bacteria. There were done the accomplished experiments of sorption of tetracycline into gram-negative bacteria in same conditions to Anthraquinone dye sorption experiments.

In this work were used in KTU laboratory newly synthesized Anthraquinone dyes RB 4-35 and RB 5-37, different in one nitro- and one methyl- functional groups. The experiments were done with 6 different gram-negative bacteria cultures: native *E. coli* KMY-λ, *S. enterica* DS88, SL 1347 and *S. enterica* mutant species L664, ΔacrB, ΔtolC.

The research was accomplished in use of spectrophotometric analysis.

The results of this research confirmed hypothesis that anthraquinone dyes are AcrAB-TolC efflux pump substrates. It was also established that the dye sorption in different gram-negative bacteria depends on the dye chemical composition. Comparative experiments with tetracycline showed that thisantibiotic not AcrAB-TolC efflux pump substrates.The equilibrium amount of tetracycline sorbed to *S. enterica* bacteria independently to their mutations was from four to more than ten times higher than the amount of anthraquinone dyes.

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P.P.23.
Biochemistry of Prokaryotes

Base flipping by methyl-directed restriction endonucleases

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Bacterial methyl-directed restriction endonucleases, specific for 5-methylcytosine (5mC), 5-hydroxymetylcytosine (5hmC) or glucosylated hydroxymethylcytosine (5ghmC) are promising molecular tools for the studies of 5mC and 5hmC distribution in eukaryotic genomes.

We have focused our research on elucidating the mechanism of modified DNA recognition by two groups of methyl-directed endonucleases: MspJI family enzymes specific for 5mC and 5hmC in various sequence contexts and PvuRts1I family enzymes that recognize 5hmC or 5ghmC. Recently, several structures of enzymes belonging to the MspJI and PvuRts1I families were solved. Comparison with known protein structures revealed that DNA-binding domains of these proteins are similar to the eukaryotic SRA domains that use base flipping for the recognition of 5mC. Despite similar structures, direct evidence for base flipping by MspJI and PvuRts1I-like enzymes is still missing.

We probed base-flipping by MspJI and PvuRts1I-like enzymes using two methods: a cytosine derivative pyrrolocytosine (pyC), which becomes fluorescent upon extrusion from DNA double helix, and a chemical assay, which is based on extrahelical thymine oxidation with KMnO4. Though pyC fluorescence assay was successfully applied to demonstrate base-flipping by another restriction enzyme McrBC, no increase in fluorescence was observed with MspJI and PvuRts1I endonucleases. Presumably, the protein pocket of these enzymes can not accommodate the bulky pyC residue. On the contrary, the chemical assay clearly indicated that MspJI and PvuRts1I-like enzymes flip out the target base. We conclude that modified cytosine recognition mechanism is conserved among eukaryotic SRA domains and prokaryotic restriction endonucleases: all these proteins flip out the modified base from duplex DNA.

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P.P.24.
Biochemistry of Prokaryotes

The antibacterial activity of cotton fabrics treated with silver compounds and zirconium chloride solution against Escherichia coli and Staphylococcus aureus bacteria

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The mechanisms behind the activity of nanoscaled silver onbacteria are not fully elucidated, the three most common mechanisms of toxicity proposed to date are: uptake of free silver ions followed by disruption of ATP production and DNA replication, silver nanoparticle and silver ion generation of ROS, and silver nanoparticle direct damage to cell membranes [1]. Inorganic compounds are interesting for their wide variety of physical and chemical properties and also for their antibacterial activity. The metal oxide nanoparticles such as TiO_2, MgO, and ZnO are reported to be superior antimicrobial agents in terms of safety, durability and heat resistance comparing with conventional organic antibacterial agents [2].

There was studied the antibacterial activity of cotton fabrics treated with suspension of nanoscaled silver, silver nitrate and zirconium chloride solutions. It was also evaluated the antibacterial activity of cotton samples covered with organic-inorganic MTX sol, containing silicon, zirconium, carbon. The antibacterial activity of fabrics samples was estimated for *E. coli* and *S. aureus* bacteria according to the AATCC 100-1999 standart method and using agar diffusion plate test (ISO20645:2004) method. According to the both methods, it was found that cotton specimens treated with silver nitrate have stronger antibacterial activity against *E. coli* and *S. aureus* bacteria than specimens treated with nanoscaled silver suspension. It was estimated that the antibacterial activity of specimens treated with silver suspension and covered with MTX sol was increased against both bacteria comparing with treated non-covered specimens. However, the antibacterial activity of specimens treated with silver nitrate and covered with MTX sol was decreased for both bacteria. The antibacterial activity of the samples treated with zirconium chloride was higher value than those of treated with silver nitrate.

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P.P.25.
Biochemistry of Prokaryotes

ATP-dependent endonuclease of a stress-sensitive restriction-modification system from Corynebacterium glutamicum

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(Putative) stress-sensitive restriction-modification (RM) system CglI from *Corynebacterium glutamicum* is organized of three genes: methyltransferase (M.CglI), putative restriction endonuclease (R.CglI) and predicted DEAD-family helicase/ATPase (N.CglI). Here we report preliminary biochemical characterization of the R.CglI and N.CglI proteins. First, we overproduced in *E.coli* isolated individual R.CglI and N.CglI proteins. Next, we co-expressed both proteins using a single vector and isolated the R.CglI-N.CglI complex. Size-exclusion chromatography revealed that R.CglI and N.CglI proteins form dimers. We show that R.CglI and N.CglI proteins assemble in a complex with R2N2 stoichiometry. Next, we show that N.CglI has ATPase activity that is absolutely dependent on double stranded DNA and is stimulated by R.CglI. On the other hand, we demonstrate that the functional N.CglI ATPase and ATP hydrolysis (~175 ATP/s/monomer) are absolutely required for the site-specific DNA cleavage by the R.CglI restriction enzyme. We demonstrate that ATP-dependent DNA cleavage by R.CglI occurs at fixed position 6-7 nucleotides downstream of the recognition sequence 5'-GCCGC-3'. The CglI RM system is more similar to the Type I rather than the Type II RM system. However, it differs from the typical Type I system by the subunit organization, cleavage pattern, and the catalytic mechanism employed for DNA cleavage.

P.P.26.
Biochemistry of Prokaryotes

Biochemical testing is not always reliable for the identification of Staphylococcus spp. isolated from companion animals

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The aim of this study was to determine the ability to identify *Staphylococcus* species isolated from companion animals using two commercially available biochemical testing systems. *Staphylococcus* isolates from riding horses (n=30) as well as from dogs and cats (n=30) were randomly selected for biochemical identification using Microgen Staph ID (Microgen) and RapID^{TM} STAPH Plus (Thermo Scientific) systems. Identification was performed according to the instructions and the results were interpreted using manufacturers software. DNA based methods – PCR for *Staphylococcus* genus (16S) and species-specific thermonuclease genes, as well as sequencing of 16S rRNA were used for reference verification.

All *Staphylococcus* strains were correctly identified up to the genus level by both commercial identification systems. All strains of *S. aureus* (n=10) as well as *S. schleiferi* (n=4) were correctly identified by both systems as well. However, *S. haemolyticus* (n=8) was correctly identified in 87.5% by STAPH Plus, whereas Microgen system all strains identified correctly. Both systems were unable to identify *S. equi* (n=3) while *S. pseudintermedius* (n=20) was correctly identified only in 70% and 75% using Microgen and STAPH Plus respectively. Moreover, software in both systems presented this species as *S. intermedius* regardless recent reclassification this species into three separate species. Certain biochemical properties of some species between the databases of both systems varied substantially. For instance, Microgen describes that *S. hyicus* breaks urea in 66% of the isolates whereas the positive percent in Microgen system is presented as 0%. Those discrepancies quite often led to unequal identification of some tested species – *S. lentus*, *S. hyicus* and *S. felis*.

According to the results obtained it could be outlined that identification of *Staphylococcus* species isolated from companion animals should be performed using DNA based methods and not solely rely on biochemical testing.

P.P.27.
Biochemistry of Prokaryotes

Characterization of Streptococcus thermophilus Csm complex mediated CRISPR interference in vitro

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CRISPR-Cas systems are an adaptive prokaryotic defence mechanism set against viral infection and horizontal gene transfer. The sequence-specific degradation of invasive nucleic acids (NA) is carried out by ribonucleoprotein effector complexes. While the cleavage is executed by protein subunits, the recognition of specific target nucleic acid is performed mostly by an RNA molecule. The effector complexes of Type I (Cascade) and II (Cas9-dual RNA) cleave foreign DNA while RNA is degraded by Type III-B Cmr complexes. Although genetic evidence suggests that Type III-A Csm complex targets DNA, there is no biochemical data on the interference mechanism. Moreover, while the active sites have been identified for the Type I and II effector complexes, no such data has been reported for the Type III systems in general. Here we aimed to establish the complex assembly and silencing mechanism of the Type III-A system Csm-machinery using *Streptococcus thermophilus* as a model system. We expressed the Type III-A CRISPR-Cas locus in *Escherichia coli*, isolated the Csm effector complex and characterized its activity *in vitro*. Further, we demonstrated that Csm complex may be reprogrammed to cleave the NA substrate of interest.

P.P.28.
Biochemistry of Prokaryotes

Antimicrobial susceptibility of coagulase-negative staphylococci isolated from human population

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Coagulase-negative staphylococci (CNS) are normal inhabitants of human and animals. They have long been dismissed as culture contaminants, but now the potentially important role of CNS as pathogens and their increasing antibiotic resistance has been recognized.

The aim of this study was to determine antimicrobial susceptibility of CNS isolated from human population and species distribution between antibiotic resistant isolates.

During 2013-2014 years 189 specimens were collected from nasal membranes of human. Isolation of staphylococci was performed using Mannitol Salt Agar and Plasmacoagulase test was performed as well. Species identification was performed using Microgen Staph ID (Microgen Bioproducts). Antimicrobial susceptibility was performed using “Sensititre” (Trek Diagnostic Systems) plates. Interpretation of results was performed according to EUCAST recommendations.

86 samples from 179 tested were positive for *Staphylococcusspp*. and demonstrated resistance to one or more antibiotics (48%). The resistances of 77 isolates were observed to tetracycline (18.2%), erythromycin (57.2%), clindamycin (14.3%), ampicillin (35.1%), and penicillin (61.1%). Nine isolates were ampicillin resistant and demonstrated complete resistance to other beta-lactamic antibiotics and tetracycline (61.1%). The most common species of resistant staphylococci included *S. warneri* (6.9%), *S. hominis* (5.8%), *S. saprophyticus* (5.8%),*S. haemolyticus* (4.7%), *S. epidermidis* (4.7%), that are more human-associated, but prevalent in animals’ population as well.

The presented data suggest that coagulase-negative staphylococci are wide spread in human population and can serve like resistance genes reservoir for spreading to pathogens like *S. aureus.*

P.P.29.
Biochemistry of Prokaryotes

Comparative Analysis of the Activity of MDR Pumps in Salmonella enterica using Methods of Fluorescence Spectroscopy and Potentiometry

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Salmonella enterica ser. *typhimurium* is the common pathogen, convenient for multidrug resistance (MDR) pump activity examination. We performed efflux pump activity measurements in real time using potentiometric and fluorimetric assays. We demonstrated here that the efflux pump substrate ethidium (Et^+) in combination with membrane-active antibiotic polymyxin B (PMB) can be used for monitoring of the activity of RND-type efflux pumps in *S. enterica*. The fluorescence of Et^+ after binding to DNA was used to monitor the influence of efflux pump inhibitor phenylalanyl-arginyl-β-naphtylamide (PAβN) on the pump activities at different metabolic states and conditions. It was demonstrated that the addition of another pump substrate tetraphenylphosphonium (TPP^+), also used as a membrane voltage (ψ) indicator, increases the efficiency of PAβN inhibition and a stronger fluorescence of Et^+ was observed. We showed that at slightly acidic pH conditions the fluorescence level of Et^+ is lower, although the potentiometric measurements did not indicate any difference in Et^+ accumulation and the efflux pump activity. Under higher ionic strength conditions rapid decrease of the fluorescence after addition of PMB was observed. In such medium we can see also the release of accumulated Et^+ as was determined using Et^+-selective electrode. EDTA-permeabilized cells after initial strong accumulation of Et^+ were able to activate the efflux pumps for extrusion of this indicator compound.

P.P.30.
Cancer Cell Biology and Biomedicine

Human carcinoma cell response to cytotoxic treatment in vitro

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Purpose Chemotherapy is a conventional and effective type of cancer treatment, but in some cases, it faces serious challenges, such as cancer resistance. In contrast, photodynamic therapy (PDT) is based on the use of light-sensitive molecules called photosensitizers and some of them do not invoke cancer cell resistance to PDT. Due to a specific mode of action, PDT is an attractive mode to the conventional strategies of cancer treatment. We have explored cellular response to treatment with conventional chemotherapeutic drugs 5-fluorouracil (5FU) and oxaliplatin and aluminium phthalocyanine tetrasulfonate ($AlPcS_4$)-mediated PDT. The cytotoxicity and expression of several molecules, modulating survival pathways in treated cells were revealed.
*Methods*Thehuman carcinoma cell lines A-431, HCT116, and5-FU resistant cancer cell line HCT116/100FU were used for the study. The cytotoxicity of the treatments was assessed by staining with crystal violet. Gene expression on mRNA and protein levels was revealed by qPCR and ELISA.
Results It was shown that HCT116/FU cells are less sensitive to 5FU (about 100 fold) and more sensitive to oxaliplatin than HCT116 cells. 5FU stimulated the expression of chemoresistence promoting upstream cytokine interleukin 1 (IL-1) in HCT116/FU and HCT116. Oxaliplatin caused different gene expression profiles of Hedgehog and Notch signaling proteins in HCT116/FU and HCT116 cells. It was determined that both hypoxia inducible factor 1, alpha subunit (HIF1A) and IL1-alpha are positive regulators of $AlPcS_4$-PDT induced vascular endothelial growth factor (VEGF) expression.
Conclusions Activation of survival pathways by treatment with cytotoxic agents maylead todevelopment of chemoresistance. Combination of chemotherapy with cell survival pathways targeting therapy might be an effective therapeutic strategy.

P.P.31.
Cancer Cell Biology and Biomedicine

Genome-wide DNA methylation profile of mesenchymal stem cells and cancer

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Alterations in DNA methylation and consequent transcriptional gene silencing have been found to be widespread and associated with hundreds of genes in many cancer types. Tumor cells becomepoorly differentiated during disease progression, therefore somatic reprogramming in cancer has similarities to processes occurring in stem cells during differentiation.

To elucidate gene methylationprofile of cell dedifferentiation, high-throughput DNA methylation profiling, using Agilent microarrays, was performed. DNA methylation signature of non-differentiated cells was identified through comparison of adipose-derived mesenchymal stem cells (ADSCs) and ADSCs differentiated into osteogenic lineage. In parallel, DNA methylation signature of prostate cancer was analyzed.

More than 27 800 CpG islands (CGI) and 5 000 CpG-rich regions not defined as CGIwere evaluated (covered with more than 230 000 features in total). According to preliminary analysis, lower global methylation level was observed in prostate tissues as compared to ADSCs. Aberrant methylation of 4500 features was detected in prostate cancer in comparison tonon-cancerous tissue samples, whereas 300 features indicated changes in methylation in ADSCs differentiated into osteogenic lineage as compared to controls. Aberrant methylation of some particular features (related to genes *LTBP1*, *KLF9*, *LEF1*) wasidentified both in ADSCs and prostate tissue while the majority of them appeared to be attributable to either one or the other.

P.P.32.
Cancer Cell Biology and Biomedicine

Expression and epigenetic regulation of stemness genes in mesenchymal stem cells

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Mesenchymal stem cells maintain their shape by activating pluripotency and differentiation genes. Expression of these genes is controlled by epigenetic modifications on regulatory regions. Gene expression can be silenced by the methylation of CpG islands on genes promoters. This could help to understand mesenchymal stem cells epigenetic control mechanism.

In this work DNA methylation marks were analysed in human adipose tissue-derived mesenchymal stem cells (AT-MSCs) and synovial fluid-derived stem cells (SF-MSCs). In all, 27 samples of AT-MSCs and 45 SF stem cells were analysed. Epigenetic 5mC mark was analysed in stemness and differentiation (*SOX2, OCT4, NANOG, TERT, TBX2* and *ESR1*), tumor suppressor (*p14*, *p16*, *RASSF1*, *GSTP1*, *DAPK1*, *RARB*) and imprinted (*H19*, *ZAC1*) genes by methylation specific PCR method. No methylation change were identified in tumor suppressor and imprinted genes in AT-MSCs, but was detectable in some lines of SF-MSCs. Stemness and differentiation genes showed frequent methylation changes, and in some of the AT-MSCs and SF-MSCs samples promoters of *TBX2* and *OCT4* were hypermethylated.

Also, 384-well format TaqMan® Array Human Stem Cell Pluripotency Panel was used to identify pluripotency and differentiation-related gene expression levels in MSC. Six AT-MSCs, 6 synovial membrane-derived and 4 SF-MSCs were analyzed using TaqMan® Low Density Array method. As a result, the expression of stemness genes was noticeable in all MSC, and the increase in expression of differentiation markers was noticed in MSCs differentiated to adipogenic and osteogenic lineages.

P.P.33.
Cancer Cell Biology and Biomedicine

Changes in salivary biochemical composition for the early diagnosis of oral cancer

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Early diagnosis of oral cancer remains the key to improved patient survival. Direct contact between saliva and malignant lesions turns saliva to an attractive and unique potential prognostic tool. The changes of salivary biochemical composition may reflect the ongoing malignant changes in oral tissues.

The aim of our study was to compare the levels of lactate dehydrogenase (LDH), tumor necrosis factor (TNF-α), copper (Cu) between healthy people and patients with diagnosed oral cancer. Evaluate and compare the effect of saliva of healthy and oral cancer patients on J744 macrophage cell line.

Unstimulated 5ml whole saliva samples were collected in the morning (between 8-10 h). Patients were asked no to eat or drink before filling the test-tube by spiting method. We analyzed the samples of 18 oral cancer patients from the department of maxillofacial surgery of clinics of Lithuanian university of health sciences (LUHS) and 20 healthy patients - randomly chosen students from LUHS, having no diagnosed inflammatory or oncological diseases. Concentration of copper was measured with Perkin Elmer Zeeman/3030 atomic absorption spectrophotometer.Activity of LDH was estimated with spectrophotometric method by evaluating the oxidation of NADH in 340 nm wavelength.Levels of TNF-α were evaluated with immunenzyme assay using ELISA kit.The effect of saliva on J744 macrophage cell line.Cells where incubated for 48 or 72 hours in 37°C 5% CO_2 adding control and/or study group saliva. After incubation the effect of saliva on viability of J744 macrophage cell line was evaluated. Our results showed, that concetrations of copper ions in control were 0,104 ± 0,019 μg/ml per mg of protein, lower than in the saliva of study group: 0,201 ± 0,039 μg/ml per mg of protein. Activity of LDH in control was 0,29 ± 0,06 (U/min per mg of protein). The activity of LDH in study group was increased - 1,24 ± 0,29 U/min per mg of protein. Levels of TNF-α in control and study groups were respectively 61,42 ± 5,42 pg/ml per mg of protein and 163,84 ± 26,46 pg/ml per mg of protein. The saliva of healthy patients did not effect

the viability of J744 macrophages. The saliva of patients with oral cancer reduced the viability of above-mentioned macrophages.

In conclusion, the concentration of copper in study groups was increased 1,93 times, activity of LDH - 4,3 times, level of TNF-α - 2,7 times in comparison with control samples; the saliva of oral patiets reduced the viability of J744 macrophages.

P.P.34.
Cancer Cell Biology and Biomedicine

Differential proteomic analysis of RH1 resistance in MH22A cells

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RH1 (2,5-diaziridinyl-3-(hydroxymethyl)-6-methyl-1,4-benzoquinone) is a novel antitumour prodrug designed to be activated by diaphorase, an enzyme, expressed at high levels throughout many solid tumors. However, acquired resistance of tumour cells to drug treatment is a major problem in chemotherapy. The purpose of this study was to elucidate the mechanism of acquired RH1 resistance. First, RH1-resistant murine hepatoma MH22A cell line was established. We have performed high-throughput differential proteomic analysis of the RH1-sensitive and sensitive MH22a cells. Total cell lysates were analyzed by high definition mass spectrometry (HDMS) using data-independent acquisition strategy. ~1700 proteins in total were identified and quantified in MH22A cell proteomes. Over 400 proteins displaying significantly altered expression levels between the cell lines were identified. During bioinformatic analysis of proteomic data these proteins were clustered by functional annotation (DAVID Bioinformatics Resources) and by protein-protein interaction data (STRING) into 20 groups that include cell cycle, response to stress, xenobiotic metabolism, DNA repair, energy metabolism, RNA processing and translation, cytoskeleton organization, endosome, cell signaling, proteasome complex proteins. The proteomic data highlights biological processes that determine drug resistance. For example, the decrease in xenobiotic metabolism enzymes leads to diminished prodrug RH1 activation in resistant cells. The reduced level of cell cycle positive regulators is consistent with prolonged cell cycle which may provide a longer period for DNA damage repair. Increased expression level of proteins, involved in response to stress, probably serves to reduce cell damage from RH1. Resistant cells are also distinguished by altered energy and protein metabolism. These results provide the basis for studying of RH1 resistance mechanisms for future screening of resistance biomarkers and their targeting.

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P.P.35.
Cancer Cell Biology and Biomedicine

THE ROLE OF STRESS-ACTIVATED PROTEIN KINASES AFTER OCHRATOXIN A INDUCED DEATH PROCESS IN BLOOD AND KIDNEY-DERIVED CELLS

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Ochratoxin A(OTA), a mycotoxin produced by severa l*Aspergillus* and *Penicillium* species, is a harmful contaminant in a variety of food products. This mycotoxin as stress inducts orprimarily affects the blood and kidney cells. MAPKs (mitogen-activated protein kinases) are serine/threonine protein kinases that participate in intracellular signaling during proliferation, differentiation, cellular stress responses, and apoptosis. JNK and p38 aremembers of this family known as the stress-activated protein kinases (SAPK), buttheir role incelllife is not unambiguous.

The aim of this work was to evaluateOTA-induced toxic effect in blood and kidney-derived cells cultures and to determine the role of stress-activated kinasesin these different cells after exposure with OTA.

For this purpose, blood-derived HL-60 and kidney-derived BHK21 cells were chosen. OTAtoxicity assessment was performed using a standard MTT test, cell viability was recorded by fluorescence microscopy after the exposure with ethidium bromide andacridineorange mixture. JNK and p38rolewas evaluated using Western blot analysis.

After cell exposure with OTACL_{50} (concentration which kill shalf of the cells), we have found that blood cells were killed at the first hours, while kidney-derived cells began to die only after 48 hours. As other authors, we have found that OTA induced apoptotic cell death. According to our data, in BHK21 cells, JNKandp38protein levels did not change, but activation of both these proteins began to increaseslightly from 9^{th} hour.In HL60 cells, JNK protein level and activation began to increase already even after the first hour.TheHL-60 cell death after OTA exposure was also significantly faster.

Therefore, blood-derived cellsare much more (about 20 times) sensitive to the ochratoxin Ain comparison with kidney-derived cells. Protein kinase JNK is involved in OTA-induced HL-60 cell death; however, SAPKsdid not playa decisiverole in BHK21 cell deathprocess.

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The impact of oxidative stress for quercetin-induced MH-22A cell death

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Flavonoids are usually recognized as antioxidants which can protect the cell from the oxidative stress. However, at higher concentrations, flavonoids can be cytotoxic to cell. One of the most widely distributed flavonoids in the plant kingdom is quercetin. It demonstrates apoptotic effect on colon, breast, lung, hepatoma cancer cells *invitro*. Some evidence indicates that quercetin exerts this apoptotic effect in a selective manner, modulating a number of key elements in signal transduction pathways linked to apoptotic processes only in cancer cells. However, the precise mechanism of action of this flavonoid remains to be unclear.

The aim of this work was to investigate the impact of oxidative stress for quercetin-induced cytotoxicity in murine hepatoma cell line MH-22A. To assess the possible flavonoid-induced oxidative stress action, we used two antioxidants - *N,N'*-diphenyl-*p*-phenylenediamine (DPPD), and desferrioxamine (DFOA). The obtained results were compared with the cell viability after exposure to oxidative stress inducer, H_2O_2. The expression and activation of MAPK signaling pathway molecules ERK and JNK was also studied.

In MH-22A cell culture, the concentrations of quercetin causing 50% cell survival was about 140 μM while that of H_2O_2 – 300 μM. Antioxidants DPPD and DFOA protected the cells against the action of tested flavonoid. This effect demonstrated the involvement of oxidative stress in the quercetin induced cell death process. On the other hand, MAPKs expression and phosphorylation after treatment with quercetin differed from H_2O_2-induced JNK and ERK pathway changes.

Therefore, flavonoid quercetin has displayed higher cytotoxic efficiency than H_2O_2. In MH-22A cell culture, the impact of oxidative stress for the cytotoxicity of quercetin is not the only decisive. It is likely that other factors in these cells also play an important role.

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P.P.37.
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The role of JNK in cisplatin induced apoptosis of adult muscle stem cell-derived adipocytes

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Recent evidence shows that tumor microenvironment composed of heterogeneous stromal cells may be involved in tumor cell resistance to anticancer therapy. It is proposed that targeting components of tumor microenvironment together with conventional treatments of cancer cells may be an effective strategy in cancer therapy. Among different cells surrounding tumor adipocytes are known to be important contributors influencing cancer development and progressions as well as response to chemotherapic treatment.As mature adipocytes isolated from organism remain viable and functionally unchanged in culture only for approximately 24 h, toxicity studies as well as crosstalk with the cancer cells require*in vitro* models of adipose cells.

In the present study we demonstrated that adult rabbit muscle-derived cell lines with unlimited proliferative potential *in vitro* treated with gama-linolenic acid differentiate toward adipogenic lineage. Adipogenic differentiation of cells was confirmed by accumulation of intracellular lipid droplets. We have assessed the susceptibility of differentiated cells to cisplatin being one of the most commonly used anticancer drugs for treatment of a wide variety of tumors including metastatic breast cancer. Further, we evaluated the role of stress-activated protein kinase JNK in regulating cisplatin induced apoptosis of differentiated cells. Our experiments show that JNK acts as anti-apoptotic molecule in adipogenically differentiated cells. JNK antiapototic action in differentiated cells may be associated with negative regulation of intrinsic/mitochondrial apoptosis pathway. The results of our study prove that JNK is involved in regulation of expression of major regulators in the mitochondrial apoptosis pathway, i.e. antiapoptotic Bcl-2 and proapoptotic Bax protein.

P.P. 38.
Cancer Cell Biology and Biomedicine

Ursolic acid enhances the cytotoxic effect of temozolomide in glioblastoma cells

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Glioblastoma (GBM) is the most prevailing brain tumour and isusuallytreated with surgery, followed by radiotherapy and chemotherapy with temozolomide (TMZ). However, therapy with TMZ, an alkylating agent, increasesthe survival of patientsonly slightly (12-14 months). Therefore, there is a high demand to develop new chemotherapeutic drugsorintroduce combination therapies using more than one pharmacologically active substance. Thus, the aim of our work was to assess the potential of ursolic acid (UA), a natural triterpene, to decrease the viability of cancerous cells in a combination with TMZ.Rat C6 cells were exposed to a range ofconcentrations of UA (1-80 μM) and TMZ (5-500 μM) for 24 h. The viability was evaluatedusing the MTT assay and microscopically using propidium iodide and Hoechst 33342 double staining. We have found that UA effectively induced cytotoxic effectafter 24 h incubation with$EC_{50}$of 16.7 ± 2.0μM. In addition, an increased number of apoptotic cells was observed. However, TMZ was able to reduce cell viability

only at the highest tested concentration value (500 µM).Importantly, a synergistic effect of TMZ and UA on cell viability was observed already at 100 µM of TMZ and5 µMof UA.Therefore, our results suggest that combinational treatment with UA and TMZmight be beneficial for glioma therapy.

P.P.39.
Cancer Cell Biology and Biomedicine

Ethanol affects energy metabolism of pancreatic stellate cells

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Pancreatic stellate cells, one of several resident cells in the exocrine pancreas, play the key role in the pathophysiology of the major disorders of the exocrine pancreas, such as pancreatic cancer and chronic pancreatitis. Alcohol consumption is responsible for about 70%–90% of cases of chronic pancreatitis. It is also known, that chronic pancreatitis is one of the predisposing factors in pathogenesis of pancreatic cancer.

Mitochondrial oxygen consumption in stellate cells was measured at 37^{o}C using Oxyhraph-2K (Oroboros) in 2 ml cell culture medium without fetal calf serum and antibiotics, supplemented with 5 mM glutamate + 5 mM malate as substrates. Generation of reactive oxygen species (ROS) was estimated as the release of H_2O_2 from stellate cells fluorimetrically using Thermo Scientific fluorometer. Amplex Red (5 µM) and horseradish peroxidase (2 U/mL) were added, and fluorescence (excitation at 544 nm, emission at 590 nm) was measured. Amplex Red fluorescence response was calibrated by adding known amounts of H_2O_2. Statistical analysis: The results are presented as means ± S.E.M (n=4 in each group). Statistical analysis was performed using Student's t test and $p < 0.05$ was taken as the level of significance.

The results showed that oxygen consumption in control stellate cells (without ethanol pretreatmen) was 14,7 ±1,6 pmol/sek/mln cells. After pretreatment of cells with 0.1% of ethanol, the oxygen cosumption remained unchanged (14,3 ±2,5 pmol/sek/mln cells) as compared to control. Pretreatment of cells with higher concentration (0.5%) of ethanol, caused the increase in oxygen consumption of pancreatic stellate cells by 34% as compared to control. Moreover, higher concentrations (1%) of ethanol decreased the pancreatic stellate cells respiration rate by 15% and 37% as compared to control and to 0.5% ethanol group, respectively. Therefore we conclude that pretreatment of cells with 0.5% of ethanol may activate ATP production in stellate cells, i.e. activated energy metabolism. H_2O_2 generationin control stellate cells was 0.298 µmol/min/mln cells. After pretreatment of cells with 0.5% of ethanol statistically significantly increased H_2O_2 generation by 27.5 %.

In conclusion, our results showed the increase in pancreatic stellate cell energy metabolism under preatretment with 0.5% ethanol. The activation of pancreatic stellate cells under alcohol intoxication could lead to progression of pancreatic fibrosis.

P.P.40.
Cancer Cell Biology and Biomedicine

Small Aβ$_{1-42}$ oligomers increase intramicroglial Ca^{2+} in NMDAR-sensitive manner

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One of the key features of Alzheimer's disease is accumulation of Aβ peptide in the brain. We have previously demonstrated that, among various aggregate forms, small (1-3 nm) soluble Aβ oligomers at submicromolar concentrations cause neuronal death in mixed neuronal-glial culture [1]. The amount of necrotic neurons was lower in pure neuronal cultures indicating the involvement of glial cells in Aβ-induced neuronal damage; however, the mechanism is not clear. In this work, we examined whether small Aβ oligomers may signal via increase in microglial Ca^{2+} level in pure microglial culture.

Primary mixed glial cultureswere prepared from the cerebral cortices of 7-day-old Wistar rats and used to make pure microglial cultures. The free intracellular Ca^{2+} was detected with Fluo-3-AM ester by a fluorescence microscope.

Intramicroglial Ca^{2+} significantly increased after 15 min incubation with 1 µM Aβ$_{1-42}$ small (1-3 nm) peptide oligomers and remained in similar level after 30 and 90 min. Monomeric, long oligomeric or fibrillar form of the peptide did not influence microglial Ca^{2+} in 90 min time. The effect of the small oligomers was reversed by NMDA receptor blockers memantine, MK801 and D-AP5, suggesting that Aβ$_{1-42}$ at early oligomerization stages may modulatemicroglial Ca^{2+}via NMDA receptors.

P.P.41.
Cancer Cell Biology and Biomedicine

Keratinocytes response to photodynamic therapy: cornification, apoptosis, autophagy

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Hallmarks of cell death triggered by tetrahydroxyphenyl chlorine (mTHPC)-mediated photodynamic treatment (PDT) were followed in primary human epidermal keratinocytes HEKa and human epidermoid carcinoma cell line A-431 *in vitro.* PDT induced the similar reduction of cell viability in either of cell line. In primary epidermal keratinocytes HEKa, PDT induced the expression of cornification markers: keratin 10, involucrin and procaspase 14, and no signs of cornification were observed in epidermoid carcinoma A-431 cells. Apoptosis, involving caspase-3 activation, was registered in both cell lines after PDT as well as accumulation of the autophagosomal marker LC3-II and increase of autophagic flux, which was studied using inhibitors of lysosomal peptidases.

P.P.42.
Cancer Cell Biology and Biomedicine

miRNA expression analysis of prostate tumors and urine sediments
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Prostate cancer (PCa) is the most prevalent and second leading cause of cancer-related death among men in Lithuania and world-wide. Detection methods, like PSA test, DRE or biopsy, are widely used in diagnostics of PCa, but their clinical value is reduced by high rate of false positive results and discomfort made for patients. Identification of the most aggressive cases of PCa is not possible by these methods, new molecular biomarkers are needed for timely identification of progressing PCa cases. Because of their significance in gene regulation, abundant expression and sufficient stability, miRNAs are the promising biomarkers for early detection and prognostication of outcomes in PCa.
miRNAs expression was evaluated by means of low density Taqman arrays (TLDA, Type A+B) in 42 tumor and 12 noncancerous prostate specimens. 755 human miRNAs markers were examined in total. 319 miRNAs fulfilled requirements for gene expression analysis and were chosen for further analysis. Comparison of benign and malignant tissues identified almost 100 miRNAs significantly disregulated in PCa. BCR and no-BCR cases were reliably distinguished by 46 up- and one down-regulated miRNAs, whereas *TMPRSS2-ERG* positive and negative tumors – by 56 miRNAs.
9 miRNAs were chosen for validation in a larger group of tumor samples. The validation step revealed over-expression of miR-19a and miR-19b as a significant biomarker of PCa. Furthermore, the fusion gene-positive and negative cases were reliably distinguished by over-expression of miR-183*, higher Gleason scores – by miR-195 and -340. Selected miRNAs were evaluated in urine sediments from 22 PCa patients. Expression of all selected miRNAs was detectable in urine from the patients, and some of them showed significant diagnostic potential. Among them, miR-31 can reliably distinguished BCR from no-BCR cases ($p = 0.019$), miR-19b – pT2 stage tumors from pT3 ($p = 0.004$). Therefore miRNA can not only be detected in urine sediments of PCa patients, but also could become a noninvasive mean for timely identification of aggressive forms of PCa.

P.P.43.
Cancer Cell Biology and Biomedicine

APC and TBX2 DNA methylation in prostate tumors
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Prostate cancer is the most commonly diagnosed oncological disease in Lithuania. In 2011 it amounted around 15 % of all malignant cancers diagnosed. Mortality rate of prostate cancer is really high in comparison to other cancers (it is second after respiratory system cancers). Therefore, the task of modern molecular genetics is to find new non-invasive, applicable for early diagnosis and prognosis biomarker or a set of biomarkers. DNA hypermethylation of regulatory regions of tumor suppressor genes is known to be one of the mechanisms silencing those genes and thus causing cancerogenesis while hypomethylation could result in activation of the oncogenes.
Changes in epigenetic regulation of *APC* is known to be one of the first markers of few types of cancer, such as colorectal, prostate or bladder. This gene encodes a tumor suppressor protein that participates in cell apoptosis, adhesion mechanisms, Wnt signaling pathway and degradation of β-catenin, which activates cell proliferation. *TBX2* is a cell differentiation gene which was previously associated with other types of cancer.
In order to explore significance of epigenetic changes of *APC* and *TBX2* in the pathogenesis of prostate cancer we investigated the methylation status of *APC* and*TBX2* in 42 cases of prostate cancer and 9 non-tumor samples. DNA methylation was studied by methylation specific PCR. In 38 of prostate cancer samples *APC* was found to be strongly methylated, while in remaining 4 methylation was partial. In 5 non-tumor samples *APC* was found unmethylated, 3 non-tumor samples were partially methylated and only one non-tumor sample was strongly methylated in the promoter region of *APC* gene. In all cancerous and non-cancerous samples *TBX2*was found to be methylated.
Considering the results and previously conducted researches epigenetic changes of *APC* could be one of the most effective biomarkers of prostate cancer.

P.P.44.
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IDENTIFICATION OF BIOMARKERS FOR DIAGNOSIS OF GLIOMA MALIGNANCIES

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Glioma is one of the most common and highly malignant brain tumors. The degree of glioma malignancy at the early stages of the tumor gliomagenesis is sometimes difficult to evaluate by using conventional morphological criteria. Recent studies show that certain phenotypes of gliomas such as glioma malignancy, resistance to therapy and relapses are associated with the methylation frequency of tumor-specific genes. However, the methylation effects on the particular gene function and the connection between the gene expression and tumor phenotype are still unclear.

The aim of the study was to identify genes whose expression and/or promoter methylation could be used for glioma tumor diagnosis and to estimate association between methylation/expression alteration, malignancy grade and patient clinical characteristics. It was investigated promoter methylation and expression of a set of cancer-related genes in different malignancy grade gliomas. The methylation frequency of genes was determined using methylation-specific PCR. Western blot analysis was used to characterize the expression patternof these genes in different grade glioma samples.

22 target genes have been selected on the relevance of the promoter methylation and/or expression of the target gene to neuro-oncogenesis and other type of cancers. We used 138 postoperative glioma samples as a research material. Successful gene promoter methylation analysis of 15 target genes has been carried out in 62-138 tissue samples. Gene expression experiments have been successfully performed for 9 genes in 47-96 tumor samples. Significant differences between different malignancy grades in gene methylation and/or protein expression profiles have been found in cases of 8 genes: NDRG2, SEMA3C, AREG, RUNX3, AHR, TEC, TGFB1, and CDKN2A (p16). All of these genes can be used as molecular markers for making prognosis of glioma patient survival and (except for CDKN2A (p16) gene) as an aid for determining the degree of tumor malignancy.

P.P.45.
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Analysis of influence of single nucleotide polymorphisms in RNASEL, LEPR, CRY1, IL4, CHI3L2 and AKAP14 genes on clinical course of prostate cancer

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Prostate cancer (PCa) is a common malignancy influenced by various environmental and genetic factors. The highest rates for PCa mortality in Europe were observed in Lithuania and other Baltic states. PCa has shown to be one of the most heritable cancers. Single nucleotide polymorphisms (SNPs) possibly could be associated with PCa risk and progression. The aim of this work was to determine *RNASEL* rs486907, rs627928, rs12757998 and rs627839, *LEPR* rs1137100, *CRY1* rs10778534, *IL4* rs2070874, *CHI3L2* rs2182114 and *AKAP14* rs3829719 SNPs' genotypes in peripheral blood samples from 213 prostate cancer patients who underwent radical prostatectomy and to analyze the association of these SNPs with high grade prostate cancer risk and biochemical disease recurrence. In addition, 34 blood samples from patients with benign prostatic hyperplasia (BPH) were analyzed. Genotyping was performed on genomic DNA using TaqMan® allelic discrimination assays.

We found significantly higher frequencies of *RNASEL* rs12757998 CC, rs627839 GG and *CRY1* rs10778534 CC genotypes in PCa patients compared to the patients with BPH (χ^2 = 6.1, df = 1, p = 0.01; χ^2 = 8.03, df = 1, p = 0.005 and χ^2 = 64.7, df = 1, p = 9.5×10^{-16} respectively). Moreover, *IL4* rs2070874 TT and *CHI3L2* rs2182114 CC genotypes were significantly more frequent among BPH patients compared to the PCa patients (χ^2 = 10.5, df = 1, p = 0.001 and χ^2 = 28.8, df = 1, p = 8.0×10^{-8} respectively). Most importantly, our study demonstrated that *RNASEL* rs627928 combined with *RNASEL* rs486907 as well as single *LEPR* rs1137100 variant can be used to distinguish between subgroups of PCa patients with high grade (Gleason score ≥7) and low grade (Gleason score <7) cancer. In addition, we found a significant relationship (χ^2 = 4.2, df = 1, p = 0.04) between combined *RNASEL* rs627928 and *IL4* rs2070874 polymorphisms and biochemical recurrence-free survival.

P.P.46.
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Differences in Thermal Stability and Thermodynamics of Potential Inhibitor Binding to the Native and Recombinant Target Protein

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The carbonic anhydrase (CA) inhibition has been exploited clinically for decades in various classes of diuretics, anti-glaucoma agents, anti-epileptics, and anti-tumor drugs. The main function of CAs is the maintenance of pH homeostasis by catalyzing the reversible hydration of carbon dioxide. There are 12 catalytically active CA isoforms in human

differing in expression patterns, tissue localization, and cellular distribution. It has been demonstrated that abnormal levels or activities of these enzymes have been often associated with different human diseases such as glaucoma, epilepsy, and progression in many types of hypoxic tumors. Consequently, CA isozymes have become an attractive drug target.

CA VI is the only secreted isoenzyme of the human CA family which is found in saliva, tears, and milk. Gene comparison tells that CA VI is closely related to isoenzymes CA IV, a target for glaucoma, and stroke, and CA IX, a marker for hypoxic tumors. This association links CA VI with certain cancers which might be associated with salivary glands. Therefore, it is important to analyze the affinity of sulfonamide inhibitors for CA VI.

This study is focused on the analysis of observed pH-dependent thermodynamic parameters of the range of potential inhibitors as well as intrinsic pH-independent parameters of etoxolamide binding to recombinant human CA VI from *E. coli* as well as native CA VI from human saliva. Thermal stability of the protein and the thermodynamics of interaction between the enzyme and inhibitors were determinedby isothermal titration calorimetry and fluorescent thermal shift assay. It was found that CA VI purified from bacteria is a perfect model to analyze reactions between human CA VI and inhibitors and the absence of glycosylation in *E. coli* has no significant effect on the thermodynamics of inhibitor binding to this enzyme.

P.P.47.
Cancer Cell Biology and Biomedicine

Influence of lipophilic agents on the formation of multidrug resistance in cell cultures

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Multidrug resistance (MDR) is recognized as one of the most common causes for failure of cancer chemotherapy. The aim of this study was to determine the correlation between gene expression and functional activity of MDR pumps in wild-type and lipophilic agents-induced human breast carcinoma MX-1 and colon adenocarcinoma CX-1 cell lines.

Methods: Functioning of P-gp and other MDR pumps was evaluated by spectrofluorimetric assay using a fluorescent dye – ethidium (Et^+) bromide. Interaction of the cell with MDR pump inhibitors and arsenate was tested potentiometrically. Multi-drug resistant cell sublines were developed exposing the parental cells to stepwise increasing concentrations of lipophilic cation tetraphenylphosphonium (TPP^+) or anticancer drug doxorubicin (DOX). Levels of the resistance were assessed in terms of IC_{50} values using the crystal violet assay. The level of *MDR1* expression was estimated using quantitative real-time reverse transcription-polymerase chain reaction (QPCR). *MDR1* gene methylation status was determined using methylation specific polymerase chain reaction (MSP). A comparison between wild-type and drug resistant cell lines was performed using gene expression microarrays.

Results: Reserpine, an inhibitor of MDR pumps, diminished Et^+ efflux out of cells. Competition for the interaction with MDR pumps was registered between the pump substrates TPP^+ and Et^+. The presence of arsenate in the incubation medium did not have any considerable effect on the activity of MDR pumps in MX-1 cells. Two MX-1 and one CX-1 cell sublines were established. MX-1/DOX subline revealed a 3-fold increase of the resistance to DOX compared to the parental cell line but MX-1/TPP^+ cells showed considerably higher resistance to DOX than MX-1/DOX ones. Both, MX-1/TPP^+ and MX-1/DOX sublines showed an increased activity of the pumps while efficiency of the efflux was rather low in the case of CX-1/DOX cells. However, the level of Et^+ efflux not always correlated with the expression of MDR pump genes. *MDR1* mRNA expression was weak in MX-1 cells and slightly increased in MX-1/DOX subline, while expression was high in CX-1 cells but decreased in CX-1/DOX subline. In both, parental and DOX resistant MX-1 and CX-1 cells, *MDR1* genewas partially methylated. Gene expression profiling by microarrays suggests that drug metabolizing enzymes can be involved in induced cell resistance to DOX.

P.P.48.
Cancer Cell Biology and Biomedicine

The effect of anthocyanins on C6 glioblastoma cells exposed to oxidative stress

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The intensified intracellular production of reactive oxygen species (ROS) and the decreased capacity of organism to cope with this situation lead to the excess level of ROS. Such a condition can contribute to the formation of cancer, aging, cardiovascular and neurodegenerative disorders. Therefore, there is increasing evidence suggesting that anthocyanins and other food-derived antioxidants may be useful in the prevention and treatment of lesions. However, the knowledge of their mechanisms of action is still scarce.

Thus, the aimof our study was to investigate a protective effect of several anthocyanins (cyanidine 3-O-rutinoside and 3-O-glucoside, malvidin 3-O-glucoside, and pelargonidin 3-O-glucoside) on rat C6 cells from oxidative stress. Our results have shown that anthocyanins did not affect C6 cell culture viability after 2 h incubation. After 24 h a significant decrease in cell viability (in comparison with control – 0.2% DMSO) was observed only in the presence of very high concentrations (80 μM and above) of Cy3R. Noteworthy, 500

au/ml of catalase did not abolish the cytotoxic effect of Cy3R. Furthermore, the strongest cytotoxic effect of H_2O_2 on cell viability with the determined EC_{50} value 158 ± 1.5 µM was observed after 24 h incubation. Next, we compared the effects of anthocyanins on H_2O_2-increased intra cellular oxidative level. We found that both anthocyanins, Cy3R and Cy3G, decreased the level of intracellular ROS with the same efficiency. Moreover, Mv3G was found to be less effective than Cy 3-O-glycosides, whereas Pg3G was not efficient at all. Thus, our analysis revealed that the effectiveness of investigated anthocyanins to reduce the level of intracellular ROS decreased in thefollowing order: [Cy3G = Cy3R] > Mv3G > Pg3G. A possible mechanism of such a protective action of Cyand Mv 3-O-glycosides is under experimental investigation.

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P.P.49.
Cancer Cell Biology and Biomedicine

The influence of protonation effects upon sulfonamide inhibitor binding to carbonic anhydrases

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Inhibition of enzymes is important in drug design. Sulfonamide inhibitor interactions with carbonic anhydrases (CA) depend on ionization properties of the inhibitor functional groups and the active site residues in the enzyme. Changes in the protonation of protein, ligand and buffer affect the observed thermodynamic parameters of binding, such as the Gibbs free energy Δ*G*, enthalpy Δ*H*, and entropy Δ*S*. Each binding reaction should be dissected in order to determine the intrinsic thermodynamic parameters that are independent on experimental conditions.

The carbonic anhydrases are established as therapeutic targets. There are 12 catalytically active CA isozymes in human body. At least 30 CA sulfonamide inhibitors have been used as drugs to treat glaucoma, epileptic seizures, altitude sickness, and as diuretics. However, most of them exhibit poor selectivity towards target isozymes and result in various side effects.

In this study a class of 4-substituted-benzensulfonamides and 4-substituted-2,3,5,6-tetrafluorobenzensulfonamides as inhibitors of CA is reported. The binding affinity of compounds towards CA isoforms I, II, VII, XII and XIII were measured using thermal shift assay, isothermal titration calorimetry, and stopped-flow CO_2 hydration assay. Futhermore, intrinsic parameters of binding were calculated. The combined use of these methods have provided a detailed picture of protein-ligand interactions and confirmed that fluorinated sulfonamides bind to CA stronger than non-fluorinated compounds. The presence of fluorine substituent decrease the pK_a of sulfonamide group and this correlates with an increase in the CA inhibitory properties. Several fluorinated sulfonamides are selective towards CAI.

P.P.50.
Cancer Cell Biology and Biomedicine

Optimization of a Quantitative Polymerase Chain Reaction for the Detection of mRNA of Carbonic Anhydrase XII

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Human carbonic anhydrase XII (CA XII) is a transmembrane protein, recently identified potential biomarker of tumour cells. The enzyme is overexpressed in cancer cells under hypoxic conditions and is involved in tumour progression by creating appropriate intracellular and extracellular conditions. The aim of the current study was to optimize a quantitative reverse transcription real-time polymerase chain reaction (RT-qPCR) method for the detection of CA XII mRNA in tumour cell lines and cervical cancer clinical samples with known histological diagnosis. TATA box binding protein (TBP) andβ-actin (ACTB) were selected as reference genes. The expression of CA XII was not detectable in Jurkat cell line, whereas strong expression was detected in U-87, A-498 and A-495 cells and weak expression was observed in HeLa and CaSki cells.It was also determined that CA XII mRNA expression level correlated with the degree of cervical histological alterations (CIN1<CIN2<CIN3).The obtained experimental data are new as for the first time the expression of CA XII mRNR in cervical specimens was investigated. The optimized RT-qPCR assay can be considered as a promising diagnostic tool for the detection of CA XII expression in cells and tissues.

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P.P.51.
Cancer Cell Biology and Biomedicine

Different Involvement of Survival Kinases in Anoikis Regulation of Cancer and Stem Cells

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Anoikis is an apoptotic form of programmed cell death induced by the detachment from the extracellular matrix (ECM). It is emerging as a hallmark of cancer as malignant cells tend to metastasize because of anoikis resistance. Moreover, most of the stem cells die within a few days after transplantation into desired tissue because of anoikis as well. Our group previously demonstrated that multipotent adult rabbit muscle-derived stem cell line Myo9 when differentiated into myogenic, osteogenic and adipogenic

lineages, except neurogenic one, is more resistant to apoptosis inducers compared to proliferating cells. Resistance to death signals correlated with the increased level of protein kinase AKT phosphorylation. In this study we show that detached proliferating Myo9 cells are more sensitive to anoikis than those differentiated into myogenic lineage. AKT phosphorylation level is also higher in differentiated cells kept under suspension conditions for 24 hours compared to proliferating ones. Transfection with additional AKT gene increases anoikis resistance of proliferating Myo9 cells. This data suggests that the modulation of AKT phosphorylation levels of stem cells could increase its therapy efficiency.

We also demonstrate that signaling mediated by cellular adhesion molecule integrin differently affects MAP kinase ERK activation in non-small cell lung cancer A549 and proliferating Myo9 cells. Detached A549 cells upregulate phosphorylated ERK levels compared to adherent cells while Myo9 cells show a decrease in ERK phosphorylation. Cells exposed to inhibitors of MAPK kinase MEK1/2 of ERK signaling pathway PD98059 and U0126 become more susceptible to anoikis. We then hypothesize that integrin signaling-independent ERK phosphorylation could be responsible for higher anoikis resistance of non-small cell lung cancer A549 cells.

P.P.52.
Cancer Cell Biology and Biomedicine

Application of Next-Generation Sequencing for Detection of Oncogenic Mutations With Relevance to Targeted Therapy

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Malignant melanoma, colorectal and non-small-cell lung cancer are among the most common cancer types in Lithuania possessing one of the highest mortality rates. In the past decade targeted therapy, a novel promising type of cancer treatment, was introduced. Profiling of somatic mutations of oncogenes that may be relevant to targeted therapy is of high importance. This study aimed to evaluate clinical application of high throughput new-generation sequencing (NGS) technology.

In this study NGS was performed to detect mutations in BRAF, EGFR, KRAS, KIT, NRAS and PIK3CA oncogenes using DNA from formaldehyde-fixed, paraffin-embedded samples of colorectal (CRC) and non-small-cell lung cancer (NSCLC).

Results: Sixteen clinical samples (8 NSCLC and 8 CRC cases) were analysed. KRAS mutations were most recurrent in both CRC (7 cases) and NSCLC (3 cases), all in codons 12,13. L858R EGFR mutation was detected in one NSCLC patient. A notable finding is that we detected a remarkably rare KRAS insertion G12_G13insG in a CRC patient and V600E BRAF mutation in two cases of NSCLC. Targeted NGS results highly correlated with that of current Diagnostic Gold Standart Real-time PCR (Cobas^{TM}). The study evaluated advantages of NGS for clinical applications: the ability to screen multiple mutations in multiple genes from multiple samples in a single run.

P.P.53.
Cancer Cell Biology and Biomedicine

Effect of HMTi 3-Deazaneplanocin A and HDACi Belinostat in modulating gene expression and protein levels in human promyelocytic leukemia cells

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Acute promyelocytic leukemia (APL) is a myeloid leukemia subtype, characterized by block of granulocytic differentiation and accumulation of promyelocytes in the bone marrow and blood. The complexity of APL epigenetic landscape, as well like other cancers, is enormous. In concert with histone deacetylation, histone methylation by Polycomb Repressive Complex 2 (PRC2) has been shown to exert a major impact on APL transcriptional silencing through methylation of histone H3 on Lysine 27 (H3K27).

In our study, we investigated the application of S-adenosylhomocysteine (AdoHcy) hydrolase inhibitor 3-Deazaneplanocin A (DZNep), in combination with hydroxamate-type HDACi Belinostat (Bel) and retinoic acid (RA) for leukemia granulocytic differentiation.

In order to evaluate the effect of DZNep in combination with Bel and RA on human acute promyelocytic leukemia NB4 cells, the level of granulocytic differentiation was determined using NBT test and the expression of the early myeloid differentiation marker CD11b. In addition, analysis of HDAC 1 and HDAC 2, PRC2 subunits EZH2, SUZ12 and EED, as well as PPARγ, C/EBPα, C/EBPε and p27 gene expression was performed by the RT-qPCR. Furthermore, the level of HDAC 1 and HDAC 2, EZH2, SUZ12, EED, H3K27me3, PPARγ, C/EBPα, C/EBPε and p27 was investigated using Western blot technique.

We showed that HMTi DZNep and HDACi Bel accelerate and effectively enhance granulocytic differentiation when used together with RA. Additionally, we demonstrated that combined treatments modulate HDAC and PRC2 gene expression, as well as certain transcription factors that are crucial for granulocytic differentiation. In line with this data, we found that used treatments downregulate H3K27me3, EZH2 and HDAC2 protein levels, in contrast upregulating p27 and HDAC1 protein expression.

In summary, we demonstrated that treatment with HMTi, HDACi and RA could be potentially applicable for promyelocytic leukemia differentiation therapy.

P.P.54.
Cancer Cell Biology and Biomedicine

Cytotoxicity of juglone to rat glioblastoma cells

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Glioblastome multiforme (GBM) causes approximately 50% of all brain tumors. Chemotherapy and radiotherapy are used for the treatment, however median survival of patients is about 1 year. Therefore, natural bioactive compounds are regarded as potential candidates to increase cancer cell sensitivity to chemotherapy.

Juglone is naphtoquinone naturally present in plants of the Juglandaceae family. Studies have shown that juglonehas anti-viral, anti-bacterial and anti-cancer effect, however, its anti-cancer activity on GBM cells has not been examined. The aim of this study was to evaluate *in vitro* the effect of different concentrations of juglone on viability of GBM C6 cells and to investigate the influence of intracellular and extracellular reactive species (RS) on juglone-induced cytotoxicity. Methods: MTT assay was performed to evaluate the anti-proliferative effect of juglone. The level of intracellular RS was quantified by $DCFH_2$-DA. The level of extracellular H_2O_2 was measured fluorimetrically using Amplex Red.Our results have shown thatjuglone (1-128 µM) inhibited viability of C6 cells in a dose-dependent manner after 24 h of incubation. Furthermore the determined EC_{50} value was 10.4 ± 1.6 µM. Interestingly, H_2O_2 generated in the cell medium from juglone reaction with cell medium components was not sufficient to affect the glioblastoma cell viability. Moreover, catalase added to the cell medium did not reduce cytotoxic action of juglone. Generation of intracellular RS was dependent on juglone concentration and incubation time. In conclusion, juglone in a concentration-dependent manner is cytotoxic to C6 rat glioblastoma cells and the cell death might be induced through the action of intracelular RS.

P.P.55.
Cancer Cell Biology and Biomedicine

A comparative study of bioreductive agent RH-1-induced signalling pathways in human lung cancer cell line A549

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In this study we aim to dissect cell death signalling pathways in DT-diaphorase-rich cancer cell line **A549** apoptosis induced by aziridine-substituted polyphenol derivative **RH-1**.

To characterize the role of oxidative stress in apoptosis induction caused by DT-diaphorase (NQQ1) bioreductively-activated quinone RH-1 we studied the influence of various antioxidants, mainly N-acetyl-cysteine (NAC). Reactive oxygen species (**ROS**) may initiate cell death processes through affecting mitogen-activated protein kinase (**MAPK**) signalling cascades. The expression and phosphorylation/activation of MAP kinases ERK, p38, and JNK were studied at the drug concentrations inducing apoptotic cell death in 24 hours (16-25 µM of RH-1). All three MAP kinases retained their constant expression at protein level during RH-1 treatment and all of them were activated during apoptosis induction, as determined by Western blot method. By means of specific small-molecule inhibitors involvement of JNK was revealed in cell death. Non-specific antioxidant NAC protected a fraction of A549 cells from deadly RH-1 impact too. Nevertheless, MAPK involvement in RH-1 induced A549 cell apoptosis seems to be unrelated to oxidative stress, as 1-5 mM NAC did not alter their phosphorylation. The sequence and relations between the mentioned signalling pathways are being examined.

A quinone moiety substituted with aziridine is known to be a potent alkylating agent which can damage DNA, thus this is another possible mechanism of cell death induction by RH-1. The tumor suppressor p53 and protein kinases ATM/ATR play a central role in sensing damaged **DNA**. Here, in A549/RH-1 model, ATM phosphorylation and p53 activation is clearly demonstrated, pointing to DNA damage dependent apoptosis induction. Attenuation of apoptosis by specific ATM inhibitor KU55933 and p53 inhibitor pifithrin-α confirmed this hypothesis.

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P.P.56.
Cancer Cell Biology and Biomedicine

The role of different connexins in formation of tunneling nanotubes and transfer of small RNAs between HeLa cells

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Membranous intercellular tunneling tubes (TTs) are recently discovered new forms of communication between remote cells implicated in the cell migration; intercellular electrical coupling and Ca^{2+} flux; transfer of proteins, organelles and genetic material.

In the present study, we employed the dual cell patch-clamp, fluorescence microscopy, time-lapse imaging and immunolabeling to examine the role of different connexins (Cx36, Cx40, Cx43, Cx45, and Cx47) expressed in HeLa cells in cell migration as well as formation and electrical and permeability properties of TTs. We demonstrate that HeLa cells expressing Cx36 exhibited the highest mobility properties compared with WT or other Cxs expressing HeLa cells. Thus, Cx36 which is highly expressed in nervous system and pancreas may be involved in controlling of cell migration and development of processes in these tissues.

In WT or any Cx expressing HeLa cell culture, 2 types of TTs were identified – "thick" ones (from 300 nm up to 6.31 µm in diameter), containing F-actin and α-tubulin, and "thin" ones (diameter less than 300 nm), actin rich only. Thick TTs were formed during cytokinesis or by lamellipodium outgrowth mechanism, while thin ones were formed by filopodia extensions or protrusions. TTs established either open-ended or connexin-based, or close-ended connections between remote cells.

TTs which were open-ended or connected the remote cells through Cx40, Cx43 or Cx47 GJs, were capable of transmitting while TTs containing Cx36 and Cx45 GJs were impermeable to the double-stranded small interfering RNA (siRNA/AF488).

This newly described pathway of siRNA or miRNA direct delivery to the remote cells suggests the possible mechanism of cancer metastasis.

Finally, we demonstrate that HeLa cells are capable of establishing the intercellular communication through TTs and Cx43 GJs with human mesenchymal stem cells which might be employed in gene silencing-based cancer therapy.

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P.P.57.
Cancer Cell Biology and Biomedicine

Generation and characterisation of RH1-resistant cancer cell lines

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Resistance to chemotherapy still is a remaining problem in cancer treatment. Cancer cells may acquire resistance to chemotherapeutic agents and revive after treatment. Furthermore these newly developed or selected cells may not only be resistant to used drugs but also can develop a cross-resistance to other drugs even if they work in different mechanisms. It is considered that over 90% of unsuccessful metastatic cancer treatment is related to drug resistance phenomenon, so it is very important to overcome the cancer drug resistance in order to increase the efficiency of chemotherapy. The diversity of mechanisms in drug resistance can be grouped in several levels of action such as increased drug efflux, drug inactivation, alterations in drug targets, repair of drug- induced damage or evasion of apoptosis. The increasing number of anticancer agents together with advanced proteomic technology which suggest new predictive biomarkers opens new opportunities to understand and overcome the drug resistance of cancer.

In this work chemotherapeutic agent RH1 which is present in phase II clinical trials was selected for development of RH1-resistant cell lines in vitro. New drug-resistant cell lines were created and characterized in 6 months. We showed that RH1-resistant cell lines were not cross-resistant to cisplatin treatment. This characterization could be useful in clinical treatment and could allow the prediction of RH1-resistant cell behavior in vivo and select the best combination of drugs for individual patient treatment.

P.P.58.
Cancer Cell Biology and Biomedicine

Modulation of Cx36 gap junction conductance by intracellular pH and Mg^{2+}

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Gap junction (GJ) channels formed by connexin (Cx) proteins provide a direct pathway for electrical and metabolic intercellular communication. We examined the effect of intracellular pH (pH_i) on the conductance (g_j) of Cx36 GJs, which are expressed in neurons and β-cells of pancreas. These GJs recently have been shown to be very sensitive to intracellular magnesium ions ($[Mg^{2+}]_i$), therefore we also investigated the impact of $[Mg^{2+}]_i$ on the g_j dependence on pH_i (g_j/pH_i). To measure g_j between Cx36EGFP transfected HeLa cells, we used dual cell patch-clamp setup and modified the pH_i by extracellular application of ammonium chloride (10 mM) or sodium acetate (100 mM) which increased or decrease pH_i to about 8.2 or 6.0, respectively.

Under control $[Mg^{2+}]_i$ (1 mM), the acidification slightly reduced (~5%) while alkalization caused ~3 fold decrease of g_j. At high concentration of $[Mg^{2+}]_i$ (5 mM) the blocking potency of alkalization increased, while the g_j reducing effect of acidification was reversed to stimulation. At low $[Mg^{2+}]_i$ (0.01 mM), the blocking potency of acidification increased and the blocking potency of alkalization decreased.

The acid dissociation constant of the cysteine side chain is close to physiological pH values, suggesting its importance in pH_i-dependent regulation of Cx36 GJ channels. In order to determine the Cx36 protein domains involved in modulation of g_j by pH_i, we generated single, double, triple and quadruple substitutions of 4 cysteines present in the Cx36 protein. To minimize the local volume changes, we used structural modeling which suggested the folowing substitutions: C87S, C92V, C168A and C264S.

Our data show that, indeed, cysteines are involved in pH_i-dependent regulation of Cx36 GJ activity and among them the major role is played by C264, present in the 4th transmembrane domain of Cx36.

In addition we show that E8 and E12 may be the candidates for $[Mg^{2+}]_i$-dependent allosteric modulation of g_j/pH_i.

P.P.59.
Cancer Cell Biology and Biomedicine

Studies on the prevalence of oncogenic HPV types among Lithuanian women with cervical pathology

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Human papillomavirus (HPV) is the main cause of cervical cancer. Therefore, the detection of oncogenic HPV types is important in predicting the risk of cervical cancer. The aim of the current study was to estimate the prevalence of 16 carcinogenic and potentially carcinogenic HPV types in the study group of Lithuanian women with various grades of cervical pathology in comparison to healthy women. A total of 824 cervical specimens were investigated for HPV DNA: 547 specimens of women with abnormal cytology and 277 specimens of healthy women. Cytological diagnosis was confirmed by histology. For the detection of HPV infection, HPV DNA was amplified by PCR using three different primer systems. HPV DNA-positive samples were investigated for the presence of 16 HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 66, 73 and 82) by multiplex PCR. HPV DNA was detected in 67.6% of specimens collected from women with abnormal cytology and 24.2% of specimens collected from healthy women. The frequency of HPV-positive specimens correlated with the severity of cervical pathology: it ranged from 50.0% in ASC to 80.6% in cervical cancer. In cases confirmed by histology the frequency of HPV-positive specimens ranged from 68.6% in CIN I to 89.2% in CIN III/CIS. The most common HPV type was HPV16 (detected in 42.3% of HPV-positive specimens) followed by HPV31 (10.1%), HPV33 (8.2%) and HPV56 (5.7%). The prevalence of HPV16 among HPV-positive specimens ranged from 27.6% in ASC to 60.0% in cervical cancer as diagnosed by cytology and from 37.5% in CIN I to 61.3% in cervical cancer as diagnosed by histology. In contrast, the frequency of HPV18 was lower as compared to other countries. HPV18 was identified in 4.1% of HPV-positive women with various grades of cervical abnormalities and in 11.3% of those with cervical cancer.

P.P.60.
Cancer Cell Biology and Biomedicine

Novel inhibitory monoclonal antibodies against tumour-associated carbonic anhydrase XII

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Human carbonic anhydrase XII (CA XII) is a single-pass transmembrane protein with an extracellular catalytic domain. This enzyme is being recognized as a potential biomarker for different tumours. It is involved in tumour progression by acidification of the extracellular milieu and regulation of intracellular pH. CA XII is aberrantly overexpressed in breast, cervix, brain cancers, renal carcinomas. The current study was aimed to generate monoclonal antibodies (MAbs) neutralizing the enzymatic activity of CA XII. Bioinformatics analysis of CA XII structure revealed surface-exposed sequences located in a proximity of its catalytic centre. Two MAbs against the selected antigenic peptide spanning 167-180 aa sequence of CA XII were generated. The MAbs were reactive with recombinant catalytic domain of CA XII expressed either in *E. coli* or mammalian cells. Inhibitory activity of the MAbs was demonstrated by a stopped flow CO_2hydration assay. The study provides new data on the surface-exposed linear CA XII epitope that may serve as a target for inhibitory antibodies with a potential immunotherapeutic application.

P.P.61.
Structural biology and Bioinformatics

Comprehensive analysis of DNA polymerase III α subunits and their homologs in bacterial genomes

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Bacterial replicative DNA polymerases are multicomponent protein machines, in which the actual DNA synthesis is performed by the catalytic α-subunit. The analysis of almost 2000 bacterial genomes revealed that they all, without a single exception, encode one or more DNA polymerase III α-subunit homologs. Classified into C-family of DNA polymerases they come in two major forms, PolC and DnaE, related by ancient duplication. While PolC represents an evolutionary compact group, DnaE can be further subdivided into at least three groups (DnaE1-3). We performed an extensive analysis of various sequence, structure and surface properties of all four polymerase groups. Our analysis reveals important differences between extant polymerase groups. Among them, DnaE1 and PolC show the highest conservation of the analyzed properties. On the other hand, DnaE3 polymerases apparently represent an 'impaired' version of DnaE1. The analysis of polymerase distribution in genomes revealed three major combinations: DnaE1 either alone or accompanied by one or more DnaE2s, PolC + DnaE3 and PolC + DnaE1. The first two combinations are present and in *Escherichia coli* and *Bacillus subtilis*, respectively. The third set (PolC + DnaE1) is found in clostridia. This intriguing

replication system, harboring two apparently highly efficient replicative polymerases, has not been experimentally characterized so far. Polymerases of the DnaE2 group are not required for chromosomal DNA replication and appear to be important for induced DNA mutagenesis. Interestingly, DnaE2 seems to be typical for oxygen-using bacteria with large GC-rich genomes.

P.P.62.
Structural biology and Bioinformatics

Viral DNA replication: new insights and discoveries from large scale computational analysis

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Ability to replicate is essential for all living entities. Duplication of genetic information is carried out by replication proteins. DNA replication is well studied in T7, T4 phages and herpes viruses, however information about replication apparatus from other groups of viruses is „gappy" or missing. Double-stranded (ds) DNA viruses infect cells from all domains of life, evolve fast and are very diverse. Their genome size varies from 5 to 2500 kbp. To better understand viral DNA replication we identified replication proteins in dsDNA viruses using current state-of-art homology detection methods. Over 150000 proteins from 1574 genomes were analyzed. We found out that the composition of replication machinery depends on virus genome size. Small viruses (<40 kbp) use protein-primed DNA replication or rely on proteins from the host. Large viruses (>140 kbp) have their own RNA-primed replication apparatus often supplemented with processivity factors and DNA topoisomerases to increase replication speed and efficiency. Latter insight led us to a search for a „missing" replication components in a large genomes. This has resulted in a discovery of single-stranded DNA binding (SSB) proteins in largest eukaryotic viruses. Surprisingly these proteins were homologs of SSB proteins previously thought to be specific for T7-like phages. Other surprise came from the analysis of a herpesviral helicase-primase. We found that its component (UL8) is inactivated and highly diverged B-family DNA polymerase.

P.P.63.
Structural biology and Bioinformatics

Structural domains involved in the regulation of connexin 36 gap junction channels by general anaesthetics

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Investigation of mechanisms of general anaesthetics action emerges a great challenge in the design of novel drugs acting on the central nervous system (CNS). Connexin 36 (Cx36) gap junction (GJ) channels are expressed in the CNS and may be involved in general anaesthesia.

In order to examine binding of general anaesthetics to Cx36 GJs, protein-ligand docking, coarse-grained molecular dynamics, site-directed mutagenesis and patch-clamp recording of GJ conductance (g_j) were used.

Protein–ligand docking showed a similar accumulation pattern of hexanol and isoflurane which involved the unique intrasubunit cavity for both molecules only, situated inside of each Cx36 *wt* subunit in the vicinity of N terminus and transmembrane domains TM1 and TM2 (with residing C87 and C92). The second intrasubunit cavity, predicted in the proximity of the unique one, contained mainly hexanol and isoflurane molecules as well as low quantity of nonanol. The highest accumulation of nonanol was predicted in the intersubunit cavity involving TM2 domain of one Cx36 *wt* subunit and TM1 and TM4 domains of the neighboring subunit. Cysteines of TM2 and TM4 domains were predicted to be possible elements of the intra- and inter- subunit cavities which could contribute to general anesthetics binding.

Patch-clamp recordings of *HeLa* cells transfected with Cx36 *wt* and cysteine mutants confirmed the ligand docking results: hexanol and isoflurane stimulated Cx36 *wt* GJ coupling (g_j), while nonanol blocked it. C92V (TM2) weakened the g_j stimulatory effect of hexanol and isoflurane while C264S (TM4) reversed it to inhibition. The blocking effect of nonanol was reduced only in C92V (TM2) and C168A (intracellular loop) mutants. Finally, coarse-grained molecular dynamics showed that C264S mutation, in contrast to Cx36 *wt*, caused hexanol to be adsorbed in the vicinity of major parts of amino acids of N terminus, TM1 and TM2 domains which formed intra- and inter- subunit cavities.

Our results implicate potentiation of Cx36 GJ channels by general anaesthetics via unique intrasubunit cavity and inhibition upon binding to intersubunit and/or second intrasubunit cavities.

P.P.64.
Structural biology and Bioinformatics

The PPI3D web server for searching, analyzing and modeling protein-protein interactions in the context of 3D structures

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Easy-to-use computational tools for structural analysis of protein-protein interactions are valuable for studies of molecular mechanisms of biological processes. We have recently developed the PPI3D web server, which provides a

possibility to query experimentally determined 3D structures of protein complexes, to analyze the identified protein-protein interactions and to generate homology models of protein complexes. Structural data for experimentally determined protein-protein interactions are represented by biological assemblies available from the Protein Data Bank. All the protein-protein interactions accessible through PPI3D are clustered according to both sequence and interaction interface similarity. This removes the redundancy of structural data while preserving alternative protein binding modes. The server enables users to explore interactions for individual proteins or interactions between a pair of proteins (protein groups). In both modes, structural data on protein-protein interactions are detected using sequence search with either BLAST or PSI-BLAST depending on the desired level of similarity. The PPI3D output enables users to interactively explore both the overall results and every detected interaction. The server reports the total protein-protein interface area, the sortable list of interface residues and their individual contribution to the interface area. The interface residues can be inspected in the sequence alignment or in the context of 3D structure. In addition, the server provides a possibility to construct a homology model for the protein complex. The server is freely accessible at http://www.ibt.lt/bioinformatics/ppi3d/.

P.P.65.
Molecular biology and Functional genomics

Dissection of the apple (Malus × domestica) nuclear proteome during interaction with apple scab (Venturia inaequalis) pathogen using 2D DIGE and LC-MS/MS analysis

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The domesticated apple is one of the main economically important fruit crops of temperate regions. Fungal pathogen apple scab causes large economical losses in apple orchards. An inherent apple scab resistance is characteristic to wild species of genus *Malus*, and apple breeding programs are aimed at introgressing apple scab resistance genes into domesticated cultivars using pyramidization of resistance genes. Therefore a better understanding of mechanisms of the plant-pathogen interaction and associated signalling pathways could provide genetic solutions for durable disease resistance. Apple has been designated as one of three model species of the *Rosaceae* family and extensive genome information became available for genomics analysis, recently. However, information about the molecular basis of apple scab pathogenesis is vague. This study presents the first comprehensive description of the proteomic anatomy of apple cell nucleus during interaction of resistant and susceptible apple genotypes with the fungal pathogen. Nuclear proteins isolated from *V. inaequalis* infected leaves of three different apple genotypes, including two clones of 'Antonovka' showing different resistance phenotype and susceptible cultivar 'Puikis', were subjected to 2D DIGE analysis. Statistical analysis revealed differential expression of 174 ($P < 0.01$) proteins among the different genotypes. Protein identification using LC-MS/MS analysis revealed transcription factors, RNA-binding, stress-inducible and ubiquitin-dependent proteins among the up- or down-regulated proteins. Most of the proteins identified were stress response, plant defence, catabolic process and transcription regulation-related proteins. The results suggest a complex network of nuclear proteins that play role in stress response processes and constitute targets for further reverse genetics research, as well as, potential candidates for development of markers useful in marker-assisted breeding programs.

P.P.66.
Molecular biology and Functional genomics

Prevalence of the genes encoding resistance to tetracycline in Staphylococcus spp. isolated from poultry and wild birds

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The aim of this study was to determine differences in dissemination of genes encoding resistance to tetracycline in bacteria genus *Staphylococcus* prevalent in poultry and wild birds. For this purpose faecal samples of slaughtered broilers from three large poultry farms (n=174) as well as feaces from seagulls (*Larus ridibundus*) collected at Kaunas city dump (n=68) were obtained using sterile cotton swabs. For *Staphylococcus* isolation Mannitol Salt Agar (Liofilchem, Italy) as well as Columbia Agar with Sheep Blood (E&O Laboratories Ltd, Scotland) were used. Genus identification was performed using gram-staining, morphology characteristics, production of catalase, susceptibility to furazolidine. PCR with genus specific (16S) primers was used for genus verification. Resistance to tetracycline was performed using Kirby-Bauer method. Interpretation of the results assessed by EUCAST clinical breakpoints. Detection of *tetK* and *tetM* genes in resistant isolates was performed by PCR.

The rate of *Staphylococcus* spp. isolation was 150 (86.2%) and 58 (85.3%) from the poultry and wild birds respectively. Phenotypical resistance to tetracycline was 52.6% in broiler isolates whereas from the wild birds – 20.7%. Both *tetK* and *tetM* genes were detected in the isolates from broilers (68% and 14% respectively). Isolates of the wild birds harboured the only *tetK* gene that was detected in the 75% of the isolates, while *tetM* was not detected. This study demonstrates high resistance rates to tetracycline from

bacteria genus *Staphylococcus* not only in poultry but in wild birds as well. This fact indicates that wild birds are potential source of resistant bacteria independently of the absence of direct contact with antimicrobials. Further studies are needed for the better understanding of possible resistance transmission routes associated not only with domestic animals but with the wildlife as well. Certain resistance genes (i.e. *tetM*) are still present only in extensive farming environment.

P.P.67.
Molecular biology and Functional genomics

Phusion U – uracil-tolerant high fidelity DNA polymerase
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Biotechnology progress drives continuous improvement of the existing molecular techniques and the enzymes. One of examples is Thermo Scientific™ Phusion™ DNA polymerase. This enzyme is recognized as the best choice for many PCR applications, but, like all other proofreading DNA polymerases, Phusion High Fidelity DNA polymerase is inhibited by the presence of uracil in DNA template strand and therefore cannot be used for such applications as amplification of bisulphite-converted or damaged (e.g. ancient) DNA, UDG-based PCR carry-over contamination prevention and other similar applications. A proprietary mutation introduced into the so-called dUTP binding pocket of Phusion enzyme allowed us to create the Phusion U DNA Polymerase, which not only retains all power of Phusion enzyme, such as great accuracy, speed, ability to amplify long amplicons up to 20 kb and a high specificity, but also is able to read through uracil present in DNA templates.

P.P.68.
Molecular biology and Functional genomics

Escherichia coli phage vB_EcoM-VpaE1 – a new member of Felix01-like myoviruses
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Highly lytic *Esherichia coli* phages are promising tools for the investigation of virulence and have high potential for phage therapy. Several new genera comprising poorly characterized lytic *E. coli* myoviruses have been proposed during the last few years. In this study, we present the genome analysis as well as morphological and physiological characterization of a novel *E. coli* bacteriophage vB_EcoM-VpaE1 (VpaE1). Based on transmission electron microscopy, VpaE1 belongs to the family *Myoviridae* (A1 morphotype) and is characterized by an isometric head of ~77 nm in diameter and a contractile tail (~120 x 16 nm). The phage has a clearly defined neck, baseplate and baseplate-associated long tail fibers.
VpaE1 can infect most of the common laboratory *E. coli* B strains but fails to infect K-12 derivatives. The efficiency of plating (e.o.p.) test of VpaE1 revealed that the phage has an optimum temperature for plating around +37°C. Adsorption tests showed that a high percentage (96%) of VpaE1 particles adsorbed the *E. coli* BL21-DE3 cells after 5 min of incubation. The single-step growth experiments showed that the latent period of VpaE1 is 32 min while the eclipse period - 20 min.
VpaE1 genome is a double stranded DNA molecule (88.403 bp) with a G+C content of 38.94%. With an average ORF size of 600 bp, ~90% of the VpaE1 genome is coding - it encodes 132 putative protein-encoding open reading frames and 20 tRNAs. While most VpaE1 genes were found to initiate from ATG (123 out of 132 ORFs), 6 ORFs were found to initiate with TTG, and only 3 with GTG. The genome analysis revealed that all 132 VpaE1 ORFs code for proteins that have reliable identity with matches to those in other sequenced phages genomes (the percentage of amino acid identity ranged from 78% to 100%). In keeping with the typical genome architecture of members of the family *Myoviridae*, VpaE1 genome appears to have a modular organization, with genes for DNA packaging, structure/morphogenesis, host lysis and replication/regulation clustered together. More than 70% of VpaE1 ORFs encode small hypothetical proteins with no known functions. The phylogenetic analysis of the major capsid protein showed that VpaE1 is phylogenetically related to nine phages belonging to a newly proposed genus "Felix O1-like viruses" of the *Myoviridae* family.

P.P.69.
Molecular biology and Functional genomics

Acinetobacter baumannii toxin-antitoxin systems higBA and splTA are required for plasmid maintenance
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Acinetobacter baumannii is an opportunistic pathogen causing nosocomial infections. Due to its ability to persist in the clinical environment and rapidly acquire antibiotic resistance, multidrug-resistant *A. baumannii* clones have spread in medical settings of many countries in the last decade. The molecular basis of the emergence and spread of the successful multidrug-resistant *A. baumannii* clones is not yet clearly understood.
Bacterial toxin-antitoxin (TA) systems are abundant genetic loci found on mobile genetic elements as well as on chromosomes. Among cellular activities shown for TA modules, they have been found to confer antibiotic resistance by inducing persistence and to stabilise mobilome of pathogens. The role of TA systems in the stable inheritance and horizontal spread of genetic elements in *A. baumannii* is not known. We have recently shown that *higBA*

and *splTA* TA systems are the most prevalent plasmid-borne TA systems in *A. baumannii* isolates from Lithuanian hospitals belonging to pandemic lineages (Jurenaite M. et al. *J. Bacteriol.* 2013;195(14):3165-72). *A. baumannii* plasmid pAB120 conferring resistance to carbapenems (Povilonis J. et al. *J. Antimicrob.Chemother.* 2013;68(5):1000–6) is the first sequenced plasmid encoding both of these TA systems and is the most prevalent plasmid among clinical *A. baumannii* ECII isolates from Lithuanian hospitals.

In this study we have analysed the role of newly found plasmid-borne *A. baumannii* TA systems in the plasmid maintenance. We tested if the TA systems can act as plasmid stabilisation mechanisms using plasmid pO1273 (a derivative of the plasmid pWH1266 (Hunger M. et al. *Gene*. 1990;87(1):45-51)) exhibiting faulty inheritance in *Acinetobacter*. The incubation of bacteria carrying pO1273 under non-selective conditions resulted in more than 50 % plasmid loss every 12 h. When *splTA* was cloned into pO1273 the plasmid retention was reconstituted to 100% up to the end of 80 h incubation. Similar result was observed for *higBA*. We then analysed if *splTA* and *higBA* TA systems play a role in the maintenance of pAB120 plasmid. A deletion of *higBA* in pBA120 reduced the plasmid copy number in the cell.

higBA and *splTA* systems are functional *A. baumannii* TA systems required for stabilisation and maintenance of mobile genetic elements. Such systems could therefore be involved in rapid accumulation and stabilisation of genetic elements, responsible for antibiotic resistance in *A. baumannii.*

P.P.70.
Molecular biology and Functional genomics

Evaluation of the Effects of Lead, Nickel and Zinc Ions on δ-Aminolevulinic Acid Dehydratase Activity

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δ-ALAD is sulfhydryl-containing enzyme that catalyzes the condensation of 2 delta-aminolevulinic acid molecules to form porphobilinogen which is a heme precursor.Due to the structure of δ-ALAD, heavy metals such as Pb and Ni capable to displace Zn or oxidize sulfhydryl groups, changing activity of this enzyme.The purpose of this study was to assess how Pb and Ni affect the activity of δ-ALAD and to determine whether Zn is able to protect the enzyme from toxic metal exposure.

Experiments were done on outbred white laboratory mice using intraperitoneal injections of Pb $(CH_3COO)_2$, $NiCl_2$ and/or $ZnSO_4$ solutions. The δ-ALAD activity was measured with dimethylamineborane by spectrophotometry at 555nm wave length. All results were expressed as the mean±standard error of mean. The level of significance was set at $p<0.05$.

According to the results, single and repeated exposures to Pbcaused a statistically significant decrease of δ-ALAD activity in mice liver by 76% and 73% respectively. Zn pre-treatment diminished the suppressing effect of Pb and increased the enzyme activity in both time points of both metal exposures, respectively by 20% and 10%, as compared to the Pb treated mice.

Pb exposure drastically reduced the δ-ALAD activity in red blood cells after single and repeated Pb administration, respectively by 99% and 92%, as compared to the control mice. Zn pre-treatment diminished the suppressing effect of Pb and increased fivefold the δ-ALAD activity only after single both metal administration.

Ni administration inhibited liver δ-ALAD activity by 38% and 32% respectively. After single exposure to both metals, Zn partially protected the enzyme activity, increasing this rate by 22%, as compared with the Ni treated mice group. However, after 14 days Zn couldn't provide protective effect against Ni-induced δ-ALAD inhibition in the liver of mice.

The single dose of Ni reduced δ-ALAD activity in the blood by 56% as compared to the control group. Repeated doses of Nihad no effect on the enzyme activity. Zn pre-treatment protected δ-ALAD activity from the inhibitory effect of Ni after single both metal administration, but had no effect on δ-ALAD activity after 14 days of both metal treatment.

P.P.71.
Enzymology and Metabolism

Methylated azopyridine as electrochemical mediator for NAD+ regeneration

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A new mediator N'N'-dimethyl-4,4'-azopyridinium sulphate (MPD) was synthesized as described in [1]. To investigate the possibility of use of MPD as electrochemical mediator for NAD^+ regeneration its electrochemistry and reactivity with the NADH were determined. The mediator shows reversible electrochemistry at pH 7.2, the redox potential equal to 185 mV *vs* Ag/AgCl 3 M NaCl. The dependence of the mediator redox potential on pH in the range from pH 5.9 to pH 8.0 shows 2 electrons and 2 protons take part in the electrochemical conversions of MPD.

The reactivity of the mediator with NADH was investigated spectrophotometrically by monitoring the change of absorbance of NADH (at 340 nm). The bimolecular constant of cross reaction of MPD and NADH is equal to $(2.2\pm0.1)\times10^5$ $M^{-1}s^{-1}$ at pH 7.2. This is very high value, higher than that of other compounds used as mediators for NADH oxidation [2].

The use of MPD in NAD^+ regeneration was investigated by applying the electrochemical mediator oxidation method. The reduced form of mediator (MPD_{red}) is produced in the reaction oxidized mediator form (MPD_{ox}) with NADH. MPD_{red} can be oxidized on the working electrode surface, resulting in the anodic current increase. The total turnover numbers of mediator (MPD) and cofactor (NADH) in alcohol dehydrogenase (ADH) catalyzed ethanol oxidation depend on substrate concentration and time of electrolysis, and the yield of the reaction is limited by the enzyme (ADH) inactivation.

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P.P.72.
Enzymology and Metabolism

The N-heterocyclic quinonoid compounds: their synthesis, quantum mechanical characteristics and enzymatic reactivity

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In this work, the N-heterocyclic ortho- and para-quinoidal compounds (N-HQCs), i.e., quinoline-5,8-dione (QD), 6,7-dichloro-quinoline-5,8-dione (Cl_2QD), 2-methyl-6,7-dichloro-quinoline-5,8-dione (2-Me-Cl_2QD), 6,7-dichloro-isoquinoline-5,8-dione (Cl_2IQD) and 1,10-phenanthroline-5,6-dione (PD), were synthesized and their electron accepting potency and other molecular characteristics were assessed by quantum mechanical computation (QMC) and electrochemical methods. The preliminary study of the enzymatic reactivity of N-HQCs was performed with respect to NADPH:cytochrome P-450 reductase (P-450R, EC 1.6.24) and NAD(P)H:quinone oxidoreductase (DT-diaphorase, NQO1, EC 1.6.99.2) as single- and two-electron transferring flavoenzymes, respectively, which are known to be the main participants in the cellular redox cycling and bioreductive activation of redox active cytotoxic agents including quinonoid compounds. The determined apparent second order rate constants (k_{cat}/K_m) of reduction of N-HQCs by P-450R varied in the range of 2.7×10^4 M^{-1} s^{-1} (quinoline-5,8-dione) and 2.6×10^6 M^{-1} s^{-1} (6,7-dichloro-5,8-quinolin-5,8-dione and 1,10-phenanthroline-5,6-dione). The k_{cat}/K_m values of NQO1-catalyzed reduction of N-HQCs were markedly higher than those of P-450R, and spanned in the range of 4.4×10^5 M^{-1} s^{-1} (quinoline-5,8-dione) and 1.2×10^8 M^{-1} s^{-1} (1,10-phenanthroline-5,8-dione). N-HQCs underwent a direct (non-enzymatic) reduction by NADPH; the defined second order rate constants were in the range of 1.3 M^{-1} s^{-1} (6,7-dichloro-isoquinoline-5,8-dione) and 65.0 M^{-1} s^{-1} (1,10-phenanthroline-5,6-dione). So far, a more thorough study has been carried out upon 1,10-phenanthroline-5,6-dione (PD) as one of the most reactive N-HQCs. The time course of non-enzymatic as well as enzymatic oxidation of NADPH by PD proceeded in a non-stoichiometric fashion, and the initial rate of NADPH oxidation was close to that of catalase-sensitive O_2 uptake. The reaction was accompanied by the reduction of cytochrome c (200 % of NADPH oxidation rate) that was suppressed by superoxide dismutase (SOD) by 20-25 %. This is most likely to be related to the single-electron oxidation of two-electron reduced PD by cytochrome c to PD radical as intermediate being oxidized by O_2 producing superoxide. When applying DFT-B3LYP/6-311G(D,P) method, the QMC showed that the electron-accepting potency of N-HQCs follows the range: 1,10-phenanthroline-5.8-dione < quinoline-5,8-dione < 2-methyl-6,7-dichloro-5,8-dione < 6,7-dichloro-5,8-dione < 6,7-dichloro-isoquinoline-5,8-dione. These data show that in spite of the lowest electron-accepting potency, PD exhibits the highest reactivity. This is most likely to be caused by PD ortho-quinonic structure. QMC study of N-HQCs within the framework of density functional theory is underway.

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P.P.73.
Enzymology and Metabolism

Modified Trypsin protease and recombinant Endoproteinase Lys-C - powerful protein sample preparation tools for analysis by mass spectrometry

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Mass Spectrometry is a key detection technology, which is still currently one of the most rapidly developing techniques in the proteomics toolbox. One of the main components of mass spectrometry work-flow is the trypsin which specifically cleaves Lysines and Arginines at the C terminal. There are several key features for Mass Spectrometry Grade tryspin, such as (i) functional purity or absence of chymotryptic or other protease activity, (ii) reduced autolysis- which determines half-life of the protease in the reaction mixture under conventional or denaturating conditions, thus plays a key role in cleavage efficiency. Despite of high enzymatic activity occasionally trypsin cannot facilitate a complete digestion of some tightly folded target proteins due to the

limited accessibility to Lysine or Arginine residues. The peptide coverage can be increased by supplementing trypsin reaction mixture with up 2 M of urea or up to 0.1 % of SDS. However the most convenient way is to combine two proteases for digestion of analyzed mixture - initially digest with Urea resistant endoproteinase LysC followed by dilution and addition of trypsin for secondary digestion.

We have developed a modified MS grade trypsin from the native source of lyophilized porcine pancreatic proteins. The obtained enzyme exhibits high purity – no detectable chymotrypsin activity, exceptional selectivity – cleaves at the carboxyl side of lysine and arginine residues with greater than 95% specificity, enhanced stability – chemically modified for reduced autolytic activity.

Endoproteinase Lys-C from *Pseudomonas aeruginosa* was produced as recombinant protein in *E. coli* based expression system. The analysis of the newly developed recombinant Lys-C revealed superior enzymes performance.

P.P.74.
Membranes and bioenergetics

Studies of yeast Saccharomyces cerevisiae secreted toxin K2 interaction with bacterial cells

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It is known that toxin K2 secreted by *Saccharomyces cerevisiae*killer strainsprevents the reproduction of sensitive yeast cells. We also examined the interaction of K2 with various bacteria. It was found that K2 binds not only toyeast, but also to cell walls of gram-positive and gram-negative bacteria when the incubation medium is enriched with microelements. The most effectively addition of microelements increases the ability of K2 toxin to bind to yeast α'1 and gram-positive bacteria *Staphylococcusaureus* cells. It was determined that the toxin kills sensitive yeast cells within 2 hours of incubation.The cell death was accompanied by the leakage of intracellular K^+ ions. These results suggest that K2 toxin forms K^+-permeable channel or affects the functioning of potassium channels in yeast. Our results indicated that this toxin has also a negative effect on the viability of some bacterial cultures. We assayed the intracellular amount of intracellular potassium ions after the bacteria incubation with intact or heat-inactivated toxin. Leakage of K^+ions into the medium indicated that K2 permeabilizes the bacterial envelopes and causes depolarization of the cells. Results of our experiments indicate that the most effectively this toxin affects gram-positive *Staphylococcusaureus*and *Micrococcus luteus* cells. However, K2 toxin does not affect the K^+ gradient in gram-negative *Escherichia coli* cells. In conclusion, our results indicate thatpermeabilization of membranes could also be the main reason of bacteria killing by K2toxin.

P.P.75.
Membranes and bioenergetics

Effect of hyperthermia on oxidative phosphorylation in rat brain mitochondria

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Hyperthermia can contribute to neuronal injury under multiple environmental and clinical conditions. Certain neuro-active drugs (amphetamine) can induce or exacerbate severe heat stroke. Heat stroke occurs when the brain temperature rises above 40.5-41°C. At this temperature, the ability of the hypothalamus to coordinate thermoregulation is compromised and therefore further increase in brain temperature may occur. Likewise, hyperthermia is used to increase the effectiveness of some anti-cancer strategies and to treat certain brain cancers, however that carries the risk of neuronal injury. A better knowledge of the mechanisms by which hyperthermia damages the brain should be helpful in devising therapies with minimal brain damage. One of the key targets of hyperthermia in cells are mitochondria. We performed experiments of direct heating *in vitro* on mitochondria isolated from male rat brain, respiring with pyruvate+malate (P+M) and glutamate+malate (G+M). In both cases hyperthermia increased respiratory rate in state 2. The effect of hyperthermia on respiratory rate in state 3 (V_3) and uncoupled respiration (V_{CCCP}) was strongly dependent on oxidizable substrates: in the case of P+M oxidation hyperthermia (45°C) stimulated V_3 and V_{CCCP} by 19% and 16% respectively, whereas V_3 and V_{CCCP} with G+M was inhibited by 19%. We investigated the effects of hyperthermia on the mitochondrial pyruvate dehydrogenase (PDH) complex and glutamate dehydrogenase (GDH). The results revealed that hyperthermia did not affect PDH complex activity but strongly inhibited GDH activity: at 45°C GDH activity decreased by 81% in comparison to 37°C. This finding provides explanation for the different sensitivity of the respiration rates V_3 and V_{CCCP} to hyperthermia in brain mitochondria respiring with P+M and with G+M.

P.P.76.
Membranes and bioenergetics

The effect of hyperthermia on permeability of membranes in CHO and MH22A cells lines

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Hyperthermia is promising modality for cancer treatment that urgently requires detailed knowledge on molecular and

cellular processes for rational development of treatment protocols. It is important to establish thresholds for thermal damage in human tissues that vary among tissue species as well as among normal and diseased tissues. Comparison of thermal susceptibility of various biomolecules has revealed that the plasma membrane (PM) is the most sensitive to heating among all cellular components. The response of other cellular membranes was not yet more thoroughly studied in this respect.

The aim of this study was to determine and compare the effect of 30 min hyperthermia (42°C) on PM and inner mitochondrial membrane (IMM) permeability in Chinese hamster ovary (CHO) and murine liver cancer (MH22A) cells. The effect of hyperthermia on PM and IMM permeability was evaluated by staining cells with propidium iodide and JC-1 (5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimi-dazolylcarbocyanine iodide) dye and applying fluorimetry and fluorescence microscopy analysis. The obtained results demonstrated that 42°C hyperthermia increases permeability of PM by 13 and 26 %, and permeability of IMM – by 24 and 51%, in CHO and MH22A respectively (as compared with 37°C). The results of our study demonstrate for the first time that inner IMM is cellular component that is very sensitive to hyperthermic treatment – possibly it is even more sensitive that cellular PM. Other important observation was that both PM and IMM in cancerous MH22A cells was more sensitive to the damaging effect of hyperthermia in comparison to the same membranes in CHO cells.

P.P.77.
Membranes and bioenergetics

Effect of hyperthermia on inner membrane permeability of brain, liver and heart mitochondria

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Hyperthermia in combination with conventional therapies improves cancer treatment results. Therefore the protocols for hyperthermic treatment are under continuous development that requires basement on the knowledge of molecular and cellular processes. It is important to establish thresholds for thermal damage in human tissues that vary among different tissues. The response of cellular membranes was not yet more thoroughly studied in this respect. The thorough study of effect of hyperthermia on the inner membrane of brain heart and liver mitochondria was performed in order to establish the pattern of the hyperthermia induced changes in the membrane barrier function. We performed experiments of direct heating *in vitro* on mitochondria isolated from male rat brain, liver and heart respiring with pyruvate+malate as oxidizable substrate. Changes in the membrane permeability were evaluated by mitochondrial respiration in state 2 or by estimation of the modular kinetics of the membrane leak. The results showed that at 40°C hyperthermia increased inner membrane permeability only in liver mitochondria – respiratory rate in state 2 (V_2) increased by 16% compared to 37°C, and dependence of respiratory rate on membrane potential was shifted to lower potential region. Further increase in temperature up to 42°C and more, resulted in remarkable increase of flux through the membrane leak module in mitochondria form all types of tissue, but in heart mitochondria it was substantially stronger (at 42°C V_2 increased by 17, 28 and 56 %; at 45°C – by 89, 60 and 372% in brain, liver and heart mitochondria respectively). The comparative study showed that the response profile of hyperthermia induced changes in the inner mitochondrial membrane barrier function is strongly dependent on the tissue, and that the sensitivity of the inner membrane to hyperthermic damage is much stronger for heart mitochondria in comparison to brain or liver mitochondria.

P.P.78.
Membranes and bioenergetics

Energetic Metabolism of infected Halophilic Archaea Haloarcula hispanica

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The goal of this study was to determine the influence of SH_1 virus infection on the energetical metabolism of *Haloarcula hispanica* cells. *H. hispanica* belongs to halophiles, which are the best suited to grow in media with 0,5–2,5 M concentration salts. These halophiles accumulate K^+ ions inside the cell in order to sustain the osmotic pressure of environment. There are known only a few viruses which can infect these cells. One of them is SH_1, which was used to perform this study. SH_1 infectedcell lysis starts 5-7 h post infection and proceeds10-15 h. It is important to find substances that could accelerate the lysis to understand this process mechanism.

It was determined that the cell respiration is sensitive to classical respiration inhibitors NaN_3 and KCN. Beside this, the infection of cells with virus SH_1 also slows down the respiration rate. It was found that natural detergent sodium cholate accelerates the lysis of infected cells. The cells that survive the infection become resistant to repeated infections by SH_1.

P.P.79.
Membranes and bioenergetics

Effect of Caffeic acid phenethyl ester (CAPE) on ischemia-induced kidney mitochondrial injury

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Ischemic acute renal injury is a syndrome that develops following a sudden transient drop in total or regional blood flow to the kidney. Despite advances in preventative strategies and support measures, this disease continues to be associated with significant morbidity and mortality. Mitochondria play an important role in pathophysiology of ischemia-reperfusion induced kidney injury. In this study we examined whether caffeic acid phenethyl ester (CAPE), one of the biologically active compounds of propolis, may protect kidney mitochondria from ischemic injury.

1.5 h prior to induction of ischemia animals were pre-treated with two (22 mg/kg or 34 mg/kg) intraperitoneal injections of CAPE. After rat kidneys were exposed for 20 min of total (*in vitro*) ischemia (at 37ºC). Mitochondria were isolated by differential centrifugation method. The mitochondrial respiration (oxygen consumption) rates at various metabolic states were measured at 37ºC using Clark-type electrode with 5 mM glutamate + 5 mM malate or 15 mM succinate (+2 mM amytal) as respiratory substrates. Lactate dehydrogenase (LDH) activity in supernatants was measured spectrophotometrically according NADH oxidation rate at 340 nm. Caspase-3-like protease activity was taken as a measure of apoptosis induced by kidney ischemia. Measurement of caspase-3-like activity (the hydrolysis of caspase-3 substrate) was followed fluorimetrically (by excitation at 380 nm with emission at 460 nm). Mitochondrial Ca^{2+} uptake was measured fluorimetrically using calcium Green 5N as indicator (by excitation at 506 nm with emission at 531 nm).

Our results showed, that 20 min ischemia caused the decrease in the mitochondrial State 3 respiration rate by 52% and the respiratory control index (RCI) by 58% ($p<0.05$) as compared to control with glutamate/malate as substrate. The State 2 respiration rate increased slightly, but not statistically significant. Succinate oxidation was also decreased. Mitochondrial Ca^{2+} uptake after 20 min of ischemia was diminished by 30% and there was an increase of caspase-3 activation by 1.15-folds as compared to control. LDH activity in supernatants had tendency to decrease but after this time of ischemia it was not statistically significant. Pre-treatment of rats with different doses (22 mg/kg or 34 mg/kg) of CAPE reduced the ischemia-induced decrease in the mitochondrial State 3 respiration rate (substrate glutamate/malate) by 20% and RCI by 33% and 21%, respectively, however, CAPE had no protective effects on succinate oxidation. Mostly interesting, the pretreatment of rats with CAPE increased the mitochondrial Ca^{2+} uptake by 8-fold and by 4-folds as compared to ischemia alone. When rats were pretreated with CAPE, caspase-3-like activity was blocked.

In conclusion, 20 min of warm ischemia *in vitro* has a deleterious effect on rat kidney mitochondrial functions. A single intraperitoneal injection of CAPE 1.5 hour before ischemia reduced partially kidney mitochondrial injury of rats and blocked ischemia-induced caspase activation.

P.P.80.
Membranes and bioenergetics

The Role of Medium pH and Ca2+ Concentration in the Regulation of Efflux Pump Activity in Salmonella enterica

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Multidrug resistance efflux pumps are transporters responsible for the extrusion of toxic substances and antibiotics from cell. Because of the pumps bacteria are sensitive to increased concentrations of antibiotics. It is important to find means forregulation of these antibiotics-extruding devices.

AcrAB-TolC is the main efflux pump of *Salmonella enterica*. It belongs to Resistance-Nodulation-Division (RND) family of pumps. These devices derive energy for their activity from the electrochemical proton gradient. This gradient is a result of extrusion of H^+ ions by respiration chain across the plasma membrane. ATP synthesis is also directly dependentonthis form of energy. Calcium ions play important role in many biochemical pathways of cells. It was shown that $Ca^{2+}$are needed for the reconstruction of efflux pump mechanism [1] and that extrusion of ethidium (Et^+) ionsat low pH does not depend on energy [2]. Because of these controversial ideas we analyzed the role Ca^{2+}ionsplay in energy supply of the efflux pumps and the influence of medium pH on the efficiency of pumps.

Were performed studies by method of potentiometric analysis.Using selective electrodeswe followed the distribution of efflux pump substrate Et^+ between the cells and the incubation medium. Changes of Et^+ concentration in the medium indicated, that Ca^{2+} ions do not affect the Et^+ efflux, but stabilize the bacterial envelope and reinforce barrier to lipophilic compounds. We showed also that the driving force of efflux pumps does not depend onthe cell incubation medium pH.

1. Martins *et al.*, Internat J Antimicrob Agents 2011;37:410-414.
2. Amaral *et al.*, Internat J Antimicrob Agents 2011;38:140-145.

P.P.81.
Membranes and bioenergetics

Studies of the efficiency of AcrAB-TolC efflux pump in Salmonella enterica and Escherichia coli cells

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Bacterial resistance to antibiotics has become a serious problem of public health and this concerns almost all

antibacterial compounds. One of the main mechanisms involved in bacterial resistance is the drug efflux. The efflux pumps, classified into five families, can provide resistance to rather specific class of antibiotics or to a large number of drugs, thus conferring a multidrug resistance (MDR) phenotype to bacteria. A promising weapon, the efflux pump inhibitors (EPI), can help to overcome this type of resistance. EPIs are expected to block the activity of pumps. One of the best known inhibitors of RND-type pumps is Phe-Arg-β-naphthylamide (PAβN).

We investigated the changesof *S. enterica* and *E. coli* cell sensitivity to antibiotics in the presence of PAβN. It was found that PAβN increases the susceptibility to tetracycline and chloramphenicol both, of wild-type bacteria and AcrAB-TolC mutant strains. Efficiency of the inhibition depends on the initial concentration of cells. We studied also the interaction of indicator compounds - tetraphenylphosphonium (TPP^+) and ethydium (Et^+) ions - with various *S. enterica* mutant strains. We observed the competition of TPP^+ and Et^+ ions for the interaction with efflux pumps. During this study we analyzed also effects of PAβN on the viability of *S. enterica* and *E. coli* cells. It was found that *S. enterica*ΔAcrB, ΔTolC mutants are the most sensitive to this inhibitor.*S. enterica*L664, a strain overproducing AcrAB-TolC pump, was rated as the most resistant to PAβN.

P.P.82.
Membranes and bioenergetics

Water induced structural changes in the membrane anchoring self-assembled monolayers probed by SERS

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Planar supported lipid bilayers are useful models of biological membranes as well as matrix for construction of biosensors and development of biomedical related diagnostic tools. Self-assembled monolayers (SAMs) are widely used to tether bilayer lipid membranes to the surface of metal substrate. Properties of the anchored phospholipid bilayers depend on the structure of the SAM. Water is required for biological function of membrane formed from tethered lipid bilayers. However, the effect of water on the organization and structure of underplayed SAM is purely understood.

In this work in situ surface enhanced Raman spectroscopy (SERS) was applied to determine water induced conformation and orientation changes in the membrane anchoring mixed monolayer composed from short chain hydrophilic ME (2-mercaptoethanol) molecules and long chain hydrophobic thiols WC14 [20-tetradecyloxy-3,6,9,12,15,18,22-heptaoxahexatricontane-1-thiol, C14 (myristoyl)] adsorbed on nanostructured gold substrate. The assignment of the bands was based on the experimental analysis of deuterated 2-mercaptoethanol and quantum chemical modeling of adsorption complexes. Intense bands in SERS spectrum near 679 and 597 cm^{-1} were assigned to C-S vibrational modes of adsorbed deuterated ME in trans and gauche conformation, respectively. Analysis of relative intensities revealed decrease in relative amount of trans conformers after incubation (72 h) of SAM in water. At the same time an increase in the population of adsorbed WC14 molecules in the all-trans hydrocarbon chain conformation was detected by SERS.

P.P.83.
Emerging techniques and new methodsin biochemistry

Snapshot of the PDGF-induced nuclear proteome dynamics

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Living organisms are highly complex systems, with processes occurring on a broad time scale ranging from very rapid (lasting part of a second) to very slow (lasting decades). Many processes, especially those that are relevant to developmental biology, such as proliferation and differentiation, are happening simultaneously. Every scientist would like to understand these process without limiting their research to any one phenomena. About 2 decades ago omics technologies started to emerge; since then they were able to provide a huge amount of data about system-wide protein expression, localization, interaction and modification. Nevertheless, even today most proteomic analyses are limited to one-time-point measurements and do not provide any insights into the dynamic nature of biological systems. The major challenge in proteomics, as well as in systems biology, has proven to be the interpretation of data, which is complicated due to identification of peptides and their modifications, variability, quantification and limitation of data-mining.This study focuses on the dynamics of the nuclear proteome induced by platelet-derived growth factor (PDGF). PDGF is a common mitogen, involved in wound-healing and tissue development, and is capable of activating the MAP kinase cascade. Despite the fact that MAP kinase signalling cascades and their transcriptional regulation are well studied on the molecular level, there has been no successful attempt to provide any insight into the entire nuclear proteome dynamics during PDGF receptor activation. Our study has so far detected hundreds of dysregulated proteins after treating cells with PDGF, involved in a variety of biological processes, such as gene expression, RNA splicing and RNA processing.

P.P.84.
Emerging techniques and new methodsin biochemistry

The effect of bovine serum albumin-coated zinc oxide nanoparticles on cell viability and ROS generation

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In biomedical applications, usage of cosmetics or environmental inhalation exposure of different nanoparticles (NPs), the blood circulatory system will most likely be the first interaction organ exposed to the nanoparticles or NP-based nanomaterials. Serum albumin is the principal protein contributing to the colloid osmotic pressure of the blood and also the transport protein for numerous endogenous and exogenous compounds. The interaction of NPs with proteins can lead to protein corona formation, decrease in NPs cytotoxicity, but in certain cases - to structural modification of the protein molecules and the acquisition of non-physiological functions by the proteins.

The aim of this study was to evaluate the effect of BSA-coated ZnO NPs on cell viability and ROS generation.

ZnO NPs were non-covalently (ZnO-BSA) and covalently (ZnO-covBSA) coated with bovine serum albumin (BSA) at 4 and 20°C. Prepared NPs were characterized by AFM, UV/Vis, fluorescence, synchronous fluorescence and for the first time by Raman spectroscopy. NPs analysis revealed changes in the microenvironment of aromatic acid residues and decrease in α-helix content in ZnO-bound BSA as compared to the free BSA.

BSA-coated ZnO NPs were not cytotoxic to CHO cells after 3h incubation, except for those prepared at 20°C and plain ZnO – ZnO-BSA, ZnO-covBSA, ZnO decreased viability by 6, 7 and 11%, respectively. All NPs had negative effect on colony formation: plain ZnO NPs - 27%, coated NPs (4°C) had negligible effect (5 and 8%) and coated NPs (20°C) had strong cytotoxic effect (20%).

ZnO-BSA and ZnO-covBSA NPs (4°C) haven't induced ROS generation. ROS generation was induced by plain ZnO NPs and by ZnO-BSA and ZnO-covBSA NPs, prepared at 20°C. ROS amount inversely correlated with viability.

Conclusion: coating of ZnO NP at 4°C with BSA decrease NPs cytotoxicity and ROS generation.

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P.P.85.
Emerging techniques and new methodsin biochemistry

Influence of beta-adrenergic stimulation on alternans formation in rabbit heart during acute regional ischemia

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Although beta-adrenergic receptor antagonists are widely used for treatment of heart failure, the role of beta-adrenergic receptors in arrhythmogenesis is not clearly known. It is shown that stimulationofbeta-adrenergic receptors reduces action potential alternans formation and arrhythmias (Kameyama et al., 2003), but other authors propose the opposite opinion (Myles et al., 2012). Here we evaluate the role of a non-selective beta-adrenergic agonist isoprenaline (ISO) on alternans formation during ischemia in rabbit heart.

The experiments were performed in isolated Langendorff-perfused rabbit hearts (n=3). Electrical activity of the heart was monitored using voltage-sensitive dye di-4-ANBDQBS and optical mapping equipment. Regional ischemia was induced by occlusion of left anterior descending coronary artery using custom made air-balloon. Pacing period was 300 or 200 ms (changed periodically every minute of ischemia).

Results showed that during regional ischemia the action potential duration in ischemic zone gradually decreased while activation time increased. Shortened pacing period at 6^{th} min of ischemia evoked optical action potential (OAP) amplitude and duration alternans. After 15 min of ischemia artery reperfusion was started and data showed that ischemia-induced changes of OAP parameters were reversible. In order to evaluate the role of beta-adrenergic receptor stimulation on alternans formation ISO was added to perfusion solution. ISO (1 μM) decreased activation time and duration of OAP. Occlusion of the artery in the presence of ISO decreased OAP duration more radically than in ischemia without ISO. But there were no alternans formation or alternans were of less magnitude in these conditions. Thus, our experimental data show that beta-adrenergic stimulation reduced alternans formation and may protect from arrhythmia during acute ischemia.

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P.P.86.
Emerging techniques and new methodsin biochemistry

Optical upstroke morphology investigation using optical mapping and transmural microelectrode recordings

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Last decade optical mapping technique became a widely-used instrument in cardiac electrophysiology. Optically recorded action potentials (APs) in single cardiac cells precisely match electrically recorded ones. However, this relationship becomes not so direct in myocardium since

optical AP (OAP) is the depth-weighted average of propagating AP and is affected by optical-biological processes (absorption, scattering and reflection). The best way to achieve informative results of electrical activity in the heart is to use both, optical approaches in combination with microelectrode technique.

In the Langerdorff-perfused rabbit heart APs were recorded with glass microelectrodes transmurally across the left ventricle (LV) wall from subepicardium towards subendocardium (3-5 mm, 20-50 steps). Simultaneously we used near-infrared (NIR) voltage sensitive fluorescent dye di-4-ANBDQBS to obtain OAP recordings using EMCCD camera (128x128 pixels, 500 frames/s). OAPs and electrical APs were recorded using pacing from epicardium and endocardium.

In our experiments we found, that AP activation time dependence on myocardium depth had a different shape, depending on pacing the type, distance, and LV geometry at recording site. The shape of the curve showed electrical transmural inhomogeneity of myocardium, which possibly was caused by histological inhomogeneity and different fibers orientation in different layers of myocardium. For the upstroke of OAPs evaluation, optical signals were compared with electrical AP, recorded and summated transmurally through LV wall depth. Recordings allowed us to evaluate the role of light scattering and how much tissue inhomogeneity was reflected in the morphology of the upstroke of OAP.

The obtained data could assist for better understanding of the mechanisms of the excitation wave propagation and for proper evaluation of optical signal in LV of the rabbit.

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P.P.87.
Emerging techniques and new methodsin biochemistry

Optical action potential alternans and its electrical origin in rabbit heart during acute regional ischemia

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Beat-to-beat alternation of the action potential at increased heart rates is extensively used as an empirical ECG-predictor of ventricular arrhythmias and sudden cardiac death. However, the mechanistic link between alternans and arrhythmias is not yet fully understood. Until recently the research was focused on action potential duration alternans as a cause of conduction blocks and reentrant arrhythmias. Here we demonstrate a possibility of an alternative scenario when alternans is just a manifestation of 2:1 conduction blocks. Experiments were performed in Langendorff-perfused white New Zealand rabbit hearts stained with near-infrared voltage-sensitive dye di-4-ANBDQBS. Regional ischemia was produced by occlusion of left anterior descending coronary artery.The heart was paced endocardially at basic cycle length (BCL) 300 ms. To induce alternans BCL was reduced to 200 ms. The alternans was monitored using optical mapping and two glass microelectrodes that were used to record action potentials from subepicardial and deeper myocardial layers. Results show that in optical recordings alternans develops inside the ischemic region 6-8 min after coronary occlusion and disappears during the 9-11th minute of occlusion. Optical action potential amplitude (OAPA) and duration (OAPD) alternans emerge and disappear almost simultaneously. During its peak amplitude, OAPD alternans occupies the area of the myocardium exceeding that of OAPA and is detectable beyond the ischemic region. Simultaneous microelectrode recordings obtained from different depths in the optical alternans regions show various degrees of conduction block which are not detectable in optical recordings. The correlation of OAPA and OAPD alternans with intramyocardial 2:1 conduction block during acute regional ischemia suggests that the alternans in optical action potential can serve as a marker of intramural conduction blocks.

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P.P.88.
Emerging techniques and new methodsin biochemistry

New reagent for gentle, efficient and cost effective protein extraction from E.coli and other bacterial cells

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Due to simple cultivation and easy genetic manipulation Gram-positive *Bacilli*, *Lactobacilli* and Gram-negative *E. coli* based constructs still remain the most favorite for recombinant protein production. In prokaryotic based recombinant cell factories the accumulation of target product could be directed to the cultivation media-secreted or accumulated in the cell cytoplasm. In Gram-negative bacteria such as *E. coli* there is an alternative intracellular accumulation option, in which the target protein is delivered to periplasmic space of the cell. The recovery of intracellular products is achieved by introducing cell disruption step which follows after cell concentration. The liberation of soluble or insoluble intercellular content with desired product is achieved after the brakeage of the bacterial cell envelope.

New reagent for bacterial cytoplasmic protein extraction combines enzymatic and chemical components, allowing efficient, but mild extraction of soluble proteins and isolation of inclusion bodies from frozen and fresh Gram-positive and

negative cells. B-PER Complete cell lysis reagent contains lysozyme, universal nuclease and detergent in Tris buffer, all in a single formulation with the long term storage at 4°C. The reagent is suitable for laboratory scale cell lysis and reduction of viscosity of bacterial crude extracts, thus the need for mechanical and physical disruption is eliminated. In this work we present data to demonstrate the performance of the newer B-PER Complete reagent.

P.P.89.
Emerging techniques and new methodsin biochemistry

The use of Cas9-RNA complexes for selective isolation of desired DNA sequences

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Selective isolation of multiple double-stranded DNA (dsDNA) fragments of interest technique is based on the ability of *in vitro* assembled ternary ribonucleoprotein complexes Cas9/crRNA/tracrRNA to recognize and bind dsDNA containing nucleotide sequences complementary to guiding crRNA. The 3' end of tracrRNA contains biotin tag which facilitates the whole complex dsDNA/Cas9/crRNA/tracrRNA isolation from the reaction mixture using streptavidin-coated magnetic beads. The technique may be applied for enrichment of desired or depletion of unwanted DNA sequences from mixtures of dsDNA fragments. Here we show that this technique works using either *Streptococcus thermophilus* or *Streptococcus pyogenes* Cas9 proteins. We demonstrate the ability of Cas9-RNA complex to bind specifically to the target DNA undermagnesium ions-free conditions and the possibility to catch this biotinylated Cas9-RNA and its target DNA complex with magnetic beads. The enrichment or depletion of desired DNA sequence is initially demonstrated in model system and later in selective isolation of chloroplast DNA from *Arabidopsis thaliana* total DNA mixture.

P.P.90.
Emerging techniques and new methodsin biochemistry

LC-MS/MS analysis of phosphopeptides isolated from apple microshoots

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Malus sp. has been designated as one of three model species of the *Rosaceae* family and extensive genome information became available for genomics analysis of plant response, recently. Despite a comprehensive knowledge on biology and genetics of apple is available, understanding of mechanistic basis of the resistance of*Malus* sp. to fungal and bacterial pathogens remains vague. Proteomics has been proven an effective approach in studies on regulation of biological processes at protein translation and post-translational modification level. However analysis of expression of proteins involved in plant signaling pathways requires highly sensitive and robust techniques. Therefore, the aim of this study is to establish a proteomics method for analysis of post-translational modifications of apple proteins using mass spectrometry approach. Micro-shoot tissue of apple cv. Golden delicious grown under *in vitro* conditions was used in the study. Peptides were prepared by digestion with trypsin and phosphopeptides were enriched using hydroxy acid-modified metal oxide chromatography method as described [Nakagami, 2010]. LC-MS/MS analysis of two separate samples with or without TCA treatment before trypsin digestion on average identified total of 175 and 176 unique peptides using apple protein database. Peptides identified in both samples constituted 16% of total unique peptides and unique phosphopeptides represented 49%. Among the 198 identified apple proteins, 60% included phospholylated peptides. Analysis of gene ontology based cellular localization of homologous proteins demonstrated that phosphorylated peptides were more often derived from nuclear proteins as compared to identified nonphosphorylated proteins (16% and 1%, respectively). Phosphorylated proteins there less common in plastid, mitochondrial or extracellular region. It is notable, that relatively large proportion of proteins were found to be associated to plant stress and pathogen response function (14%), that was comparable to the proportion of proteins associated to metabolic (25%) and gene expression (14.5%) function.

Nakagami H. et al. (2010) Plant Physiol. 153: 1161-1174.

P.P.91.
Emerging techniques and new methodsin biochemistry

Efficient mammalian membrane protein extraction

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We have developed an efficient Membrane Protein Extraction reagent for the enrichment of integral and membrane-associated proteins from cultured mammalian cells or tissues. Integral and peripheral membrane proteins (MPs) are important for the maintenance of many cellular functions such as signal transduction, cell integrity, intracellular and extracellular transport of molecular solutes and cell-to-cell communication. In addition membrane associated proteins and receptors are the largest category of drug targets studied in the pharmaceutical industry. Methods to characterize MPs are limited by the lack of extraction protocols and reagents that allow sufficient amounts of MPs to be obtained from various cell types without cross-contamination from other protein fractions. There are many strategies to extract MPs from eukaryotic

cell lines and tissues, including subcellular fractionation, cationic colloidal silica absorption, aqueous-polymer two-phase system, Triton™ X-114 phase separation, and high-salt or high-pH buffers. Most of these traditional protocols are laborious, time-consuming and require expensive ultracentrifugation equipment.

New Thermo Scientific reagent referred as Mem-PER Plus Membrane Protein Extraction Kit enables small-scale solubilization and enrichment of integral and membrane-associated proteins from cultured cells and tissues using a simple mild reagent-based procedure and a bench-top microcentrifuge. The reagent is directly compatible for the main downstream applications: SDS-PAGE, Western Blotting, BCA, immunoprecipitation, and amine-reactive protein labeling techniques.

P.P.92.
Other subjects

Influence of the copper sulfate on the rPpL laccase catalysed benzodiazepine oxime oxidation

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The synthesized benzodiazepine oxime derivative had absorbance spectra in the UV region with the maxima at 225, 255 and 290 nm. (Fig. 1a).It was determined that this compound benzodiazepine oxime can be oxidized by laccase rPpL, because has N-OH group. During the enzymatic oxidation process absorbance decrease was observed at 225 nm and the increase at 255 and 290 nm and the new absorbance maximum at 360 and 550 nm were observed.

This communication describes a study of the oxidation of the benzodiazepine oxime catalyzed by *Polyporus pinsitus* laccase (rPpL) in the presence of copper sulfate and the aim of this study was to investigate the influence of copper sulfate on the benzodiazepine oxime oxidation spectra changes.

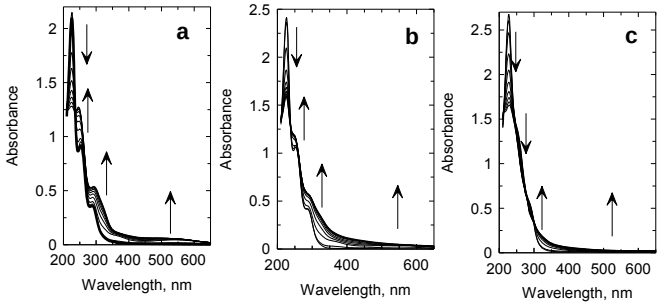

Fig. 1.Benzodiazepine oxime absorbance spectra change during rPpL laccase catalysed oxidation reaction without of $CuSO_4$ and after addition 50 μM (b) or 300 μM (c) of $CuSO_4$ to the reaction mixture.

The changes of substrate absorption spectra during the oxidation catalysed by rPpL laccase were observed when different concentrations of copper sulfate was added to the reaction mixture (Fig.1 b-c). The most noticeable changes were observed in the absorbance spectrum at the range of 220-300 nm. Addition of 50 μM of copper sulfate slightly increased rate of absorbance changes at 225 nm, but reduced at 255 nm (Fig. 1b). Whereas addition of 300 μM of copper sulfate increased rate of absorbance changes about 1,5 time at 225 nm, but rate of absorbance changes at 255 nm decreased about 3 times (Fig. 1c). During the reaction in the presence of 300 μM of copper sulfate absorbance at 255 nm not increased, but actually decreased.

Addition of copper sulfate influenced benzodiazepine oxime oxidation dependences on substrate and on enzyme concentration. The rate of absorbance changes depended on buffer solution pH in the presence and absence of $CuSO_4$ in the reaction mixture and maximal reaction rates were observed in the pH interval of 4.5-5.5.

P.P.93.
Other subjects

Analysis of atopy-associated genetic markers in the Lithuanian birth cohort

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Over the last decades, the prevalence of allergic diseases has been increasing in Europe and currently approximately 2 % of the population suffers from food allergy and 4 % – from asthma. As few effective techniques to cure allergic diseases are in use, research into predictive atopy-associated markers could provide ways to prevent the start of the atopic march. This study is the first analysis of atopy-associated genetic markers in the Lithuanian birth cohort.

Seven single nucleotide polymorphisms (SNPs) were investigated – *CD14* rs2569190, *FLG* rs11584340 and rs2184953, *FCER1A* rs2427837 and rs2251746,*IL13* rs20541 and rs1800925 by either allele-specific PCR or PCR restriction fragment length polymorphism (PCR-RFLP).

A total of 133 children (mean age 5.2 years; 57 % male) were genotyped and separated into cases and controls according to the result of a skin prick test (SPT). In the combined cohort, only *FLG* rs2184953 A/G genotype was linked to increased odds of producing a positive SPT. An association between *IL13* rs20541 T alele and at least one positive SPT was observed in the male subgroup. Within the female subgroup, positive SPT was associated with *FCER1A*rs2251746 C alele.

In conclusion, 3 SNPs were linked to allergy (positive SPT) in the analysed cohort. A significant difference in the prevalence of the atopy-associated SNPs among the genders was also observed.

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P.P.94.
Other subjects

The immunogenicity of amyloid beta oligomers and their role in macrophage-mediated inflammation

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The central molecule in the pathogenesis of Alzheimer's disease (AD) is believed to be a small-sized polypeptide – beta amyloid (Aβ) which has an ability to assemble spontaneously into oligomers. Studies concerning therapeutic and prophylactic approaches for AD are based on the immunotherapy using antibodies against Aβ which in some cases of clinical trials led to neuroinflammation. However, knowledge on the mechanisms of Aβ-induced immune responses is rather limited. Research on Aβ oligomeric antigens in complex with antibodies showed that neurotoxic effects on primary neurons are increased by Fc-dependent microglia activation. In the current study, we evaluated the dependence of immunogenicity of Aβ on the size of oligomeric particles and investigated how Aβ oligomers alone or in complexes with antibodies influence macrophage phenotype. **Results:** The analysis of serum antibodies in immunized mice revealed that 1-2 nm Aβ oligomers are highly immunogenic while larger Aβ oligomers and monomers induced a weak IgG response. Epitope mapping of Aβ-specific antibodies demonstrated that the main immunodominant region of the Aβ oligomers is located at its N-terminus, between amino acids 1 and 19. Cytokine profiles of cell growth supernatants from mice spleen macrophage and J774 cell line cultures treated with Aβ or their complexes with antibodiessuggest that macrophages are directed towards M1 phenotype. **Conclusions:** Small Aβ oligomers induce the strongest immune response in mice. The N-terminus of Aβ oligomers represents an immunodominant epitope which indicates its surface localization. Aβ oligomers in complex with antibodies form inflammatory conditions in macrophage cultures. The results of the current study may be important for further development of Aβ-based vaccination and immunotherapy strategies.

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P.P.95.
Other subjects

Characterisation of Protein Domains of Atypical Restriction-Modification Systems

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The restriction-modification (RM) systems, found in bacteria and archea, are composed of two enzymes: a DNA methyltrasferase and a restriction endonuclease. RM systems from *Corynebacterium glutamicum* (CglI) and *Neisseria gonorrhoeae* (NgoAVII) consist of three enzymes: a DNA methyltransferase, a restriction endonuclease and an ATPase. Bioinformatic analysis has revealed that the restriction endonucleases, R.CglI and R.NgoAVII (R proteins), contain phospholipase D (PLD) superfamily nuclease domains with a putative HXK active site motif and B3-type DNA binding domains. N.CglI and N.NgoAVII (N proteins) are composed of three domains: N-terminal DEAD-superfamily helicase domains with putative ATP hydrolysis and Mg^{2+} coordination sequence motifs, Z1-superfamily, which is yet to be characterised, and C-terminal domains.
Here we report cloning, expression, purification and characterisation of the domains of the R and N proteins. Size-exclusion chromatography has revealed that R proteins form dimers in solution through their N-terminal PLD domains. N.CglI is also a dimer and the dimerisation surface lies in the Z1 domain. N.NgoAVII was found to be a monomer. R and N proteins of the CglI system form a R_2N_2 restriction endonuclease complex in solution. Chromatography experiments suggest that both R.CglI domains (PLD and B3) interact with the DEAD helicase domain of the N.CglI in the complex. Gel mobility shift assay experiments confirmed that B3 domains of the R proteins are responsible for the recognition of the cognate 5'-GCCGC-3' DNA site. This study has deepened our understanding about the structural and functional organisation of the atypical CglI and NgoAVII RM systems.

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Domenic organization of DNase from halophile Thioalkalivibrio sp.

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Based on a study of secreted bacterial DNase from halophilic bacterium *Thioalkalivibrio sp.* we suggest model explaining how this enzyme is able to maintain activity at almost saturated solution of sodium chloride. The enzyme is composed from two domains: N-terminal domain which exhibits DNase activity and C-terminal domain comprising duplicate HhH motifs, which natural functions are recognition and interaction with DNA. Using deletion mutations analysis we have demonstrated that the C-terminal domain in *Thioalkalivibrio sp.* DNase is responsible

for enzyme halophilicity. Also, it was shown that the same active site in the enzyme's N-terminal domain is responsible for catalytic activity both at low and at high ionic strength. Analysis of sequence databases suggests that non - specific DNA binding domain is characteristic to many halophilic or halotolerant bacterial DNase species and it serves as a mechanism to maintain enzyme activity at high ionic strengths.

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Study of JNK signaling pathway in apoptosis regulation of neuro-differentiated cells

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Advances in stem cell culturing methods have enabled scientists to expand a range of healthy cell lines grown *in vitro* for long periods of time and to apply these cultures in various branches of Cell Biology and toxicology. Stem cell capability to differentiate into almost any cell type makes them even more attractive and provides ability to test drug action mechanisms both in proliferating and in terminally differentiated cell cultures.
In this work the role of stress-activated protein kinase JNK was studied in neuro-differentiated muscle-derived Myo stem cell death induction by a popular anticancer drug cis-diamminedichloroplatinum(II), so-called cisplatin. Studies revealed apoptotic mode of cell death. In differentiated along the neurogenic lineage Myo cells cisplatin induced prolonged both JNK and its target c-Jun phosphorylation, as determined by Western blot method. By use of JNK inhibitor SP600125, the opposite role of JNK signalling in proliferating and differentiated Myo cell death regulation after cisplatin treatment was shown. The stress kinase JNK is involved in protection of neurally differentiated Myo cells from cisplatin-induced cytotoxicity, as inhibition of JNK decreased viability of these cells, as measured using MTT reduction method. Involvement of JNK in the regulation of mitochondrial apoptosis pathway in Myo cells was shown at Bax and Bcl2 protein level.
Probably the most studied anti-apoptotic signalling molecule is a kinase AKT. In various cancer and stem cell models AKT plays essential role in protecting cells from apoptosis. Here we demonstrate that inhibition of JNK downregulates AKT phosphorylation, pointing to cross-talk between these signalling pathways.

P.P.98.

Yeast producing pulcherrimin. Formation of Liesegang rings. Investigation of biocidal effect.

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Yeast *P. guilliermondii*, *C. lusitaniae* secret products with antimicrobial and biocidal properties. Yeast when grown on plates on agar media containing iron produce pulcherrimin that could be observed in form of red Liesegang rings. The method suitable to observe Liesegang rings formation after 2-3 days was created.The first ring limits group biocidal effect. It was shown for the first time that individual colonies also could form obvious Liesegang rings. Their formation depended on iron amount in the media. Determination of iron was carried out by inductively coupled plazma optical emission spectrometry (Optima7000DV, Perkin Elmer USA). With excess of Fe^{3+} ions yeast killer zones were formed immediately, Liesegang rings were not formed and pulcherrimin was accumulated in the cells. With deficiency of iron, the rings were formed since pulcherrimin precursor was secreted. Under certain conditions, Liesegang rings can be formed inside the yeast colonies as in eukaryote tissues (important for medicine). These processes differed from those of control killer *S. cerevisiae* and *M. pulcherrima*. Electrochemical measurements at yeast-modified electrodes also revealed the difference among pulcherrimin-producing and non-producing yeast strains. Moreover, Liesegang rings were observed with *Monoraphidium* spp. (isolated from microalgae communities of *Gonyostomum semen*) secreting X material. These experiments allow rapid and effective investigation of pulcherrimin formation, its biochemical properties and importance for yeast vital processes.

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Other subjects

Biochemical Blood Parameters of Lithuanian Cattles of Organic and Usual Dairy Farming

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Organic farming represents advantages, importance and benefit of ecological production to human health. However it is not reflected how organic farming influence cattle‘s wellness and variations of blood biochemical parameters. The main aim of this trail was to establish and compare the values of serum protein, calcium, magnesium, phosphorus, aspartate aminotransferase (AST), alanine aminotransferase (ALT) of fifteen cows of organic farm in Klaipėda region and fifteen cows of non-organic farm in Plungė region.
Accordingly to the result, the quantity of protein ranged from 66.5 to 98.9 g/L in the blood of organic farming cows, whereas the quantity of protein in the blood of usual farming

cows ranged from 66.6 to 84.5 g/L. The quantity of calcium ranged from 1.4 to 3.06 mmol/L of blood serum of organic farming cows and from 2.15 to 2.88 mmol/L of usual farming cows. The quantity of phosphor varied between 1.62 and 2.9 mmol/L and 1.03 and 2.62 mmol/L and the quantity of magnesium ranged from 0.78 to 1.12 mmol/l and from 0.82 to 1.27 mmol/L in the blood of organic farming cows and usual farming cows blood serum, accordingly. Values of AST (84-220 TV/L) in blood of organic farming cows increased comparing to AST values which varied between 66.4 and 130.2 TV/L in usual farming cows blood. The activity of ALT ranged from 19 to 42.6 TV/L and from 18.5 to 52.6 TV/L in the blood of organic farming cows and in usual farming cows blood, accordingly. The values correspond to physiological values of cattle blood biochemical parameters with the small deviation.

It might be concluded that values of AST, ALT, calcium, magnesium, phosphorus and protein are similar for cattle's of organic and non-organic farms. But the case of increased values of some parameters of the organic farming cattle's comparing with physiological limits requires more investigations.

P.P.100.
Other subjects

The Influence of Feed Additive BIOGROM® on Blood Biochemical Parameters of Lithuanian Black-and-White Cattles

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Lithuanian Black and White cattle asan independent breed were recognized in 1951. Now they account for about 60% of bred cattle in Lithuania. Improving the Lithuanian Black and White's,the most important tasks are to increase the milk production, milk fat andprotein content; to improve the uddermorphological and physiological characteristics and to preserve meat quality.

Biogrom® is the multifunctional feed additive for ruminant sinfluencing strengthening of immunity, an increase inmilk yield and amount of selenium in milk, and a reduction of somaticcells amount in milk, risk of acidosis and cases of diarrhea, also.

The main aim of this trail was to investigate the influence of the feed additive Biogrom® on main biochemical blood parameters and minerals, of Lithuanian Black and Whitecattle.

The experiment was conducted in Practical Training and Testing Centre of LHUS and at the Department of Biochemistry. The cattle were divided into 2 groups of 10 cows each (Control and Experimental). The cattle had a standard, commonly balanced ration with the 40 g supplement of additive Biogrom® for the Experimental group daily, duringthe next90 days.Bloodsampling from the caudalvein were selected to determine the concentrations of chosen biochemical parameters every 30 days.

Components of theused additive fed to cows were useful and valuable, while their concentrations of calcium, phosphorus, magnesium and proteins in blood were influenced positively. The obtained results are within the normal range of recommended levels and are higher to compare with results of the Control group. There was equivalentin crease in alkaline acidity and concentration of carotene observed, what leads to improved animal well-being and positive synthesis ofvitamin A. Concentration of ketone bodies does not exceeded recommended quantity. In conclusion, Biogrom®leads tothe prevention ofacidosis and ensures the positivedynamics of biochemical parameters in cattle blood.

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Other subjects

Factors affectingthe toxin K2 inhibition zone diameter

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Toxin K2 secreted by *Saccharomyces cerevisiae*killer strainsprevents the reproduction of sensitive yeast cells. The mechanism of cell killing by this toxinstill requires more detailed investigation. We determined that K2 toxin is the most stable when secretedintopH 4.4medium. Toxinreleased to pH 6.0 medium forms the largest inhibition zone. The reason of this can bethat the higher amount of toxin is secreted to medium at theseconditions.We determined that for evaluation of K2 activity the best is to use lawns with no more than $1x10^7$cells. It is known that diameter of the inhibition zonesof this toxin is proportional to logarithm of the toxin activity. The number of toxin-affected cells on the lawn is directly related to the activity of toxin suspension. We determinedthe linear regressionof the numberof toxin treated cells to the diameter of inhibition zone with the equation $y=0.885x + 1.499$ ($R^2=0.994$). These results allow us todetermine the optimal volume of the K2 toxin suspension required for the inhibition of sensitive cell growth.

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Screening of monooxygenases by using indoline as a chromogenic substrate

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Oxidative biotransformations that usually involve monooxygenases or dioxygenases are increasingly exploited in the synthesis of many highly valuable synthons. In order to identify novel activities and to improve these enzymes for engineering approaches, suitable screening or selection methods are necessary. Various surrogate fluorogenic and chromogenic enzyme substrates, including indole and its derivatives, as well as post-reaction derivatization approaches have been tested to create and improve the screening systems for oxygenases.

A novel medium for the efficient screening of oxidoreductases-producing microorganisms was developed using indoline derivatives as chromogenic substrates. Screening of soil samples allowed the identification of more than one hundred isolates capable of forming violet pigment on 5-bromoindoline. Five bacterial isolates active towards 5-bromoindoline, yet not producing indigo blue from indole were selected for further work. The analysis of 16S RNA-encoding genes revealed that K125 and 14-470 isolates were related to *Pseudomonas* spp., KVIA17 and NOR43 were homologous to *Arthrobacter* species, whereas the isolate 33-3 belonged to *Rhodococcus* genus.

Genomic fragments encoding 5-bromoindoline oxidation were cloned from the libraries constructed using DNA of the selected strains. The length of the cloned DNA fragments ranged from 8 to 14 kb. Sequence analysis showed that all DNA fragments encoded oxygenases. Moreover, both mono- and dioxygenase genes were identified. Further studies on enzyme properties including substrate specificity are in progress.

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Other subjects

NOVEL DEAMINASES FROM THE METAGENOMIC LIBRARIES

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Modified nucleotides present in the nucleic acids occur in almost all classes of RNAs in great chemical diversity and are critical for their folding, stability, and protein translation process. Biosynthesis of pyrimidine nucleotides such as uracil or cytosine and their modifications is well studied, however, little is known about the cellular degradation of these compounds and corresponding enzymes. Present work focuses on the degradation of isocytosine (2-aminouracil), an isomer of cytosine. Enzymes capable of removing the amine group from pyrimidines are well known, e.g., cytosine deaminase, a hydrolase that catalyses the conversion of cytosine to uracil during the metabolism of pyrimidines. Enzymes that are capable of catalysing similar reactions in catabolism of other pyrimidine bases ought to exist. One way of detecting those enzymes is through screening of the metagenomic libraries. A strict uracil auxotroph-based selection strategy was created to find the genes responsible for conversion of isocytosine to uracil. Five genes encoding potential deaminases were obtained from metagenomic libraries. One of these deaminases shows similarity to 8-oxoguanine deaminases and hydroxydechloroatrazine ethylaminohydrolases. The enzymatic activity and substrate specificity of newly discovered enzymes will be further analysed in order to establish their biochemical and cellular function.

P.P.104.
Other subjects

Toxin of type II TA system of Escherichia coli O157:H7 exhibits novel mechanism of growth inhibition

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Toxin-antitoxin (TA) systems are small genetic loci widespread in bacterial and archaeal genomes. TA systems are typically composed of two elements – a toxic protein and its antidote. Due to the addictive nature of TA genes they stabilize mobile genetic elements. Moreover numerous studies propose that TA systems could control bacterial response to various forms of stress. With a few exceptions a majority of currently known toxins target translation through various mechanisms, most commonly by degrading mRNAs. We have recently discovered a new TA system whose toxin possesses an acetyltransferase domain. We have shown that AtaR/AtaT genes behave like a TA system. Toxin and antitoxin proteins were co-purified from protein lysates showing that they form a complex which renders toxin inactive. Based on ^{35}S-Methionine incorporation experiments, we showed that the toxin blocks translation. Mutations introduced to a predicted active site and Coenzyme A binding pocket disrupts toxicity. Therefore we propose that toxicity is specifically associated with the acetylation activity. AtaT toxin belongs to GNAT family of proteins that acetylate various substrates, including proteins by either terminal Nα-acetylation or Nε-acetylation of internal lysines. We are underway to test which type of acetylation is exhibited by AtaT. In search of acetylation target we have constructed a genomic library to find multicopy suppressors that restore viability upon expression of the AtaT toxin. Preliminary library screenings suggest that AtaT could target folate metabolism, similarly to the sulfonamide class of antibiotics. We are currently testing this hypothesis.

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Other subjects

Quantitative evaluation of human carbonic anhydrase XII expression in prostate, lung and breast cancer

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Human carbonic anhydrase XII (CA XII) is an enzyme known as a potentional cancerbiomarker. It has been reported that CA XII is overexpressed under hypoxic terms and it affects tumour growth by making optimal intercellular conditions for cancer cells. CA XII expression in various organs and its relation with patient survival prognosis is intensively investigated in many laboratories.

The aim of the current study was to examine tissue specimens collected from patients diagnosed with various types of cancers and to compare CA XII expression levels between normal and cancer cells. Previously optimized quantitative reverse transcription real-time polymerase chain reaction (RT-qPCR) method was used to analyze CA XII mRNA expression in normal and tumour tissues. In total, 36 lung cancer, 15 breast cancer and 47 prostate cancer specimens were investigated and compared with the respective normal tissue specimens. The obtained results showed that CA XII was overexpressed in cancer tissues, while normal tissues were characterized by low levels of CA XII expression.

In conclusion, this study confirmed that CA XII is overexpressed in prostate, lung and breast cancers at mRNA level. RT-qPCR for CA XII mRNA detection is a promising molecular diagnostic tool whichcan be applied for the diagnostics of various types ofcancers.

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P.P.106.
Other subjects

LYSOZYME-INDUCED CHANGES IN LACTOCOCCUS LACTIS AND RELATIVE CELL ENVELOPES

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Lysozyme is a muramidase founded in all eukaryotic organisms. This protein is the first component of immune system fighting the invaded pathogenic bacteria. Lysozyme has two different activities - enzymatic and membrane-active, but the latter one needs to be better explored. *Lactococcus lactis* cells were used for studies of the mechanisms of lysozyme action. These nonpathogenic bacteria are widely used in milk and meat industry.

Three forms of lysozyme-intact, heat-treated and chemically-inactivated - were studied. It was shown that all these forms of lysozyme induce leakage of K^+ ions from cytosol and decrease the respiration rate of *L. lactis, Streptococcus thermophiles* and *Streptococcus agalactiae* cells. High concentrations of lysozyme (1 mg/ml and higher) caused an immediate increase of the cell membrane permeability. At lower concentrations of lysozyme the increase in permeability was observed only after a certain lag period. Some differences in the responses of *L. lactis* cell to the modified lysozyme were observed. Intact and heat-treated lysozyme caused a faster permeabilization of the cell envelope and a sharper decrease of the respiration rate than chemically-inactivated one. The response of *L. lactis* mutant strains to lysozyme was also examined. A later starting leakage of K^+ from the cell and a slower decrease of respiration rate were observed in the cases of *L. lactis*mutants resistant to lysozyme. *St. thermophilus* wild-type and mutant strains were not sensitive to lysozyme but *St. agalactiae* mutant were more sensitive to this enzyme than wild-type cells.

P.P.107.
Other subjects

Cell culture model to investigate cellular response to ionizing radiation

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Radiotherapy is one of the most important modality for cancer treatment. However, the relationship between molecular cell response to ionizing radiation and radiotherapy efficiency is still not well defined. Therefore the aim of this study was to investigate the response of mouse Lewis Lung Carcinoma (LLC1) cell line to different ionizing radiation treatments.

We have employed cell culture model to investigate cellular response to ionizing radiation. Single or fractionated dose irradiation of 2Gy, 10Gy and 5x2Gy has been used. Global gene expression, cell survival and cell cycle changes after irradiation have been analysed in Lewis lung carcinoma (LLC1) cell line cells.

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