RBC 2012

Relax-Be snooping-Carpe diem

Regional Biophysics Conference 2012

Kladovo-Belgrade, Serbia

September 03-07, 2012

BOOK OF ABSTRACTS

Organized by Biophysical Society of Serbia

EBSA Biophysics in Europe

IUPAB Connecting the World of Biophysics

ESN European Society for Neurochemistry

CMJ Croatian Medical Journal

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The RBC is a series of biennial symposia intended to bring together biophysics researchers from Austria, Croatia, Hungary, Italy, Serbia, Slovenia and Slovakia and also includes internationally renowned guest speakers. The first such meeting took place in March 2005 in Terme Zrece, Slovenia. The language of the conference is English.

http://rbc2012.biofizikasrbija.com/

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12:35-12:55 **Ferenc Vonderviszt** (Hungary), *Flagellin-based self-assembling building blocks for creation of filamentous nanostructures*

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12:20-12:40 **Denis Knyazev** (Austria), *Gating of the bacterial protein translocation channel SecY*

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10:30-10:50 **Tanja Dučić** (Germany), *X-ray microscopy on cellular level.*

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12:10-12:30 **Nevena Puač** (Serbia), A*pplications of nonequilibrium plasmas in biology and medicine.*

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15:05-15:25 **Božidar Casar** (Slovenia), *Stereotactic radiosurgery (SRS) with linear accelerator for treatment of intracranial lesions.*

15:25-15:45 **Aleš Iglič** (Slovenia), *Electrostatics and mechanics of the interactions between osteoblasts and titanium implants.*

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16:35-16:55 **Lenart Giradon** (Slovenia), *Using cells and biomaterials for regeneration of diseased and damaged tissues.*

16:55 -17:15 **Sofija Andjelić** (Slovenia) *Calcium changes and contractions of human anterior lens epithelial cells*

17:15-17:35 **Petar Marinković** (Germany) *Imaging axonal transport in health and disease*

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20:00 **Farewell Dinner**

Friday, 7/9

Departure

PLENARY LECTURES

1.

NMR metabolomics and fluxomics

Emirhan Nemutlu[1,3]
Song Zhang[1]
Nenad O. Juranic[2]
Andre Terzic[1]
Petras Dzeja[1] and
Slobodan Macura[2]

[1] *Division of Cardiovascular Diseases, Department of Medicine, Mayo Clinic, Rochester, Minnesota 55905, USA,*
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[3] *Department of Analytical Chemistry, Faculty of Pharmacy, University of Hacettepe, 06100 Ankara, Turkey*

Detailed knowledge of metabolic processes involved in cellular production, function and degradation holds immense potential for understanding mechanism of many diseases, their early diagnosis and therapy monitoring. For a comprehensive characterization of metabolic networks and their function, quantitative knowledge of metabolite concentrations (metabolomics) and metabolite fluxes (fluxomics) is required. Analytically, this could be perceived as a determination of concentrations and turnover rates of a large number of small molecules (metabolites) from tissue or body fluids. Thus, metabolomics and fluxomics methods mostly rely on the information-rich analytical technique, most notably NMR spectroscopy and mass spectrometry. Recently we have demonstrated that ^{18}O-assisted ^{31}P NMR provides phosphorous-containing metabolite levels and their respective turnover rates in tissue and blood samples (1). The ^{18}O labeling is based on incorporation of the ^{18}O nuclei (from $H_2{}^{18}O$), into phosphate group with each act of ATP hydrolysis and the subsequent distribution of ^{18}O-labeled phosphoryls among phosphate-carrying molecules. All major phosphometabolites and their turnover rates can be quantified using ^{18}O-assisted ^{31}P NMR spectroscopy and significantly accelerated by the use of mass spectrometry. On selected examples we'll demonstrate that ^{18}O-assisted ^{31}P NMR/mass spectrometry is a valuable tool for phosphometabolomic and fluxomic profiling of transgenic models of human diseases.

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1. Nemutlu E, et. all., Physiol Genomics. **2012**, 44(7):386-402; Anal Bioanal Chem. **2012**, 403(3):697-706

2.

Harvesting the sun…safely and efficiently

Roberta Croce,

VU University Amsterdam

Photosystem I and Photosystem II of higher plants are large multi-protein complexes composed of many subunits and coordinating between 170 and 250 chlorophylls per reaction center. This complexity makes it very difficult to extract details about excitation energy transfer and trapping. Recently we managed to obtain homogeneous preparations of both Photosystems with different antenna sizes and compositions (1,2). These complexes were analyzed by Time-resolved fluorescence spectroscopy. The comparison of the results obtained on the different complexes allows to disentangle the role of the individual antenna complexes in excitation energy transfer and trapping (3,4). By integrating biochemical, structural and spectroscopic data, a picture of the flow of excitation energy in the supercomplexes and in the thylakoid membrane is obtained.

1. Wientjes E., Oostergetel G.T., Jansson S., Boekema E.J. and Croce R. (2009), J. Biol. Chem. 284, 7803–7810.
2. Caffarri S., Kouril R., Kereiche S., Boekema EJ and Croce R. (2009), EMBO Journal. 28, 3052-3063.
3. Wientjes E., van Stokkum IHM, van Amerongen H. and Croce R. (2011), Biophys. J. 101, 745-754.
4. Croce R. and van Amerongen H. (2011), J. Photochemistry and Photobiology B, 104, 142–153.

3.

Tripartite synapse: exocytosis in astrocytes

Vladimir Parpura

Department of Neurobiology, Center for Glial Biology in Medicine, Atomic Force Microscopy & Nanotechnology Laboratories, Civitan International Research Center, Evelyn F. McKnight Brain Institute, University of Alabama, Birmingham, AL 35294, USA; vlad@uab.edu

Astrocytes are an integral element of the tripartite synapse. These cells release the excitatory transmitter glutamate which modulates synaptic transmission in nearby neurons. Astrocytic glutamate release can occur through Ca^{2+}-dependent exocytosis, which can operate under physiological conditions. The intercellular Ca^{2+} necessary for this glutamate release comes from two sources. The majority originates from internal stores, but entry of external Ca^{2+} is also involved. Transient receptor potential (TRP) proteins, which form channels that are activated by depletion of internal Ca^{2+} stores to allow Ca^{2+} entry from the extracellular space, mediate the entry of external Ca^{2+} in glutamate release from astrocytes. Vesicular glutamate transporters (VGLUTs) are responsible for vesicular glutamate storage and exocytotic glutamate release from astrocytes. Over-expression of individual isoforms of VGLUTs in solitary astrocytes shows that VGLUT-3, but not VGLUT-1 and -2, enhances glutamate release from astrocytes without affecting their intracellular Ca^{2+} increase. Inhibition of glutamine synthetase activity by L-methionine sulfoximine in astrocytes, which raises cytoplasmic glutamate levels greatly increase the exocytotic glutamate release. Taken together, VGLUTs and cytoplasmic glutamate levels in astrocytes regulate exocytotic release from these cells.

The mechanism underlying Ca^{2+}-dependent release of various transmitters from astrocytes is exocytosis. Astrocytes express the protein components of the SNARE complex, including synaptobrevin 2, syntaxin and SNAP-23, but not SNAP-25. Using astrocytes expressing synapto-pHluorin, exoctytotic sites can be fluorescently imaged. Fusions of synapto-pHluorin labeled vesicles with the plasma membrane can be observed using total internal reflection fluorescence microscopy; the time course of fusion events (burst vs. sustained), their type ("kiss-and-run" vs. full fusion) and spatial relationship between different fusion sites is discussed. Single molecule investigations of the SNARE complex using force spectroscopy show that ternary complexes containing SNAP-23 have a shorter spontaneous lifetime than those containing SNAP-25B. Thus, the spatio-temporal characteristics of astrocytic exocytosis might be in part due to intrinsic properties of the ternary SNARE complex in astrocytes.

4.

RETRO-Images from infected cells

Ashwanth Christopher Francis

University of Trento, CIBIO, Laboratory of Molecular Virology, Trento, Italy.

Most HIV-1 particles infecting the host cell do not reach the nucleus and few of them productively integrate in the cellular genome. So far investigations on the viral fate from the time of entry to integration had to rely on techniques that do not allow following individual virions to discern those that successfully reach the nuclei to integrate. We recently developed a fluorescent microscopy experimental system to detect single viral particles up to the nuclear compartment. The analysis of nuclear pre-integration complexes (PICs) revealed that PICs preferentially localize in specific regions of the nuclei occupied by eu-chromatin in the periphery of the nuclear compartment. To deepen our understanding of HIV-1 nuclear biology we measured individual viral particle movements in live cells. From the analysis of viral trajectories we discovered that PICs move in the nuclei by active transport and that nuclear actin is a possible molecular motor mediating PICs nuclear trafficking. Finally, new recent development in HIV-1 as well as MLV visualization systems will be presented.

SECTION LECTURES

1.S1

Molecular modeling of nucleic acid - small molecule interactions

Sanja Tomić,
Marina Grabar,
Ivo Piantanida

Ruđer Bošković Institute, Bijenička 54, Zagreb, Croatia

Specific interactions of small molecules with nucleic acids have attracted significant scientific interest due to their possible medicinal, biochemical and biological importance. However, often the small molecules interact with the nucleic acid non-specifically and according to the binding mode they are considered as intercalators, groove (minor or major) binders and external electrostatic binder. For the purpose of developing new more selective drugs and biochemical markers we combine computational, molecular modeling approach with the experimental, mostly spectroscopic results. I will speak about results of our recent molecular modeling studies on two different sets of small molecules specifically interacting with nucleic acids.

The first is related to guanidiniocarbonyl-pyrrole-pyrene conjugates which show distinctively different binding mode to ds-DNAs in comparison to ds-RNAs, accompanied with specific spectroscopic response, thus behaving as nucleic acid sensors. The second are benzimidazole translation inhibitors of the hepatitis C virus RNA.

2.S1

Predictions of bacterial transcription start sites - a biophysical approach

Marko Djordjevic

Institute of Physiology and Biochemistry, University of Belgrade, Faculty of Biology, Belgrade, Serbia

Transcription start sites (TSS) in bacterial genomes are locations where RNA polymerase binds and initiates transcription. Accurate knowledge of TSS is important not only for bioinformatic applications (e.g. gene and operon predictions), but also as the first and the rate limiting step in understanding transcription regulation. TSS prediction is a classical bioinformatics problem, where available methods show poor accuracy. We here approach this problem from a biophysics perspective, in order to develop a more accurate method for TSS prediction. The main idea is to combine accurate alignments of promoter elements [1] with a biophysical model of transcription initiation that we recently developed [2,3]. In this talk I will discuss both theoretical modeling of transcription initiation, and our recent advances in understanding promoter specificity. I will also present how the modeling and the analyzed sequence specificity are combined in a biophysics based algorithm for TSS detection.

1. Djordjevic M, J Bacteriol. 193:6305, 2012
2. Djordjevic M, Bundschuh R., Biophys J. 94:4233, 2008
3. Djordjevic M, submitted to J. Theor. Biol., 2012

3.S1

Unusual tRNA structure ensures fidelity of translation

Rachel Schmidt*
Kaitlyn M. Peterson*
Ivana Simonović
Miljan Simonović

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*(*Equally contributed to this study)*

Fidelity of gene translation critically depends on the accuracy of aminoacyl-tRNA synthetases (aaRS), the enzymes that couple each proteinogenic amino acid with the cognate tRNA. While the selection of amino acids is dictated by the architecture of the catalytic site, the tRNA recognition relies on an elaborate mechanism in which a set of conserved identity elements in the isoacceptor tRNAs is recognized by the enzyme. Moreover, the direct aminoacylation reaction in which an amino acid is attached to the tRNA is a prevalent route to synthesis of the correct aminoacyl-tRNAs (aa-tRNA) in all domains of life. Here, two extraordinary examples that challenge the long-established view about the formation of aa-tRNAs will be presented. In the first, an essential amino acid is synthesized *via* an indirect aminoacylation route that critically depends on a tRNA structure distinct from other elongator tRNAs, whereas in the second, a single aaRS acts on two natural isoacceptor tRNAs despite the fundamental differences in their main recognition element. The distinct mechanisms presented herein play an important role in preserving the accuracy of the translational machinery.

4.S1

Function of uncoupling proteins

Elena E. Pohl

Institute of Physiology, Pathophysiology and Biophysics, University of Veterinary Medicine, Vienna

Uncoupling proteins (UCP) form a subfamily inside a mitochondrial anion carrier family. Its best investigated member, UCP1, is expressed in brown adipose tissue. It uncouples substrate oxidation from mitochondrial ATP synthesis, catalyzing thereby energy dissipation as heat and playing a major role in non-shivering thermogenesis. In contrast, the function and even the localization of other uncoupling proteins (UCP2-UCP5) are controversially discussed over many years. The most attractive hypothesis deals with their participation in reactive oxygen species (ROS) regulation. Our recent research concentrated on the transport characteristics, tissue distribution and functions of uncoupling proteins initially associated with brain: UCP2, UCP4 and UCP5. Using antibodies evaluated with recombinant proteins and knock-out mouse we revealed that only UCP4 is present in neurons and neurosensory cells at protein level (1, 2). In contrast, UCP2 is up-regulated in cells with a high proliferative potential, such as lymphocytes, stem and cancer cells. Neither one of these proteins was up-regulated under experimental conditions which modeled the increased ROS production in mitochondria.

1. Smorodchenko, A., Rupprecht, A., Sarilova, et al. (2009). Biochim. Biophys. Acta 1788, 2309-2319
2. Smorodchenko, A., Rupprecht, A., Fuchs, et al. (2011). Mol. Cell. Neurosci. 47(4), 244-53.

5.S1

Dynamic structural adaptation in functional protein-protein complexes

Péter Závodszky

Institute of Enzymology, Research Centre for natural Sciences, Hungarian Academy of Sciences, Budapest H-1518 P.O.B.7.

Most enzymes operate optimally at the edge of their conformational stability. The reason for this is the requirement for dynamic interaction between the protein matrix and the substrates. It is obvious that protein molecules undergo fluctuations of internal energy at room temperature. These fluctuations of energy are also reflected in conformational fluctuations. The dominant fluctuations of a native, globular protein are overlapping, noncooperative motions. Although the existence of such conformational dynamics is well supported with experimental evidence, the functional significance is still disputed.

Certain biological functions (ligand binding, complex formation, receptor recognition etc.) of proteins are associated with significant conformational changes. X-ray diffraction visualizes the initial and final conformations only, NMR studies can reveal the conformational dynamics at the level of individual groups of atoms however, the size of the protein prone to such studies is limited. Hydrogen-deuterium exchange can report about gross conformational flexibilities however, the topological assignment – in this case – remains unresolved. All this techniques in combination with molecular dynamics simulations may disclose the mechanism: how conformational fluctuations underline a particular biological function. In the case of an autoactivating serine protease dimer of the complement system (MASP2), the mutual recognition, the shift of zymogen conformation to active like conformation, the cleavage step and the release of the activated substrate can be visualized in terms of mutual, fluctuational adaptation of the two zymogen proteases.

6.S1

Effects of interfacial water structure on protein conformation and function

András Dér

Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

A phenomenological theory of salt-induced Hofmeister phenomena is presented, based on a relation between protein solubility in salt solutions and protein–water interfacial tension. As a generalization of previous treatments, it implies that both kosmotropic salting out and chaotropic salting in are manifested via salt-induced changes of the hydrophobic/hydrophilic properties of protein–water interfaces. The theory is applied to describe the salt-dependent free energy profiles of proteins as a function of their water-exposed surface area. On this basis, three classes of protein conformations have been distinguished, and their existence experimentally demonstrated using the examples of bacteriorhodopsin and myoglobin. The experimental results support the ability of the new formalism to account for the diverse manifestations of salt effects on protein conformation, dynamics, and stability, and to resolve the puzzle of chaotropes stabilizing certain proteins (and other anomalies). It is also shown that the relation between interfacial tension and protein structural stability is straightforwardly linked to protein conformational fluctuations, providing a keystone for the microscopic interpretation of Hofmeister effects. Implications of the results concerning the use of Hofmeister effects in the experimental study of protein function are discussed.

7.S1

Molecular Aspects of the Regulation of the Actin Cytoskeleton

Miklós Nyitrai

Department of Biophysics, Medical School, University of Pécs, Pécs, Szigeti str. 12, H-7624, Hungary

Fluorescence spectroscopic methods are powerful tools to study the conformational, dynamic and structural properties of cytoskeletal proteins. In this presentation we gave examples regarding the advantages of these methods through the results of our investigations of actin-binding proteins. Actin is one the key components of the cytoskeleton. We studied how formins, tropomyosins and myosins modify and regulate the conformational transitions of actin. Formins are conservative proteins with important roles in the regulation of the actin based microfilamental system in eukaryotic cells. They have several domains including FH1, FH2, GPB and DAD. In the interaction between actin and formin the FH2 domain plays a key role. The 'mammalian Diaphanous-related 1' constitutes one of the subfamilies of the formins. The data obtained in steady-state and time correlated fluorescence spectroscopic assays showed that these mDia1 formin fragments made the actin filaments more flexible in a concentration dependent manner. We also investigated whether the mDia1-FH2 affects the nucleotide exchange on the actin filaments. Steady-state fluorescence anisotropy and photometric coupled assay measurements showed that the ATP-ADP conversion was accelerated in the presence of formins, and the effect was stronger at greater formin concentrations. Further experiments revealed that two abundant actin-binding proteins - tropomyosin and myosin - could reverse the formin induced conformational changes in actin. These observations indicate that actin-binding proteins play an important role in the regulation of the conformation of the actin filaments.

8.S1

Flagellin-based self-assembling building blocks for creation of filamentous nanostructures

F. Vonderviszt[1,2]
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V. Szabó[1]
M. Meiczinger[1]
A. Muskotál[1]
H. Jankovics[1]
B. Tóth[1,3]

[1] *Bio-Nanosystems Laboratory, Research Institute of Chemical and Process Engineering, University of Pannonia, Veszprem, Hungary*
[2] *Research Centre for Natural Sciences, Budapest, Hungary*
[3] *Centre for Agricultural Research, Martonvásár, Hungary*

The aim of this work is to furnish various proteins with polymerization ability by creating fusions with flagellin, the main component of bacterial flagellar filaments. The hypervariable D3 domain of flagellin, situated at the outer surface of flagellar filaments, is not required for filament formation. The concept in this project is to engineer flagellin to give it various functionalities by replacing the D3 domain with suitable foreign proteins without adversely affecting polymerization ability, and to assemble these chimeric flagellins into tubular nanostructures of high stability.

The prototype of flagellin-based polymerizable enzymes has been created by replacing the hypervariable central portion of the polypeptide chain of flagellin with the amino acid sequence of the xylanase A enzyme. A polymerizable GFP variant has been also developed which exhibited intensive fluorescence and was capable of efficient filament formation. Work is in progress to produce flagellin-based binding proteins capable of efficient recognition of target molecules.

Our results demonstrate that polymerization ability can be introduced into various proteins, and building blocks for rationally designed assembly of filamentous nanostructures can be created. Multifunctional filaments obtained by directed co-polymerization of flagellin-based fusion constructs offer potential applications in medical diagnostics, environmental monitoring or biotechnology.

The support of the National Development Agency (CK77819, NANOFLAG, TÁMOP-4.2.2/B-10/1-2010-0025) is gratefully acknowledged.

9.S1

Marine biopolymers and nanoparticles interaction

Vesna Svetličić[1]
Galja Pletikapić[1]
Ivana Vinković Vrček[2]
Vera Žutić[1]

[1] *Division for Marine and Environmental Research, Ruđer Bošković Institute, Zagreb, Croatia*
[2] *Analytical Toxicology and Mineral Metabolism Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia*

This study highlights the capacity of AFM for investigating nanoparticle (NP) algal cell interaction with a subnanometer resolution. We designed a set of AFM experiments in order to: (i) characterize the state of NPs in terms of size, shape and structure; (ii) visualize changes in cell morphology induced by NPs and (iii) characterize NP interaction with the extracellular polymers (EP), attached to the cells or released in the culture medium.

In natural seawater used throughout this study the single Ag NPs adopted truncated tetrahedron morphology with particle heights of 10, 20, 30 and 40 nm. The EP production has been shown to increase as a feed-back response to Ag NP exposure and may contribute to detoxification mechanisms. Imaging EP at high resolution revealed the incorporation of Ag NPs and their aggregates into the EP-gel matrix proving their detoxifying capacity. However, this nanospecific interaction (direct NP interaction with the EP polysaccharide component) could have significant environmental implications.

10.S2

Electrical behavior of dendritic spines

Marko Popovic

Department of Physiology, Yale University School of Medicine, New Haven, USA.

The objective of the study was to determine whether spines play an electrical role in signal processing based on postulated isolation of the spine head from the parent dendrite by elongated spine necks. There is no direct information on the electrical resistance of the spine neck, the critical functional variable in this context, because electrode recording of membrane potential signals from spines is not realizable due to their small size. Estimates from indirect evidence from prior voltage-imaging studies and from modeling are inconclusive. We investigated electrical signaling in spines by combining patch-electrode recordings with the wide-field epi-fluorescence recording of membrane potential transients from spines and parent dendrites of cortical layer-5 pyramidal neurons loaded with membrane impermeable voltage-sensitive dye in brain slices. We advanced the sensitivity of optical recording by: (a) using monochromatic excitation light at a near-optimal wavelength and (b) increasing the excitation light intensity from a laser above the level that can be reached with conventional arc-lamps. With these improvements, our measurements were approximately 100-200 fold more sensitive compared to previously reported SHG and confocal recordings. We first tested whether steady-state membrane potential changes are attenuated in proportion to the length of the spine neck as they propagate across the neck into the spine head. The results from ~50 spines characterized by different length of the spine neck did not reveal significant correlation between the neck length and the voltage attenuation of a steady-state hyperpolarizing membrane potential signal. We carried out similar measurements of backpropagating action potential voltage transients which are more sensitive to the hypothetical filtering effect of the dendritic spine necks, compared to steady-state membrane potential changes. The results showed that bAPs propagated into the spine heads without detectable changes in their time course or amplitude, independently of the length of the spine neck.

11.S2

MEG studies on dynamic cortical networks: Integrated view on processes of perception and cognition

Ana Sušac
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Magnetoencephalography (MEG) is a non-invasive neurophysiological technique that measures the magnetic fields generated by neuronal activity of the brain with milisecond temporal resolution. MEG recordings and spatio-temporal source localization were used to investigate dynamics of cortical networks activated by different tasks. Our results indicated integrated effects of perceptual and cognitive processes even during the earliest evoked activity. The goal of the first study was to determine the generators of the visual mismatch-negativity response (vMMN) to an infrequent change in a repetitive sequence of images. The peak latency of the vMMN response was between 100 and 160 ms; neuromagnetic sources of the vMMN were localized in the occipital cortex. The aim of second study was to explore the earliest modulation of cortical activity by spatial attention in the visual modality. The results of our multi-dipole source localization analysis provided new evidence that even the earliest cortical visual neuromagnetic responses are modulated by attention. Neuromagnetic activity was also explored while subjects solved simple algebraic equations. All participants became more accurate and faster during the time course of the experiment. In addition, mathematics students noticed the patterns in the responses which helped them to improve their performance. Preliminary results suggested that early visual perception is very important in complex cognitive tasks such as equation solving.

12.S2

Facilitation of Long-Term Potentiation by Muscarinic M1 Receptors in the Hippocampus

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Activation of mAChRs facilitates the induction of LTP in the hippocampus and is critical for various forms of learning and memory. Nevertheless, the specific muscarinic receptor subtype involved and the critical intracellular signaling pathways engaged have remained controversial. Using whole-cell patch clamp recording, we have shown that a recently discovered and highly selective allosteric M1 receptor agonist 77-LH-28-1 facilitates long-term potentiation (LTP) induced by theta burst stimulation at Schaffer collateral synapses in the hippocampus. In addition, we have found that M1 receptor activation enhances N-methyl-D-aspartate receptor (NMDAR) opening during theta burst stimulation, indicating that this is the mechanism for LTP facilitation. However, our data argue strongly against a direct action of M1 receptor activation on NMDARs. Instead, M1 receptors were found to enhance NMDAR activation by inhibiting SK channels that otherwise act to hyperpolarize postsynaptic spines and inhibit NMDAR opening. Thus, we describe a mechanism where M1 receptor activation inhibits SK channels, allowing enhanced NMDAR activity and leading to a facilitation of LTP induction in the hippocampus.

13.S2

Purinergic signaling during development of auditory brainstem neurons

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In the auditory system, neuronal activity is generated in the cochlea and from there it is conducted along the afferent auditory pathways. During early postnatal development, i.e. before the ear canal opens and the processing of acoustic information commences (~P12 in rats, mice and gerbils), the pattern of respective discharges is modulated by extracellular ATP. To examine the physiological impact and the developmental time course of purinergic signaling in the central auditory brainstem neurons of Mongolian gerbil, extracellular recordings *in vivo* were combined with simultaneous iontophoretic drug applications of P2 receptor agonists or antagonists. Our results show that P2X responses are prominent prehearing and gradually down regulated after hearing onset. Activation of P2X receptors can *per se* evoke action potentials (AP), but also increases firing rate driven by the glutamatergic input from the cochlea. Endogenously released ATP facilitates AP generation by depolarizing membrane potential and increasing $[Ca^{2+}]_i$. The source of ATP remains to be determined yet, since the corelease with glutamate from excitatory terminals or with GABA/glycine from inhibitory synaptic terminals appears unlikely. In conclusion, by attuning the activity of specific neuron types within auditory brainstem nuclei, purinergic signaling could be engaged by some developmental mechanisms such as trophic regulation, regulation of synaptic strength, or structural reorganization of neuronal circuits.

14.S2

Retzius nerve cells of the leech as a model for investigation of ionic mechanisms of epileptogenesis

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Leech Retzius neurons generate rhytmic bursts of impulses in the presence of Ca^{++} channel blocker Ni^{++}. Ca^{++} channel blockade reduces Ca^{++}-activated K^+ current, which demasks persistent Na^+ inward current, leading to generation of rhythmical plateau depolarizations.

Each cycle consists of a prolonged plateau-like depolarization that drives a burst of impulses, a rapid repolarization and a slowly depolarizing ramp of potential that gives rise to the next burst generation plateau. It is proposed that oscilations result from two basic processes: depolarization due to opening of voltage-dependent, noninactivating Na^+ channels and repolarization resulting from activation of an electrogenic Na^+-K^+ pump. In the Na^+-free Ringer solution epileptiform activity induced by Ni^{++} becomes eliminated, suggesting that sodium influx plays a major role in its generation. Rhytmical bursting activity is strongly disrupted by lowering extracellular K^+ concentration, suggesting that repolarization segment of the oscilation cycle may involve activation of an electrogenic Na^+-K^+ pump. Ethanol supresses Ni^{++}-induced bursting activity of Retzius cells by lowering the frequency of bursts, and reducing the duration and amplitude of plateaus of depolarization, as well as number of spikes per plateau. Our experience and that of other authors indicate that leech Retzius neurons should be used as a usefull model for studying cellular mechanisms of epilepsy.

15.S3

A dual role for Rac1 GTPases in the regulation of cell motility

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Rac proteins are the only canonical Rho family GTPases in *Dictyostelium*, where they act as key regulators of the actin cytoskeleton. In order to monitor the dynamics of activated Rac1 in *Dictyostelium* cells, a fluorescent probe was developed that specifically binds to GTP-bound form of Rac1. The probe is based on the GTPase- binding domain (GBD) from PAK1 kinase, and was selected on the basis of yeast two-hybrid, GST pull-down and fluorescence resonance energy transfer assays. PAK1_GBD localizes to leading edges of migrating cells and to endocytotic cups. As in vertebrates, activated Rac1 therefore appears to control de novo actin polymerization at protruding regions of the cell. Additionally, we found that the IQGAP-related protein DGAP1, which sequesters active Rac1 into a quaternary complex with actin-binding proteins cortexillin I and II, localizes to the trailing regions of migrating cells. Notably, PAK1_GBD and DGAP1, which both bind to Rac1-GTP, display mutually exclusive localizations in cell migration, phagocytosis and cytokinesis, and opposite dynamics of recruitment to the cell cortex upon stimulation with chemoattractants. Moreover, cortical localization of PAK1_GBD depends on integrity of the actin cytoskeleton, whereas cortical localization of DGAP1 does not. Taken together, these results imply that Rac1 GTPases play a dual role in regulation of cell motility and polarity in *Dictyostelium*.

16.S3

Exploring the permeation pathway of nonselective TRPC channels

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Canonical transient receptor potential channels represent the first identified mammalian relatives of the *Drosophila* TRP protein. TRPC signalling was shown to be involved in various physiological processes such as development of neuronal and cardiac tissues, immune cell maturation and blood vessel constriction as well as in several pathophysiological events such as cardiovascular hypertrophy and hypertension. To date, little is known about the architecture of these channel proteins and the structure–function relations. Guided by a molecular homology-modelling approach, we combined site directed mutagenesis with patch-clamp measurements to identify critical residues within the permeation pathway of the lipid-gated TRPC member TRPC3.

We identified a central negatively charged glutamate residue (E630) that is essential for divalent permeability representing a key element of the channel's selectivity filter (Poteser et al., PNAS 2011). Further cysteine scanning mutagenesis indicated that E630 is located within the narrowest region of the permeation pathway, formed by the putative pore loop. An amine-based sizing approach demonstrated a decreased pore diameter in response to exchange of E630 to neutral glutamine. Interestingly, neutralization of two negatively charged residues E615 and E616 or of a positively charged lysine residue K619, located at the entrance of the permeation pathway, resulted in loss of channel sensitivity to GPCR/PLC-signalling. Basal channel activity remained fully preserved in these mutants or even enhanced in the E615Q mutation, indicating a structurally intact permeation pathway. We conclude that three charged residues located at the outer pore-vestibule are pivotal for the channel's activation machinery, most likely by enabling sensitivity to lipid mediators. These results provide first insight into the molecular basis of TRPC3 channel function and may represent an important step towards understanding the channel's role in native tissues.

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17.S3

Ionic transport in plant intracellular organelles: linking biophysics and plant physiology

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Being eukaryotic plants cells exhibit different intracellular compartments. Plant cells present two unique intracellular organelles: i) the chloroplast, ii) and the central vacuole. The chloroplasts are the compartment where photosynthesis takes place in green plants and therefore they are a primary source for biomass production. The vacuole is an intracellular compartment that can occupy up to 90% the total cell volume and is involved in multiple functions. This intracellular compartment is involved in the detoxification of the cytoplasm, accumulation of nutrients, maintaining of the pressure of turgor, plant development and signaling. The size of the plant central vacuole allows using electrophysiological approaches to directly study intracellular transport systems within their native environment. Using a combination of molecular and physiological tools it is possible to link the biophysical properties of the vacuolar transporters with the physiological role they play in plants. The link between biophysics and physiology has been shown in the case of the CLC (ChLoride Channels) transporter family and in the ALMT family (Aluminum activated Malate Transporters).

18.S3

Regulation of mitochondrial chloride channels

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The role of the mitochondrial chloride channels is still unclear, though it has been indicated that in cardiomyocytes, the chloride mitochondrial channels regulate the membrane potential across the inner mitochondrial membrane. The deregulation of the mitochondrial membrane potential leads to changes in plasmalemma action potential, an effect that might lead to fatal ending. In spite of the importance of these channels, their identity remains hidden. Several anion channels have been described in the inner mitochondrial membrane; however it seems unlikely that mitochondria should need so many and so similar but different anion channels. We have therefore focused on the purification of mitochondria from cardiac tissue and studied the anion channels present in the mitochondrial membranes at single channel level. The anion channels reconstituted into artificial lipid bilayer had a mean conductance of 129±18 pS in 250/50 mM KCl. Both activity and single channel conductance were affected by changes of pH of the surrounding solutions. In conflict with the properties of centum pS and IMAC channels, the presence of Mg^{2+} ions significantly enhanced the channel activity and 100μM 4'-chlorodiazepam did not cause any significant effect. Thus, the obtained results did not bring further light in the field of mitochondrial anion channels and the observed discrepancies will be discussed in context of identity and origin of the studied mitochondrial anion channels.

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19.S3

Mechanism of voltage sensitivity of the Cl^-/H^+ antiporter CLC-5

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ClC-5 is a Cl^- / H^+ antiporter which functions in endosomes and is important for endocytosis in the proximal tubule. The mechanism of transport coupling and voltage dependence in ClC-5 is unclear. Recently, a transport deficient ClC-5 mutant (E268A) was shown to exhibit transient capacitive currents. Here, we studied the external and internal Cl^- and pH dependence of the currents of E268A. Transient currents were almost completely independent of the intracellular and extracellular pH, but showed a non-trivial dependence on external chloride, strongly supporting a model in which the movement of an intrinsic gating charge is followed by the voltage dependent low affinity binding of extracellular chloride ions. Mutation of the "external" Glu-211, a residue implicated in the coupling of Cl^- and proton transport, to aspartate abolished steady state transport, but revealed transient currents which were "shifted" by ~ 150 mV to negative voltages compared to E268A. This identifies Glu_{ext} as a major component of the gating charge underlying the transient currents of the electrogenic ClC-5 transporter. The first molecular events in the transport cycle of ClC-5 emerging from these results are a movement of the side-chain of Glu_{ext} from an external to a central binding site, followed by the binding of extracellular Cl^- ions.

20.S3

Interfacial proton diffusion

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Fast lateral proton migration along membranes is of vital importance for cellular energy homeostasis and various proton-coupled transport processes. Commonly, it is treated as a succession of jumps between membrane-anchored proton-binding sites. Our experiments provide evidence for an alternative model. We released protons at the interface, and monitored their arrival at distant sites by fluorescence measurements. The kinetics of the arrival is probed as a function of distance (i) for membranes of various compositions [1] and (ii) for the decane water interface [2]. We find that long-range proton diffusion along the interface does neither require the presence of ionizable groups [1] nor that of lipids [2]. This observation suggests that the delayed proton surface-to-bulk transfer is due to the attraction by interfacial water. How to reconcile the high affinity to the interface with the high proton mobility is explained by extensive *ab initio* simulations on the water/n-decane interface. The hydroniums in direct contact with n-decane have reduced mobility. However, the hydroniums in the second layer of water molecules are mobile [2]. Their *in silico* diffusion coefficient matches the one derived from our *in vitro* experiments. We conclude that these are the protons which allow for fast diffusion along biological membranes.

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21.S3

Antifungal activity of peach defensin involves specific lipid interaction and plasma membrane permeabilization

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Plant defensins are small cysteine-rich antimicrobial peptides occurring in various plant species. They share a common three-dimensional structure, stabilized by eight disulphide-linked cysteines and composed of three antiparallel β–strands and one α–helix. Most plant defensins possess antifungal or antibacterial activity but are non-toxic to mammalian and plant cells. Plant defensins induce membrane permeabilization, resulting in Ca^{2+} uptake, K^{+} efflux, alkalinization of the medium, and membrane potential changes. The gene encoding for a peach (*P. persica*) defensin PpDFN1 was expressed and purified in *E. coli*. Defensin was tested for antimicrobial activity against fungi by Sytox Green assay. Treatment of fungal hyphae with FITC fluorescent labelled PpDFN1 indicated that the protein was not internalized by fungal hyphae but localizes on the external surface of cells. Data revealed that PpDFN1 has a inhibitory effect on spore germination and acts through membrane permeabilization. Biophysical analysis demonstrated that this protein was able to interact with membranes containing sphingolipid species and with lipids extracted directly from fungi. Interaction with fungal membrane lipid components leads to the insertion of peach defensin into the membrane resulting in membrane destabilization.

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22.S3

The mechanisms of interaction complexes of nucleic acids and nanoparticles with lipid membranes

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The transport of deoxyribonucleic acids (DNA) into the cells is of main gene therapy interest and promises to treat a variety of serious human diseases. To achieve this goal, the foreign DNA has to reach cell cytoplasm and nucleus, where the transcription and exertion of its intended action will take place. However, there are several obstacles that DNA has to face on this road. Firstly, DNA has to cross the cell membrane. In order to avoid a degradation of DNA, nucleic acids have to be complexed together with gene carriers.

In this work we have studied interaction of potential non-viral vectors - cationic poly(amidoamine) dendrimers (PAMAM) of fourth generation (G4) and their complexes with anionic polyelectrolyte dextran sulphate (DS) and DNA with 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) lipid monolayers.

The aim of this work was to explore the mechanisms of interaction of pure G4 dendrimers and G4 dendrimers modified with DS and DNA with lipid membranes via measuring the surface pressure of lipid monolayers by means of Wilhelmy method. The complexes were characterized by measuring the size and Zeta potential using dynamic light scattering method. We have shown that dendrimers can be incorporated into the monolayer at subphysiological surface pressures (< 30 mN/m), so they are expected to be useful in the design of dendrimer-based drug carriers. Dendrimer-DS and Dendrimer-DS -DNA complexes were able to induce increase of the lipid monolayer pressure, which is evidence on their incorporation into the monolayer.

Acknowledgements: This work was supported by the Slovak Research and Development Agency (contracts No. LPP-0250-09 and APVV-0410-10).

23.S3

Quantitative analysis of the clustering of ErbB receptors by number & brightness analysis and FRET methods

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The ErbB family of receptor tyrosine kinases comprises four members (ErbB1-4) which are activated upon growth factor binding. Their uncontrolled activation is often observed in human malignancies. According to the central dogma of receptor biology ErbB receptors are monomeric in their resting state, and they are induced to form homo- and heterodimers by ligand binding. Using flow cytometric homo-FRET measurements we measured the number of proteins in homoclusters of ErbB1 and ErbB2 and showed that ErbB2 formed large-scale clusters containing ~100 receptors in resting cells and that these clustered were disassembled after receptor activation. The behavior of ErbB1 was opposite to that of ErbB2 in that it formed only small clusters in unstimulated cells which grew after stimulation. Next, we used number and brightness (N&B) analysis to enumerate the number of receptors in small-scale clusters of ErbB1 and ErbB2. Our data indicate that preformed dimers of ErbB1 exist in resting cells if the expression level of ErbB1 is high. EGF stimulation leads to dimerization of ErbB1 and the formation of larger associations associated with clathrin-coated pits. ErbB2 again showed an opposite behavior namely, its clusters were larger in unstimulated cells and they shrank its size after stimulation. Conclusion: we have provided evidence that (i) receptors are not monomeric in quiescent cells; (ii) not only dimers, but also higher order aggregates, are formed.

24.S3

Gating of the bacterial protein translocation channel SecY

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To prevent the proton motif force from collapsing, the bacterial membrane must be impermeable to protons. Signal peptide binding is believed to open the SecY complex to allow for protein translocation across the bacterial plasma membrane. However, the binding site of the signal peptide only becomes accessible upon SecA binding in post-translational translocation or upon the action of an unknown trigger in co-translational translocation. To clarify the conundrum, we reconstituted the purified SecY complex into bilayer lipid membranes. We observed that the binding of SecA or of ribosomes to SecYEG is essential and sufficient to trigger the opening of the translocon even in the absence of a signal peptide. The pore has the ion conductivity of the plug deletion mutant [1]. Counting the number of reconstituted channels in the bilayer by both electrophysiological and fluorescent means indicates a very high open probability. The reversal potential remains at zero in asymmetrical salt concentration, thus ruling out ion selectivity. This finding suggests that in order to maintain cell viability, SecYEG is likely to adopt more than one conformation while bound to ribosomes or SecA.

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25.S4

Quantitative study of transcription factor binding kinetics in living cells

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Despite considerable molecular genetics and biochemical studies and challenging theoretical work, we still do not fully understand how transcription factors find their specific target sequences in a large eukaryotic genome. The main obstacle to further progress is limited number of experimental approaches that enable quantitative and non-destructive study of molecular mobility and the kinetics molecular interactions in living cells. The aim of this presentation is to introduce functional Fluorescence Microscopy Imaging (fFMI), a multimodal approach with single-molecule sensitivity based on high-resolution fluorescence imaging and fluctuation spectroscopy [1, 2]. This approach enables us to measure in living cells the functional readouts for molecules of interest: their local concentration, diffusion constants, kinetic rate constants and equilibrium association/dissociation constants. Application of fFMI to study molecular mechanisms underlying the assembly of functional transcriptional complexes and the mechanisms involved in target site recognition [3] will be presented.

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26.S4

Optically-driven microtools for microfluidic and biological applications

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The preparation of specialized tools in the micrometer size range for applications from physics to biology is a rapidly expanding field. One of the most effective and flexible method to make these structures is two-photon polymerization (TPP). With TPP practically any 3D structure can be made with even 100nm resolution and with the size of tens of micrometers. The transparency of the used materials enables the microtools to be trapped by optical tweezers thereby providing them with precise movement and positioning. The relative ease of the chemical modification of the TPP materials' surface broadens substantially their application spectrum. For instance, hydrodynamic synchronization of rotating micro objects in water such as bacterial flagella can be modeled with specially-shaped microstructures. With careful design they can be shaped into portable micro light-guides for fluorescence excitation. Coating their surface with proteins, practically of any kind, enables their association with biological surfaces, such as cell membranes. When coated with metal nanoparticles, localized metal enhanced fluorescence measurements can be performed with them on fluorescent surfaces.

27.S4

Modelling of smooth muscle contraction in normal and pathological conditions and its coherence with pharmacology and medicine

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Systems biology approach to modelling of intra and inter cellular signalling pathways that lead to force development in airway smooth muscle cells (ASMC) shall be presented. The prospective of mathematical models in pharmacological and medical applications will be outlined in three cases. First, special emphasis will be given to the modelling of coupling between eicosanoids production, taking place especially in eosinophils, and bronchi constriction/dilation. Their production is imparted in the condition called aspirin induced asthma that is manifested in some asthmatics after ingestion of non-steroidal anti-inflammatory drugs (NSAIDs). Action of different drugs used in treating asthma and their effect on eicosanoid production and force development in ASMC will be simulated and debated. In the second and the third case, the mechanisms, signalling pathways and the models of force development in ASMC will be stressed. A simulation of action of beta-agonists, known bronchodilators of airways, will be presented in the second case and referred to their differential effect on cardiac muscles. Third, a new important therapeutic target in ASMC, i.e. Rho-kinase signalling pathway, will be emphasized from theoretical and experimental perspective. The *in–vitro* and the model results of the effect of Rho-kinase inhibitors on the force development after cholinergic stimulation in ASMC will be presented and discussed.

28.S4

X-ray microscopy on the cellular level

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Significant progress has been made in the field of soft- and hard-X-ray microscopy, through developments X-ray tools i.e. source, optics and imaging methodologies, as well as through a wide range of applications in bio-imaging during last years. Combination of different imaging techniques (correlative imaging) are also greatly developing in recent times. This presentation will cover up-to-date work in the development of direct X-ray microscopy i.e. image techniques and the relevant application in neuroimaging. For example one of the imaging methods, the X-ray fluorescence (XRF) microscopy is well-suited to *in situ* investigations of trace and other macro elements distributions within whole, unstained, biological samples, e.g. single cell, with sub-ppm detection limit (Fig. 1). XRF is particularly suited for quantifying trace elements in cells; exploiting on an inherent property of matter, elemental contrast is not reliant on artificial dyes or fluorophores (see Fig. 1). The high penetration of X-rays combined with high spatial resolution, make X-ray microscopy ideal tool for 2- and 3-D for structural visualization on subcellular level.

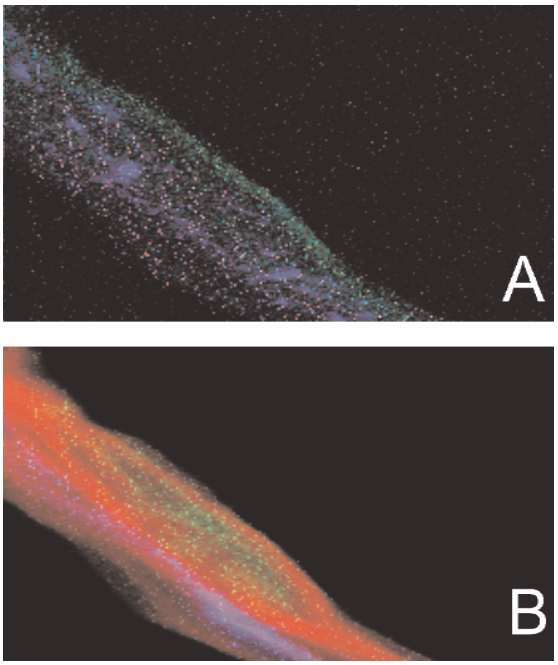

Figure 1. X-ray fluorescence of cryo-preserved myelinated axon.
Overlaying distribution of microelements (Mn-red, F-blue, Cu-green in situ (A) and the distribution of macroelements: P (red), Cl (green) and K (blue) (B) in the single isolated neuron. Image size 70x40 µm, 280x160 pixels with dwell time 150 ms per pixel. E = 7.2 keV.

29.S4

Confocal fluorescence microspectroscopy: powerful biophysical tool to resolve molecular environment

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Fluorescence microscopy (FM) provides a very sensitive mean of localizing the amount of the fluorescence light through biological sample. In order to characterize local molecular environment we upgraded FM to fluorescence microspectroscopy (FMS) with spatially-resolved detection of fluorescence emission spectra.

FMS primarily enables spectral-based identification of fluorophores, even if their emission spectra overlap significantly, in addition to spectral-based identification of multiple local environments around environmentally sensitive probes, spectral-based identification of binding state between the probe and the carrier molecule or system as well as spectral-based identification of nanoparticles even if they are smaller than the optical resolution [1]. FMS can also be used to resolve bleaching rates and autofluorescence together with spectral components characteristics. In our presentation, different experiments will be discussed, new environmental-sensitive probe design [2,3] as well as technical issues like broad-spectrum excitation source, signal-to-noise ratio influences, and high wavelength accuracy , being similar to determination of positional or localization accuracy in single molecule studies [3].

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30.S4

Serial X-ray nanocrystallography with Free Electron Laser radiation

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Single crystal X-ray diffraction snapshots can be collected from a stream of microcrystal flowing in a water jet using femtosecond pulses from a hard X-ray Free Electron Laser (FEL) (1).

Diffraction from ultra-short (<70 fs) pulses can be collected before significant changes occur to the sample (2). The recorded diffraction pattern can be indexed and summed in order to get structure factors and then to calculate the electron density map (3). Serial crystallography can therefore be a novel way to determine the structure of proteins that do not grow in crystals of sufficient size for standard synchrotron radiation measurements or are particularly sensitive to radiation damage.

Serial crystallography also opens up the possibility for time resolved structural studies. Optical pump lasers synchronized to FEL pulses (4) can be used to obtain X-ray diffraction snapshots from the excited states of proteins in nanocrystals, thus allowing the study of reaction dynamics in biological systems.

In the poster we show the results obtained using serial crystallography at the Linac Coherent Light Source FEL on different proteins nanocrystals (1,5).

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4 Aquila et al. Optics Express 20, 3 (2012)
5 R. Koopmann et al. Nature Methods 9 (2012)

31.S4

Applications of nonequilibrium plasmas in biology and medicine

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Constantly growing field of biomedical applications is a new frontier that drives the development of plasma sources. The choice of the plasma system used for treatment is usually guided by the type of samples that are treated and effect these plasmas are intended to have on the samples. Some of the samples cannot undergo vacuum and due to this fact non-thermal atmospheric pressure plasmas lately have drawn considerable attention with their enormous potential for technological applications in surface modifications and biomedical applications. The necessity to use plasma for *in-vivo* treatments/procedures has led to several requirements for plasma sources to meet. Because of its mild plasma, low gas temperature and geometry, the plasma needle is especially convenient for medical applications. We have studied effect of the plasma needle on different living cells, from plants through bacteria such as Escherichia coli and Staphylococcus aureus to the human peripheral blood mesenchymal stem cells (hPB-MSC), as a model system to predict the degree of possible damage to the human cells. The results support application of the plasma needle in treatments of light bacterial infections, such as in vivo sterilization of skin and dental cavities.

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32.S4

Proposing some new biomarking tools for cancer and ALS

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Various different experimental techniques have been used to characterize number of pathological states like cancer and neurodegenerative disorders. However, each of this techniques have disadvantages, mostly related to low sensitivity, lack of specificity or show to be major time and money consumers. Consequently, there is always a need for development of new experimental approaches which could potentially facilitate diagnosis, prognosis and prediction. Two poorly exploited techniques have been presented as potential cancer and amyotrophic lateral sclerosis (ALS) biomarking tools. As the first candidate, micro-Raman spectroscopy combined with neural network software algorithm was evaluated for the classification of a number of different types of cancer. From the experimentally obtained Raman spectra database, supported by neural network learning algorithm, fast and confident identification of specific cancer types from a target organ could be achieved. The second biomarking technique, which was evaluated for determination of specific features in cerebrospinal fluid (CSF) of ALS patients, was the cyclic voltammetry. The results, obtained from the CSF of ALS patients, show that at specific electrode potentials, the characteristic plateau appeared and the potential of oxygen evolution was shifted toward more positive values. These voltammogram features, which are not present in the CSF of controls, could also provide us valuable information about the pathology of ALS.

33.S4

Effect of perifosine on liposome membrane characteristics, transcytosis and liposome contents leakage

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A correlation between liposome membrane fluidity, liposome stability, and transcellular transport was studied on a larger series of liposomes in order to investigate, which membrane properties determine the efficiency of transcellular transport of liposome encapsulated calcein. For this purpose, physical characteristics of liposomes composed of PC, and CH, as a main lipid constituents, and variable amount of helper lipids, DOPE and alkylphospholipid OPP (perifosine) were investigated by electron paramagnetic resonance (EPR). Epithelial Madin-Darby canine kidney (MDCK) cell line was used as a well characterized model of a tight cellular barrier.

According to our results, liposome stability is an important characteristic of liposomes correlated with efficient delivery of calcein, across MDCK cell barrier. Main influence on liposome stability was found for perifosine, which changes a disordered domain type of liposome membranes in such a way, that leakage of liposome contents can occur more easily. Properties of the most disordered membrane domain types of liposomes abruptly change at about 20 mol% of perifosine in a way which might facilitate transport of entrapped hydrophilic substance through the tight MDCK cell barrier.

We report a possible novel role of perifosine as a helper lipid, facilitating transcellular transport across tight MDCK cell layer, holding prospects for improved transcellular delivery of liposome encapsulated hydrophilic drugs to cancer affected tissue.

34.S5

The use of laser technologies in biomedicine

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We present laser based microscopes for biological applications, currently being developed in our group. Non linear optical microscope uses femto second, broad band laser pulses for multi photon excitation in biological samples for generation and subsequent collection of second and third harmonics. Technique allows increased depth penetration and three dimensional localization, providing unparalleled view of biological tissues and cells. Its effectiveness depends on the light frequency and cell structure and performance of an optical system. The dominant response of a sample at second and third harmonics requires that restoring force on an electron in laser field is spatially asymmetric, like in cell boundaries, or in parts of cell where symmetry is broken by an interface.

3-D digital holographic microscopy, integrated with numerical processing, enables unique quantitative information about structures of cells and microorganisms. Numerical reconstruction of sectional images at different depths of the sample makes this technique very fast, fully non invasive and real time technique. 3-D images reconstructed from digital holograms are also very valuable for cell identification.

Both non linear and holographic microscope can be, relatively easy integrated with optical tweezers which provide a unique and flexible tool for cell manipulation. The difference on trapping force required for normal and cancerous cells can be use for quick classification.

35.S5

Stereotactic radiosurgery with linear accelerator for treatment of intracranial lesions

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Introduction Stereotactic Radiosurgery is a radiotherapy treatment technique for benign and malignant intracranial diseases, which has many important components, among them is the accuracy in the dose delivery. Prior to clinical use of Novalis Tx linear accelerator comparison of calculated and measured doses was needed and was done for two calculation algorithms and for two treatment techniques.

Materials and Methods In our study, the dose calculation performed by commercially available 3D treatment planning system iPlan (BrainLab company) was compared with the measurements carried out with small volume cylindrical ionization chamber Exradin A16, using specially designed Lucy phantom. The calculation of the doses was done for 7 Static Fields and 5 dynamic Conformal Arcs using Pencil Beam and Monte Carlo algorithms. Measured doses were determined according to procedure described in IAEA TRS 398 dosimetry protocol.

Results We found acceptable agreement between measured and calculated doses for both calculation algorithms. The differences ranged from 1.4% to 3.8% - the measured doses were always lower than the calculated ones. Table represents the results of the comparison between the calculated and measured doses at the isocenter for the average of two treatment plans (7 SF and 5 DCA) and two calculation algorithms.

Calculation algorithm	D_{calc}/D_{meas}
Pencil Beam	1.036
Monte Carlo	1.014

Conclusions The results in our study confirm the accuracy of the dose calculated by the treatment planning system iPlan for both algorithms: Pencil Beam and Monte Carlo, as the differences lie well within the recommended 5% value of the International Commission on Radiation Units and Measurements (ICRU). Superiority of Monte Carlo calculation algorithm was shown and is preferred method for clinical application.

36.S5

Electrostatics and mechanics of interactions between osteoblasts and titanium implant

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A possible mechanism of the attractive interaction between negatively charged titanium (Ti) surface and negatively charged osteoblasts is described theoretically. It is shown that adhesion of positively charged proteins with internal charge distribution may give rise to attractive interaction between the Ti surface and the osteoblast membrane. A dynamic model of the osteoblast attachment is presented in order to study the impact of geometrically structured Ti surfaces on the osteoblasts attachment. It can be concluded that osteoblasts are most strongly bound at the sharp convex edges of vertically aligned TiO_2 nanotubes. The fact that the surface with small diameter nanotubes has on average more sharp convex edges per unit area (with increased surface charge density) than the large diameter nanotube surface may explain why the osteoblast binding affinity to a small diameter nanotube surface is larger than the binding affinity to a large diameter nanotube surface or to a smooth titanium surface.

1. Gongadze E., Kabaso D., Bauer S., Slivnik T., Schmuki P., van Rienen U., Iglič A., Int. J. Nanomed., 6:1801–1816, 2011.

37.S5

Towards increased selectivity of cancer treatment by Photodynamic therapy

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The efficacy of photosensitier (pts) in inducing either apoptosis or necrosis after photodynamic action critically depends upon different parameters including: i) selective pts accumulation in tumor cells and, ii) dynamics of sub-cellular distribution of therapeutically active form of drug. Low-density lipoproteins (LDL) play a key role in the delivery of hydrophobic pts to tumor cells in PDT. Construction and characterization of new LDL-based selective nano-delivery system is discussed in the presentation. Dynamics of organelle specific sub-cellular redistribution of hypericin (Hy) plays important role in its photodynamic activity. We report the development and characterization of micrometer-sized dielectric beads with nano-metal structures attached to their surface. The metalized beads are sufficiently transparent enabling optical trapping while the presence of metal nano-islands provided the SERS. This highly efficient probe can be placed and scanned with nano-metric accuracy in living cells. Application of such optical nano-sensors is demonstrated on: (i) detection of low quantities of photoactive drug emodine, (ii) study the kinetics of drug diffusion through the cellular membrane.

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38.S5

Using cells and biomaterials for regeneration of diseased and damaged tissues

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Tissue engineering principles provide a platform for several applications, namely, tools for studying tissue development and diseases, models for drug and biomaterial testing, and finally, they can be used in the human body in order to regenerate the missing parts of the tissues. The general tissue engineering approach is to combine cells, biomaterial, and environmental cues, which should all be selected according to the specifics of the application. Several different approaches to engineer autologous bone tissue will be presented. Different types of osteogenic cells have been employed, including adult ostoblasts and mesenchymal stem cells. For supporting and enhancing the differentiation of cells towards osteogenic phenotype, specific *in vitro* microenvironments were provided to the cells, such as three-dimensional environment, specific nutrients, and mechanical stimuli. Two types of ceramics and a natural bone matrix have been used as a biomaterial, all of them being biocompatible, and osteoconductive, therefore enabling cells to attach and proliferate. All tested approaches resulted in formation of new bone tissue matrix. The functionality of bone grafts was further proved in two successful clinical applications, which resulted in repair of long bone defects, and a successful treatment of periodontal disease. New approaches to further improve the functionality of bone tissue grafts are based on increasing the complexity of the engineered tissue, meaning simultaneous culturing of all relevant cell types – besides bone cells also vessel cells, development of biomaterials mimicking native bone structure and biology, understanding the cross talk between cells and biomaterial, and defining optimal culture regimes.

39.S5

Calcium changes and contractions of human anterior lens epithelial cells

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The purpose of the study was to characterize the contractions of human anterior lens epithelial cells, which create gaps between cells, and to assess the physiological mechanisms and a possible association of the contractions with free intracellular calcium concentration ($[Ca^{2+}]_i$) changes.

Lens capsules obtained during cataract surgery were stained with fluorescent dye Fura-2. Its fluorescence, upon excitation at 360 and 380 nm, was imaged to monitor changes in cell morphology and $[Ca^{2+}]_i$ in response to pharmacological stimulation by acetylcholine (ACh) and to mechanical stimulation by flow of saline or direct contact. Contractions were also studied by scanning electron microscope.

Epithelial cells contracted in about a third of preparations after stimulation. Contractions started either before or at best simultaneously with the rise in $[Ca^{2+}]_i$. Contractions also occurred when there was hardly any change in $[Ca^{2+}]_i$ upon application of physiological saline alone.

The contractions of the anterior lens epithelial cells occur in significant portion of human lens anterior capsule postoperative preparations. They can be mechanically induced, are localized and reversible, have a fast response and did not differ among different types of cataract.

Contractions are at least partially independent of changes in $[Ca^{2+}]_i$.

40.S5

Imaging axonal transport in health and disease

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Neurons use axonal transport to shuttle organelles and vesicles essential for their function and survival between soma and synapses. It thus appears logical that intact axonal transport is an important requirement for neuronal survival. Indeed, deficits in axonal transport have been reported in many neurodegenerative diseases including Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis. However, the exact relationship between axonal transport disturbances and axon degeneration remains unclear. This is mainly due to the fact that it is difficult to follow changes in axonal transport in single axons in intact preparations and correlate this directly to alternations in axonal or synaptic stability. For example, transport assays using ligation or tracer injection techniques are possible *in vivo* but lack single axon resolution; in converse, studies in cell culture allow measuring transport in single cells, but are not suited to study long-term survival. We have recently developed methods to overcome these technical limitations. By combining *in vivo* imaging with new transgenic mice that can be crossed into disease models, we can now visualize the transport of individual organelles labeled with conventional or photo-convertible fluorophores in single, fully matured axons in their normal environment. In my presentation, I will report on the development of these tools and their application to neurodegenerative diseases.

POSTER PRESENTATIONS

P1.S1

A flagellin-based polymerizable GFP variant

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Flagellin is the subunit protein of bacterial flagellar filaments. Flagellin has ability to polymerize into long filaments under appropiate conditions. Our work aims at the construction of flagellin-based fusion proteins which possess polymerization ability and preserve the functional properties of the fusion partner as well. The hypervariable D3 domain of *Salmonella* flagellin, contianing residues 190-283, is a good target for genetic engineering studies since it can be extensively modified or removed without disturbing the self-assembling ability. In this work a fusion construct a flagellin and the superfolder mutant of the green fluorescent protein were created by replacing D3 with superfolder green fluorescent protein (GFP). The obtained GFP variant was capable of forming stable, highly fluorescent filamentous assemblies. Our results imply that other protein (enzymes, binding proteins, etc.) can also be furnished by polymerization ability in a similar way. This approach paves the way for the construction of multifunctional tubular nanostructures.

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P2.S1

Ultrafast time-resolved fluorescence of flavin adenine dinucleotide

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Fluorescence kinetics measurements were carried out on flavin adenine dinucleotide (FAD) in water mixed with different organic solvents and on flavocytochrome prepared from *Thiocapsa roseopersicina*. The fluorescence was detected by a measuring apparatus combining the technique of fluorescence up-conversion and time-correlated single photon counting, with time resolution of ~250 fs in a wide spectral range. The kinetics were analysed on a quasi-continuous set of time constants, supposing that the majority of them has negligible contribution to the reconstruction of the experimental signal.

It was found that in pure water the fluorescence kinetics can be well characterized by five discrete time constants. The fastest component (~200 fs) is clearly attributable to the solvation dynamics of the neighbouring water molecules. The rest of the time constants span the region of 1 ps – 3 ns, and probably correspond to different (planar and stacked) conformation of the FAD molecule. In the presence of ethanol, dioxane or DMSO the time constants hardly changed but the weight of the slow components markedly increased, indicating a higher preference of the planar conformation. In contrast to this, in flavocytochrome a strong quenching of FAD fluorescence took place, probably due to the interaction with the surrounding amino acid residues.

P3.S1

mDia1-FH2 affects the ATPase activity of the actin filaments

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Formins are conservative proteins with important roles in the regulation of the actin based microfilamental system in eukaryotic cells. They have several domains including FH1, FH2, GPB and DAD. In the interaction between actin and formin the FH2 domain plays a key role. The 'mammalian Diaphanous-related 1' constitutes are one of the subfamilies of the formins. These mDia1 formin fragments affect the conformation of the actin filaments in a concentration dependent manner. In the current work we have investigated whether the mDia1-FH2 affects the nucleotide exchange on the actin filaments. Steady-state fluorescence anisotropy and photometric coupled assay measurements showed that the ATP-ADP conversion was accelerated in the presence of formins, and the effect was stronger at greater formin concentrations. These observations indicate that there must be a tight coupling between the rate of nucleotide exchange on actin protomers and the conformational properties of the filaments.

P4.S1

Reduction of tumor suppressor cytochrome b561 by ascorbate and dihydrolipoic acid

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Cytochromes *b*561 (Cyts-b561) are ascorbate (ASC)-reducible proteins with 6 *trans*-membrane domains, found in a great variety of organisms. All are electron transferring proteins with 4 His residues, arranged in 4 consecutive *trans*-membrane domains, for coordinating two *b*-type hemes (one heme on each side of the membrane). One member of the Cyt-b561 protein family was shown to participate in tumor suppression (TSCytb). Both the human and the mouse TSCytb have already been cloned and expressed in yeast cells.

Mus musculus TSCytb with a C-terminal His_6-tag was cloned into a pESC(-His) vector, expressed in yeast cells (*Saccharomyces cerevisiae*) and purified by affinity chromatography. UV-Vis spectroscopy was used to characterize the affinity to and reducibility by various reductants of TSCytb, including several thiol reagents. The concentration-dependent reduction of TSCytb by dihydrolipoic acid (DHLA) is discussed in comparison to that by ASC (1). In reduced state, the two hemes have different split a-bands in the absorption spectrum in agreement with earlier results obtained for TSCytb and other Cyt-b561 proteins after reduction by ASC.

These results allow us to speculate what role TSCytb or other Cyts-b561 might play in the redox regulation of cells via interaction with both the ASC and the lipoic acid pool.

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P5.S1

Hematological changes in mice exposed to static magnetic fields of different orientations

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This study investigates influence of subchronic continuous exposure to upward and downward directed static magnetic field (SMF) on hematological parameters and spleen cellularity in mice. Male, Swiss-Webster, 6 weeks old mice were divided in three groups and continuously exposed or not exposed for 28 days to the vertically declining inhomogenous SMF characterized by the averaged field of 16 mT and averaged field gradient of 10 mT/cm. Differently oriented SMF did not alter hemoglobin and hematocrit content among the groups but did elevate serum transferrin compared to the control. Spleen cellularity in animals in the downward group was significantly higher compared to the upward and control group. Spleen lymphocytes in both of the exposed groups were significantly higher than in the control group. In contrast, spleen granulocytes in the exposed groups were significantly lower than in the control group. Significant decrease was also observed in brain and liver iron content with concomitant increase of iron in serum and spleen in exposed animals. Subchronic continuous exposure to 16 mT SMF caused redistribution of lymphocyte and granulocyte between spleen and blood. This distribution is typical for stress induced hematological changes. These results suggest that observed changes were not due to an unspecific stress response, but that they were rather caused by specific adaptation to subchronic SMF exposure.

Key words: static magnetic field, hematology, spleen cellularity, iron

P6.S1

Actin controls the I-BAR-membrane interaction

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The Inverse BAR (I-BAR) domain is the N-terminal 250 amino acid of the IRSp53 protein that induces negative membrane curvature (filopodia, lamellipodia) both *in vitro* and in cells. Generation of membrane curvature by I-BAR proteins often works together with actin dynamics. I-BAR shares its function between actin bundling and membrane binding but it is still obscured what molecular mechanisms are responsible for these functions. The aim of our project is to investigate the detailed membrane binding properties of the I-BAR of IRSp53 and its relations to the actin cytoskeleton. *In vitro* FRET experiments and fluorescence quenching studies were carried out between the I-BAR and liposomes made up from different lipid constructs. We have found that the I-BAR has preference to bind to the negatively charged lipids however it can also bind to the uncharged lipids. The fluorescence quenching studies reflected that the accessibility of the I-BAR surface was higher toward the negatively charged lipids than for the uncharged ones. TNS fluorescence assay reflected that the I-BAR domain binds to the surface of the micells rather than penetrating into its core. I-BAR membrane interaction is controlled by the polimerization state of actin where filamentous actin stabilizes, while, the globular actin disrupts their interaction. Our results suggest that the I-BAR-actin connection has an important role in the filopodia formation and regulation.

P7.S1

A fresh look at the bacteriorhodopsin photocycle

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Bacteriorhodopsin is the sole protein constituent of the purple membrane patches in the cell membrane of *Halobacterium salinarum*. This light driven proton pump forming 7 transmembrane helices binds a retinal chromophore in its hydrophobic interior and contains several strategically located amino acids and water molecules which participate either directly in proton translocation or indirectly in its regulation. Light absorption initiates a photocycle with spectrally distinct intermediates in the nanosecond to millisecond range, which can be followed by UV-VIS spectroscopy.

Time resolved multichannel difference spectra were analyzed by the chemometric method of singular value decomposition with exponential-fit-assisted self-modeling[1] and the extinction spectra of the intermediates were determined. Absorption kinetics has also been measured at several characteristic wavelengths with high time resolution. These complementary datasets have been fitted by an unprecedentedly detailed functional model of the photocycle which takes into account all known transitions of the protein structure and of the proton translocation. Experimental data at a number of temperature and pH values as well as in the presence of high concentrations of salts from the Hofmeister series provide new insight into the energetics of this prototype of ion pumps.

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1. Zimányi, L., J. Phys. Chem. B 2004, 108, 4199-4209

P8.S1

Epitope location in ordered and disordered regions of tumor-associated antigen EBNA 1

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The overexpression in cancer and spontaneous humoral immune response demonstrated against certain tumor-associated antigens (TAA), imply that they are possible targets for vaccine trials, but epitope immunodominance relation to protein 3D structure is not fully understood. Epstein-Barr virus (EBV)-associated malignancies involve the latent cycle and can be distinguished by the paterns of latent viral gene expression. EBV nuclear antigen 1 (EBNA 1) is expressed in latency I and II states of EBV-associated cancers and is possibly associated with autoimmune diseases. $CD4^+$ cells against promiscuous immunodominant HLA-II epitopes, located in the EBNA-1 region 475-552, have been generated, but not against epitopes in the region 403-428, predicted as subdominant, according to NetMHCiipan epitope program. Region 475-552 is a part of the predicted highly structured (ordered) domain, while region 403-428 appartains to the potential disorder-to-order transition element, which is proposed by ANCHOR predictor to be a region of molecular recognition. The boundaries of the region 403-428 are recognized by antibodies. Peptide 398-404 was found to elicit a lupus like disease in rabbits, while epitope 444-450 is recognized by a monoclonal antibody which also interacts with homologous peptide in MAGE-A4 cancer/testis antigen. Peptide 398-404 could be a cryptic epitope, partially masked by a molecular complex, which potentially influence epitope spreading involved in autoimmune processes.

P9.S1

T-cell epitope clustering in protein regions of cancer/testis antigens

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T-cell epitope predictions based on MHC-binding peptide affinities are considered as a valuable step in vaccine design. We have applied programs for epitope and disorder predictions on 642 proteins from various taxonomic groups. Epitope frequencies for both HLA-I and HLA-II-binding nonamer peptides were higher in ordered than in disordered protein regions. Epitopes appertaining to ordered protein regions were prevalently hydrophobic. Cancer/testis antigens (CTA) are potential cancer-vaccines candidates, because they are aberrantly expressed in several types of cancer, while normal expression is restricted to testicular germ cells which do not express HLA-I antigens. Epitope frequency in ordered and disordered protein regions was analysed for several immunogenic CTA, mostly from the MAGE-A, NY-ESO and SSX families that have been previously intensively studied in cellular immune response. Majority of predicted epitopes, presented by HLA-I and HLA-II molecules, are localized in ordered protein regions. In long disordered protein sequences epitopes are frequently flanking potential disorder-to-order transition elements. These results correspond with the locations of experimentally determined epitopes. The CD4+ response to naturally processed HLA-II-presented epitopes from cancer/testis antigen MAGE-A3 were found to be promiscuous for several DRB1 allels, and localized in the central, ordered region of MAGE-A3 antigen, comprising of amino acids 107-293.

P10.S1

Modeling of organic biocompounds

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In this paper we report the results of our research using computer modeling approach for organic compounds that are important for biophysical processes. Electronic subsystem responsible for a large range of properties of organic molecules and quantum structures of medium size can be explored on a computer in a relatively inexpensive level of theory using various available software packages, so allowing the use of relatively standard desktop computers.

Among others, it is possible to calculate those quantities that are very sensitive to structural changes, such as NMR parameters, making them perfect for the purpose of comparing similar or in some way perturbed systems. Study presented here involves three well-known but not theoretically investigated enough, the active component of -blockers: acebutolol, metoprolol and atenolol. Beside these, we also present results of boron disubstituted sumanene. Molecular bowl sumanene is model compound of fullerenes and nanotubes, synthesized in the last decade.

Stability analysis and aromaticity calculations of active components of -blockers confirmed their high stability and medium aromatic nature. Concerning boron disubstituted sumanene, quartic function of bowl depth does not only well describe the change of inversion barrier with bowl depth, but also describe well the change of nucleus independent chemical shifts with bowl depth.

P11.S1

The effect of mouse twinfilin-1 on the structure and dynamics of actin

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The actin cytoskeleton of eukaryotic cells plays a key role in many processes. The structure and dynamics of the cytoskeleton are regulated by a large number of proteins that interact with monomeric and/or filamentous actin. Twinfilin is a 37-40 kDa actin-binding protein composed with two ADF-homologous domains connected by a short linker.

The effects of the mouse twinfilin 1 (TWF1) on the monomeric actin was studied by biophysical techniques. The affinity of TWF1 to the ATP-actin monomer was determined by fluorescence anisotropy measurements (K_D = 0.015μM). The fluorescence of the actin bound ε-ATP was quenched by acrylamide in the presence and absence of TWF1. Twinfilin reduced the accessibility of the bound ε-ATP which indicates that the nucleotide binding cleft shifted toward a closed conformational state. Stopped-flow experiments confirmed that the kinetics of nucleotide-exchange of actin decreased in the presence of TWF1. The thermodynamic properties of TWF1 and the effect of it on the stability of actin monomer were also investigated with differential scanning calorimetry. The TWF1 stabilized the structure of the monomeric actin.

These results can help to understand in more details the regulation of G- and F-actin by actin binding proteins.

P12.S1

Influence of NaF and $NaClO_4$ salts on the stability of Trp-cage miniprotein: a computational study

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In a recent study, Dzubiella [1] pointed out that the effects of Hofmeister active salts on the stability of simple model peptides (like charged Ala-based helices) can be simulated using non-polarizable force fields. In this study, we investigated the effects of kosmotropic and chaotropic ions on the stability of a more protein-like model peptide with a well-defined structure, the Trp-cage miniprotein. It is a 20-residue-long polypeptide and shows several characteristic features of proteins, namely, it has a stabilizing salt bridge and a hydrophobic core. In our investigations, the Amber ff99SB-ILDN force field and the TIP3P water model together with the ion parameterizations of Joung et al. [2] and Baaden et al. [3] were used for three 600-ns-long REMD simulations (*i.e.* pure water, water with NaF and $NaClO_4$ in 1M concentration). The influence of studied salts on the structural stability of Trp-cage miniprotein is characterized by the RMSD values of backbone, and the solvent accessible surface area (SAS). These quantities show the expected shifts if we add anions with different Hofmeister activity to the solution. The changes in the protein-water interfacial region were examined in terms of total SAS and its fluctuations, and the alteration of surface tension due to the adding of ions is also calculated.

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P13.S1

An insight into statistics of fluoprescence spectra of complex natural product mixtures

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The aim of this study was to apply fluorescence spectroscopy combined with statistical fixed size window factor analysis (FSW-FA) for rapid preliminary screening of different fluorophores in bioactive complex natural product mixtures. Indeed, the water and methanolic extracts of two evolutionary simpler organisms intended to be used in pharmacy, specifically the moss *Rhodobryum ontariense* and the bryozoan *Hyalinella punctata*, were investigated.

Fluorescence spectra were collected using a Fluorolog-3 spectrofluorimeter (Jobin Yvon Horiba, Paris, France) equipped with a 450 W xenon lamp and a photomultiplier tube. For each a series of emission spectra were measured by varying excitation wavelengths with 10 nm steps. As a result of decomposition of the series of emission spectra for each of the samples, by using FSW-FA, the total number of components in an integral spectrum was obtained.

The analysis of the emission spectra has shown 13/12 and 15/15 components in the aforementioned extracts of the moss and the bryozoan species, respectively. These results indicate that fluorescent spectroscopy combined with an appropriate statistical analysis of the spectra can be used as an elegant and economic tool for observing of fluorophore units in biologically important samples

P14.S1

Effect of Hofmeister-salts on the unfolding of Trp-cage miniprotein followed by CD-spectroscopy

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Based on his observations in the 1880's, *Franz Hofmeister* discovered a series of salts that significantly alter the structural stability and solubility of proteins. The anions are ordered in the *Hofmeister-series*, as follows:

$SCN^- < ClO_4^- < I^- < ClO_3^- < Br^- < NO_3^- < Cl^- < CH_3COO^- < HPO_4^{2-} < SO_4^{2-} < F^-$

Early (chaotropic) members of the series decrease the protein-water interfacial tension, in effect, they increase the solubility ("salting in") and conformational fluctuations. By contrast, later (kosmotropic) salts increase the interfacial tension, stabilize the protein structure and normally decrease fluctuations ("salting out").

Temperature induced unfolding of Trp-cage in different salts (*i.e.* $NaClO_4$, NaCl and NaF) was studied by CD-spectroscopy in the far-UV region (190-240 nm). The transition temperature of unfolding, as well as the helicity of miniprotein were calculated from the ellipticity measured at the characteristic peak of α-helix (222 nm). The helical content was calculated also based on molecular dynamics simulations, and the results obtained by the two methods were in good agreement. The variations of the melting point and helical content clearly indicate anion-induced alterations of structural stability. As expected, the chaotropic $NaClO_4$ loosens the secondary structure, decreasing the melting point of the miniprotein. On the contrary, the kosmotropic NaF stabilizes the protein structure, thus the transition from the folded to the unfolded state occurs at higher temperature.

P15.S2

Effect of HBO treatment on expression of SOD2 after cortical stab injury in rats

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Despite the amount of work that has been done in order to better understand traumatic brain injury (TBI), there is currently no effective treatment. Substantial amount of evidence has been published indicating that treatment with hyperbaric oxygen (HBOT) can interfere with the processes that are following TBI and modify its consequences. However, the exact mechanisms by which HBOT exerts its positive effects are still elusive. Since antioxidant enzymes are activated in response to TBI, the aim of the present study was to evaluate the effect of HBOT on superoxide dismutase 2 (SOD2) expression pattern after TBI.

In this study we have used a model of cortical stab injury (CSI). The experiments were conducted on the male Wister rats, 10 weeks old, divided into control, sham, lesioned and HBO groups. Animals were exposed to 100% oxygen at 2.5 atmospheres absolute (ATA), for 60 minutes, once daily for 3 or 10 days. Cortices were processed using TRIzol method for isolation of RNA and proteins. Pattern of SOD2 expression was analysed using real-time PCR, Western Blot and double-label fluorescence immunohistochemistry. Our results indicate that CSI alters SOD2 expression in time-dependent manner, while HBOT returns its expression to physiological levels.

Based on the obtained results we concluded that beneficial effect of HBOT on recovery after brain injury may be in part due to reduction of oxidative stress via restoring SOD2 expression back to physiological levels.

P16.S2

Colocalization of AQP4 and Kir4.1 and functional properties of Kir channels in the ALS rat model

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder affecting upper and lower motoneurons. Although the exact mechanism underlying the disease is still unknown, some studies support the distinct role of astrocytes. Astrocytic endfeet ensheathing the blood vessel wall take part in the blood-brain barrier (BBB) maintenance. The aim of our study was to examine the BBB state with the focus on the expression of aquaporin 4 (AQP4) and potassium channel Kir4.1, both predominantly localized in astrocytic endfeet. Our MRI studies confirmed a compromised BBB in the transgenic $hSOD1^{G93A}$ ALS rat model. Working on brainstem slices AQP4 and Kir4.1 were fluorescently labeled and scanned on confocal microscope. Upon analysis of images for colocalization higher correlation coefficient of AQP4 and Kir4.1 in ALS was obtained as compared to WT. The functional state of the Kir channels in cultured cortical astrocytes was further studied by whole-cell patch-clamp. Kir current densities and membrane conductances were decreased in ALS astrocytes. ALS astrocytes also showed lower ability to buffer K^+. Inhibition of Kir currents with 1 mM CsCl or 100 µM $BaCl_2$ revealed decreased Cs^+- and Ba^{2+}-sensitive Kir currents in ALS astrocytes. The misbalance in the AQP4 and Kir4.1 expression in astrocytic endfeet may thus indicate possible deregulation of water flow and potassium buffering that may underlay the observed BBB dysfunction.

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P17.S2

Dexamethasone modulates synaptosomal ectonucleotidase activities in rat hippocampus

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Dexamethasone (DEX) is widely used as co-medication in the therapy of solid malignant tumors since it moderates some of the side effects of chemotherapeutic drugs. It has been beneficial in reducing apoptosis in cells of healthy-tumor-surrounding tissue after radiation therapy. Ectonucleotidases, NTPDase1,2,3 and ecto-5'-nucleotidase (5'-NT), are surface-sited enzymes in synaptic plasma membranes (SPMs) that hydrolyze adenine nucleotides ATP, ADP and AMP to neuroprotective adenosine. In the present study, the effect of chronic DEX treatment on the ectonucleotidases activities in hippocampus of adult male rats was examined. Treatments were administrated i.p. for 8 days and consisted of either DEX (100 mg/kg/day) or an equivalent volume of saline vehicle. ATP, ADP and AMP hydrolysis, as indicators of ectonucleotidase activities, were measured in hippocampal SPMs by colorimetric determination of liberated inorganic phosphate. A significant increase in NTPDase 1,2,3 activity was observed (about 21% in ATP hydrolysis, at $p<0.001$ and 55% in ADP hydrolysis at $p<0.01$) and augmentation in 5'-NT activity (about 20% in AMP hydrolysis, at $p<0.01$) when compared to controls. Our results suggest that DEX is capable to modulate the hydrolysis of extracellular adenine nucleotides and thus, might potentiate the production of adenosine in synaptic cleft and regulate the activity of various neurotransmitter systems in hippocampus.

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P18.S2

Time-dependent modulation of hippocampal apoptotic signalling following cerebral hypoperfusion

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Permanent common carotid arteries occlusion (2VO) in rats is appropriate model to investigate the effects of chronic cerebral hypoperfusion on neuronal and thus, cognitive functions. In our study, adult male Wistar rats were subjected to 2VO or sham-operation and sacrificed 3 or 7 days following the insult. Western blot is applied to detect the changes in levels of p-Akt, as well protein expressions of procaspase 3 and Bcl-2 family members (Bcl-2 and Bax) in hippocampal crude synaptosomal fractions. Degree of apoptosis induced fragmentation of DNA was determined *via* diphenylamine DNA fragmentation assay. 3 days after 2VO no relevant alternation in the quantity of investigated proteins and total/fragmented DNA ratio was detected, indicating that this period might was not sufficient for apoptotic changes to occur. Further, a significant increase in expression of Bax and procaspase 3 as well Bax/Bcl-2 protein ratio was observed after 7th day. Compared to suitable control, enhanced DNA fragmentation was noticed while the expressions of p-AKT and Bcl-2 were not significantly changed which might suggest that apoptotic processes started to develop. Our results implicate that the peak of neurodegenerative processes is at 7th day and that neuroprotective effects of certain substances, which are planned for our further experiments, should be tested in this time point.

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P19.S2

Magnesium ions reduce bursting frequency in leech Retzius neurons in concentration–dependent manner

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Aim: The aim of the present study was to investigate the effect of raising concentrations of Mg^{2+} ions on epileptiform activity induced in leech Retzius (Rz) neurons by blocking Ca^{2+} channels with nickel.

Methods: We have used classical electrophysiology to measure number of waves of depolarization per minute prior to and after the addition of different concentrations of Mg^{2+} in separate trials into the superfusing 3mM Ni^{2+} Ringer solution. Each trial was performed on at least 5 cells. All data are represented as average±SEM.

Results: Application of 3mM Ni^{2+} induces epileptiform activity in leech Rz neurons in a form of oscillatory and repetitive bursting activity. Addition of 1mM Mg^{2+} into the perfusing saline reduced the frequency of bursting from 6.40±4.82 to 4.82±0.43 waves/min (n=10, $p<0.05$). Concentration of 3mM Mg^{2+} caused a reduction from 6.08±0.62 to 3.77±0.36 (n=9, $p<0.01$). Introducing 7mM Mg^{2+} lowered bursting frequency from 5.05±0.36 to 2.28±0.38 (n=9, $p<0.01$), while 10mM Mg^{2+} containg saline superfusion reduced it from 5.74±1.04 to 0.16±0.07 waves/min (n=6, $p<0.01$). The highest concentration applied of 20mM Mg^{2+} completely abolished bursting activity, bringing its frequency from 6.10±0.64 waves/min down to zero value (n=11, $p<0.01$). CONCLUSIONS: Magnesium suppresses leech Rz neuron bursting activity in a dose-dependent manner. Mechanism of suppressive action is presumed to be competitive Na^{+} channel blockade or activation of Ca^{2+} dependent K^{+} channel by Mg^{2+} ions.

P20.S2

Box-count analysis of neuronal images: influence of scale methodology and image representation

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Box-count method, as a leading technique of fractal analysis, has found widespread application in the field of neuroscience, particularly when the two dimensional neuronal images have been quantified. Digitized images of Golgi impregnated neurons from the human cortex, mammalian retinae and spinal cord were subjected to box-count analysis. These neurons have either sparse or abundant dendritic branching. This report discusses two important issues of box-count methodology: the sequence of box sizes per grid and effects of image representation. Box sizes were scaled as geometric progression, arithmetic progression and random choice, while corresponding fits were further studied analyzing correlation coefficients and their standard errors. It is well-known that images can be presented as binary, skeletonized or outlines of images. Whereas skeletonized neuronal images, subjected to box-count analysis, can quantify the deviation of dendrites, binary or outline images quantifies both the space-filling property and the shape of the neuron. Practicality of using the binary or outline neuronal images in order to quantify both properties and each one separately is also discussed.

P21.S2

Neurons of the human dentate nucleus during prenatal development: mathematical modeling of the dendritic branching pattern

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The prenatal development of the human dentate nucleus leads to changes in morphology of its neurons, particularly to changes in their dendritic branching patterns. This study explores four important properties of the neuronal dendritic pattern, that is, the size of the neuron, winding of individual dendrites, maximal dendritic density and position of the maximal density, in order to investigate the changes in morphology of these cells during prenatal development. Since the results showed that all parameters used increased during gestation, a few suitable mathematical models (linear, power and exponential) which quantitatively describe changes in dendritic branching patterns were offered. Using the correlation coefficients and corresponding standard errors the exponential fit as the most suitable one was accepted. The evaluation of the speed of the exponential growth suggests that the size of the neuron demonstrates the highest change during prenatal development. The data were further evaluated and the positive linear correlation between the size of the neuron and the winding of its dendrites, as well as between the size and maximal density of the neurons, was obtained. The findings of the present pilot study could represent a good start for further morphometric analysis of dentate neurons in the prenatal and postnatal development. This study could also provide a better understanding of the formation of the neuronal circuitry when the human dentate nucleus is concerned.

P22.S2

Cyclic voltammetry in diagnosis of ALS

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The diagnosis of amyotrophic lateral sclerosis (ALS) based on ALS functional rating score (ALSFRS) is often shown to be unreliable and false. On the other hand, it is a fact that MRI studies of a brain of ALS patients show the presence of iron deposits in precentral gyruses of gray matter (PGGM), meaning that blood-brain barrier is compromised. Therefore, various studies have been performed with the goal to detect excess of iron in the cerebrospinal fluid (CSF) of ALS patients (e.g. EPR detection of $^{\cdot}OH$ radicals as products of Fenton reaction after supplementation of H_2O_2 to the CSF). Unfortunately, no acceptable correlation could emerge, probably caused by the presence of a range of iron complexes in CSF. Therefore, a different approach to detect iron states in CFS is required. The aim of this work was to determine if there is a specific feature in CSF that distinguishes patients with ALS from those with purely motor peripheral neuropathy (PN) and healthy control subjects. CSF obtained from ALS patients and normal controls were analyzed using the technique of cyclic voltammetry. The results show that, at potential of 1.1 - 1.2 V vs. Ag/AgCl electrode, for the ALS patients, the plateau appeared and the potential of oxygen evolution was shifted toward more positive values. These voltammogram features were not present for the control patients. The cyclic voltammetry is fast and inexpensive technique and showed to be promising candidate for evaluating new biomarkers for ALS.

P23.S2

Adenosine is released per se in normoxic conditions. After ischemia it is greatly an extracellular ATP product

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It is still unknown which part of extracellular adenosine derives *per se* from cells or from ATP. Extracellular concentrations of adenosine and ATP from the rat striatum were estimated by the microdialysis technique under *in vivo* physiological conditions and after focal ischemia induced by medial cerebral artery occlusion. Under physiological conditions, adenosine and ATP concentrations were in the range of 130 nmol/L and 40 nmol/L, respectively. In the presence of the novel ecto-ATPase inhibitor, PV4 (100 nmol/L), the extracellular concentration of ATP increased 12-fold but the adenosine concentration was not altered. This demonstrates that under physiological conditions, adenosine is not a product of extracellular ATP. The presence of the concentrative nucleoside transporter CNT2 on plasma and vesicle membranes isolated from the rat striatum was demonstrated by immunoelectron microscopy. Result favors that under *in vivo* physiological conditions adenosine is transported in vesicles and is released in an excitation-secretion manner.

In the first 4 hours after ischemia, adenosine increased to ~690 nmol/L and ATP to ~50 nmol/L. In the presence of PV4 the extracellular concentration of ATP was in the range of 450 nmol/L and extracellular adenosine was in the range of 270 nmol/L. This demonstrates that early after ischemia, extracellular ATP is hydrolyzed by ecto-nucleotidases which significantly contribute to the increase in extracellular adenosine.

P24.S2

Independent component analysis of Raman spectra of normal brain tissues: the cell-type contributions

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Raman spectroscopy assesses the chemical composition and molecular interactions in tissue samples in minimally destructive or non-destructive regime and can provide information complementary to histological. The obtained spectra reflect complex composition of tissues which makes their difficult. Independent component analysis, which performs blind source separation from complex signals, can be used to separate different contributions in spectra in various tissues. The aim of this study is revealing whether those contributions arise from particular cell types or their mixtures.

We performed micro-Raman spectroscopy of sections of selected brain tissues which are obtained from bank of frozen tissues and originated from the same cadaver. Further, spectra were obtained from pure astrocyte culture and section or cranial nerve. Ten spectra of each sample were acquired using 532 nm laser with power on surface of 10 mW. Independent components analysis (ICA) was performed on preprocessed and whitened spectra using FastICA algorithm. The obtained components were compared to spectra of pure astrocyte culture and cranial nerve. We founded that for each analyzed components one of ICs identical spectral features as spectra of pure astrocyte culture. The same was not observed when comparison was made between remaining ICs and spectra of nerve section. The obtained results imply that ICA could separate contribution of glial cells from other cellular components of brain tissues.

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P25.S2

Investigating peptide–membrane interactions in Alzheimer's disease

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Amyloid-β (Aβ) accumulation is closely related to Alzheimer Disease (AD) pathogenesis. Mature amyloid fibrils in senile plaques were previously thought to be the main cause of AD, however, recent studies suggest that soluble isoforms are the trigger of AD. Aβ pore formation cristallyzed as the main contributor to synapse disruption and uncontrollable nitric oxide release. Membrane active traits of Aβ affect cerebral and peripheral vasculature resulting in reduced blood flow, ischaemia, hypoperfusion. We conducted neutron diffraction experiments at D16 diffractometer at the Institute Laue Langevine, Grenoble to determine the position and orientation of selectively deuterated Aß in stacks of planar model membranes sprayed on quarz glasses, in a relative humidity of 98%. The diffraction pattern revealed the position of the deuterated amino acid in depth of the membrane. Moreover, Aß samples exhibited a time-dependent altering diffraction pattern and scattering density profiles with two separated peptide signals, interpreted as structurally distinctive populations of intercalated Aß. Temporal evolution in membrane organization clearly shows Aß`s destabilizing properties and toxic potential. Further the impact of cholesterol on the exact localisation and spatial occupation of Aß was systematically followed. Deuterated labels shifted into the lipid core and the peaks merged, a single uniform structured population of peptides arose i.e. cholesterol acts as a modulator of Aß behaviour.

P26.S2

Ex vivo brain imaging by µCT: visualization of ischemic lesion

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X-ray computed tomography is a nondestructive three-dimensional (3D) imaging method already in use for medical diagnostics. X-ray micro-computed tomography (µCT) scanners are designed for imaging of small laboratory animals, mice and rats. They have identical basic properties as medical CT scanners, but they allow predominantly imaging of diverse mineralized animal tissues. They are very limited in imaging of soft tissues due to the low intrinsic x-ray contrast of the non-mineralized tissue.

In order to visualize *ex vivo* brains by µCT we compared ionic and non-ionic radiological contrast agents (RCA).-. Brains were immersed in RCA for several days and subsequently scanned using 1076 µCT (SkyScan, Belgium). Non-ionic RCA Omnipaque (GE-HealthCare, Norway) showed better gray to white matter contrast and it was chosen for further studies.

In order to verify whether the µCT can be used to characterize the brain ischemic lesion, we compared the µCT results with the Nissl stained serial histological sections of the same brains. Brain ischemic lesions were induced by middle cerebral artery occlusion (MCAO). The brains were isolated 24 h later, fixed by 4% paraformaldehyde, immersed in Omnipaque for 5 days, scanned by µCT, subsequently prepared for histology, serially sectioned, and stained by Nissl stain.

Brain morphology was visible by µCT and it was possible to delineate the ischemic lesion. The volumes of ischemic lesions visualized by µCT corresponded to 3D reconstructions of Nissl stained brain sections. Moreover immunohistochemistry was possible on the brains treated by RCA and sectioned after µCT scan.

The use of µCT enhances the translational component of animal experiments for human medicine. Moreover the more affordable µCT could replace very expensive magnetic resonance imaging of brain lesions.

P27.S2

Electrophysiological phenotype of cultured rat microglia: the effect of ribavirin

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Microglia, the resident immune cells in the central nervous system, beside its protective role, acts proinflammatory in chronic inflammatory conditions. Ribavirin is clinically approved antiviral drug, with anti-inflammatory potential shown in the animal model of multiple sclerosis. Recently, the pure culture of microglia has been used as *in vitro* model system for assessment of immunosuppressive action of ribavirin (10 μM) on microglia stimulated by lipopolysacharide (LPS) (25 ng/ml). In this study we undertook whole-cell patch-clamp measurements in order to characterize physiological properties of microglial cells in the same *in vitro* model system, in resting and LPS-activated state. The recorded families of currents corresponded well to published studies, shown to be carried through inward rectifying and delayed rectifier K^+ channels. We found that resting and activated microglia outward current densities differ significantly. Activated microglia has almost double current density at 50 mV step (n=6). Our preliminary data show that cells treated with ribavirin and LPS simultaneously (ribavirin+LPS) do not display an increase of outward currents, as compared to the cells treated with ribavirin alone. The cell capacitance significantly increased from 16±3 pF (n=6) in control, to 36±3 pF (n=9) in LPS-treated cells, while in (ribavirin+LPS)-treated cells was 23±5 pF (n=8). Our preliminary data suggest that ribavirin may modulate electrophysiological properties of microglial cells.

28.S2

Ca - sensitive magnetic resonance probe influences intracellular calcium and outward currents of primary cortical astrocytes

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Recently developed calcium-sensitive magnetic resonance probe (MRP) showed remarkable longitudinal relaxivity changes upon interaction with Ca^{2+} in buffer and biologically relevant solutions[1]. The potential of MRP for non-invasive detection of changes in Ca^{2+} concentrations using MRI would be of vital importance for *in vivo* real time imaging. Our goal was to check for the possible acute effects of MRP on cell viability and intracellular calcium signaling in primary cortical astrocytes. Cell viability assays, performed after acute exposure to several concentrations of MRP (up to 1.8 mM) for 4 hours, confirmed that the substance is not toxic at the applied concentrations. Changes in intracellular calcium concentrations ($[Ca^{2+}]_i$) were measured using the Fluo-4 AM calcium-sensitive probe on a laser scanning confocal microscope. All three applied concentrations of MRP (mM: 0.6, 1.2 and 1.8) decreased $[Ca^{2+}]_i$ of astrocytes by ~65%, but the cells were able to respond to ATP with standard $[Ca^{2+}]_i$ transients even in the presence of MRP for 30-40 minutes. To determine which pools were responsible for the loss of calcium, several blockers were used: the potent inhibitor of sarco-endoplasmic reticulum Ca^{2+}-ATPases (SERCA), thapsigargin (1 μM), and two blockers of capacitative calcium entry (CCE) channels, $LaCl_3$ (10 μM) and $GdCl_3$ (10 μM). In calcium free extracellular solution, MRP induces a decay of $[Ca^{2+}]_i$ by ~45% that does not change even in the presence of thapsigargin. When SERCA is blocked in presence of external calcium (2 mM), the decrease of $[Ca^{2+}]_i$ evoked by MRP is the same as without the blocker (~65%). On the other hand, CCE blockers reduce the loss of $[Ca^{2+}]_i$ induced by MRP to the level observed in calcium free extracellular solution (by ~45%). Furthermore, we have examined the effect of MRP (1.2 mM) on membrane properties of cortical astrocytes in culture by patch-clamp technique in whole cell configuration. Preliminary data show that application of MRP for a period of 10 min decreased outward currents of examined astrocytes. Such effect of MRP was less pronounced on astrocytes pretreated with benzamil (100 μM), a Na^+/Ca^{2+} exchanger inhibitor, for a period of 5 min. As expected, MRP lowers the concentration of available Ca^{2+} near the plasma membrane, which affects its gradient. The loss of intracellular calcium triggered by MRP cannot be attributed to the action of SERCA and ~20% of the free intracellular calcium is probably lost through CCE channels.

1. Angelovski G, et al., (2008) ChemBioChem 9:1729-1734.

P29.S3

Analysis of real-time PCR data by using factor analysis with promax rotation

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Multivariate analytical methods have found application for curve resolution in many spectroscopy techniques. Real time PCR spectra represent kinetics of fluorescence intensity increase, with the form of exponential function.

After testing on simulations, we have found that factor analysis with oblique rotation Promax was particularly efficient. Algorithm efficiency was improved by changing the kappa parameter to values in range 10 – 15.

Procedure has been used for the analysis of mutations in EGFR gene. The human epidermal growth factor receptor (EGFR/HER1/ErbB1) belongs to (HER)/ErbB family of tyrosine kinase receptors. Numerous studies have shown that EGFR is mutated in a significant proportion of primary lung adenocarcinomas, emphasizing the fact that number of patients with EGFR mutations show hypersensitivity to the tyrosine kinase inhibitors gefitinib and erlotinib. Patients with found mutations were candidates for the therapy which leads to prolongation of the life span.

Detection of EGFR mutations was performed by using DxS EGFR Mutation Test Kit. This kit enables the detection of the 29 different mutations in a real-time PCR Assay based on DxS Scorpions® technology.

After factor analysis was applied on data it was found to be possible to distinguish individual mutations or even the cases if the patient has several mutations in EGFR gene. Distinguishing of mutations could be correlated with future clinical examinations.

P30.S3

Biomechanical investigations of living endothelial cells

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Cells move, interact, deform while they generate and respond to mechanical forces. Elasticity, adhesion, stress relaxation, only a few from the most important mechanical aspects of cellular behavior. The atomic force microscope (AFM) has recently evolved to a high resolution imaging tool and micro-mechanical tester. As it operates in liquid, using this powerful technique, valuable data can be obtained about elasticity, intercellular adhesion, stress-relaxation of living cells.

Brain capillary endothelial cells form the morphological basis of the blood-brain-barrier (BBB). These cells, strongly interconnected by tight and adherens junctions, build up a well defined filter-layer which preserves the homeostasis of the central nervous system (CNS). As the BBB is an important way of drug delivery into the CNS, biomechanical investigation of capillary endothelial cell is of primordial importance. Both spatial and temporal dependence of elasticity of subconfluent and 100% confluent human capillary endothelial cells will be presented. Since a lymphatic system is not present in the CNS, metastasizing melanoma cells must cross the BBB. Mechanical aspects of this process were investigated by atomic force microscopy. Furthermore, intercellular adhesion between endothelial and single melanoma cells and their stress relaxation was monitored under physiological conditions.

P31.S3

Interaction of hypericin with Protein Kinases Cα and Cδ and its Influence on U-87 MG Cells Death

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Protein kinases (PKC) participate in the regulation of early stages of apoptosis by phosphorylating key apoptotic proteins or in later events by acting downstream of caspases. PKCd has a role in the execution of the apoptotic program, while PKCa is frequently associated with cell survival and suppression of apoptosis. U-87 MG glioma cell line is characterized by higher expression of PKCa and PKCδ isoforms. The equilibrium of these isoforms is essential for life cycle of U-87MG cells. Photoactivated hypericin (Hyp) causes not only relocalization of PKCs but also time dependent mode of their activation/inhibition. The opposite role of PKCa and PKCd isoforms in apoptotic process induced by photodynamic action was examined on U-87MG glioma cells. Confocal fluorescence microscopy, flow-cytometry, fluorescence imaging and Western blot (WB) were used as experimental techniques. Hyp photoactivation strongly affects apoptotic response of the cells. The photo-damage results in mitochondria membrane depolarization, failure of nuclear membrane, relocalization of PKCα from cytoplasm to perinuclear area. Finally, the concentration ratio of PKCa and PKCd clarifies their role in photoactivated apoptotic process.

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P32.S3

Characterization of cell based drug delivery models by microelectric sensing and permeability assays

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Cell-based models are important for understanding drug penetration across the blood-brain, the nasal and intestinal barriers. Recently label-free devices were developed to monitor cellular events in real time. These systems measure impedance across microelectrodes that are covered by cells and provide quantitative information on their intercellular adherence indicating paracellular permeability.

We tested the effects of a drug candidate and pharmaceutical excipients on three cell culture-based models by microelectric sensing and permeability assays. Primary rat brain endothelial cells, RPMI 2650 human nasal and Caco-2 human intestinal epithelial cell lines were directly grown either on microelectrodes (96-well E-Plates) to measure impedance (xCelligence, Roche) or on cell culture inserts with porous membranes (Transwell, Costar) to test compound permeability.

Sucrose esters, non-ionic surface-active pharmaceutical excipients, decreased impedance in a dose and time dependent manner and increased paracellular transport of marker molecules in both epithelial cell lines. Tesmilifene, a chemopotentiating agent decreased impedance and increased permeability of marker molecules across brain endothelial monolayers.

Real-time impedance measurements on adherent cell monolayers helped to determine optimal time points and concentrations for permeability assays. Barrier integrity changes measured by microelectric sensing and permeability data were in agreement in all three models.

P33.S3

Physicochemical properties of 1-palmitoyl-2-oleoyl-sn- glycero-3-phosphocholine (POPC) monolayers – influence of charge addition and temperature

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Monolayer properties of the unsaturated phospholipids 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and its ethylated cationic derivative, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-ethylphosphocholine (E-POPC), have been investigated at the air/water and nitrogen gas/water interfaces over a range of temperatures. Analysis of the measured surface pressure by an excluded volume interaction model suggests that lipid aggregation/domain formation occurs within the monolayer even at low surface pressures. The stability of the POPC monolayer was significantly lower at the air/water interface as compared to POPC at the nitrogen gas/water interface, suggesting oxidation and breakdown of the unsaturated lipid in contact with air. Monolayer properties of pure E-POPC as well as of POPC and E-POPC mixtures were also investigated. The electrostatic repulsion between the cationic groups increases the surface pressure at a given area per molecule. Calculations of the electrostatic contribution to the surface pressure, and its comparison with the experimental data for pure E-POPC provide evidence for significant ion binding. A thermodynamic analysis of the mixed monolayers was carried out and revealed miscibility and exceptional stability of the 1:1 mixture of POPC and E-POPC.

P34.S3

The mechanism of vanadate reduction in Phycomyces blakesleeanus mycelium

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Although, fungi represent the main sites of entrance of vanadium, an essential ultra-trace element into biotic component of ecosystem, available data are far from clarifying mechanisms and place of vanadate reduction in fungal cells. In this study we investigated the interactions of physiologically-relevant vanadium redox states: vanadate (+5) and vanadyl (+4), with mycelium of fungus *P. blakesleeanus* using EPR spectroscopy and biochemical assays. The appearance of EPR spectrum after addition of V^{+5}, EPR inactive form, confirm ability of *P. blakesleeanus* mycelium to reduce vanadate. We have examined the capacities of cell surface enzyme showing ferricyanide reductase activity (FRA) in *P. blakesleeanus* to reduce V^{5+}. In comparison to the mycelium grown on full medium (control), iron-deficient mycelium (-Fe) showed almost two fold higher capacity to reduce V^{5+}, which may be related to increased expression of FRA enzyme. The supplementation of Cd^{2+} (FRA inhibitor) provoked significant concentration-dependant decrease of V^{4+} formation. Almost complete inhibition of V^{5+} reduction was observed at the higher Cd^{2+} concentration and in mycelium treated with ferricyanide (the competitor of V^{5+} for reduction). Unexpectedly, mycelium co-supplemented with V^{5+} and ferricyanide showed an additional signal which was identified to originate from Mo^{5+} in molybdenum-molybdopterin (Mo-MPT) co-factor. The involvement of FRA enzyme with Mo-MPT co-factor in V^{5+} reduction is further substantiated by V^{5+}-provoked inhibition of ferricyanide reduction and by drastically decreased capacity of Mo-deficient mycelium to reduce V^{5+}.

P35.S3

Real-time cell electronic sensing as a method to measure toxicity in cultured cells

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Determination of the toxic effects of drug candidates and novel excipients is important in drug development. Cell based assays are suitable to investigate adverse effects of substances by high throughput screening and correlate well to *in vivo* studies. Colorimetric end-point tests (tetrazolium dye conversion, measurement of enzyme release from damaged cells) and morphological examinations (fluorescent stainings of cell organelles etc.) are widely used to determine cell viability or death. Impedance based cell microelectronic sensing is a new tool to obtain kinetic data on cell changes including viability without labelling.

Our aim was to compare end-point cytotoxicity assays (MTT dye conversion, lactate dehydrogenase release, cell nuclei staining) with real-time cell microelectric sensing (xCelligence, Roche). The toxicity of surfactants sucrose esters, cremophors and Tween 80 were tested on human epithelial Caco-2, RPMI 2650 and endothelial hCMEC/D3 cell lines.

All surfactants caused dose- and time-dependent cellular toxicity. Surfactants interfered with colorimetric end-point assays at high concentrations. Impedance measurement gave kinetic information on cell viability and correlated well with the other methods. Based on our results novel cell microelectric sensing is a sensitive and reliable tool to measure real-time the toxic effect of substances at a broad range of concentrations in cultured cells.

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P36.S3

Changes in the membrane potential oscillations of Br neuron induced by magnetic field and ouabain

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Bimodal pacemaker Br neuron from the snail brain spontaneously generates bursts of action potentials (AP) accompanied by quiescent intervals. Two processes can be distinguished during the rhythmic bursting activity of Br neuron: the slow oscillation of membrane potential (OMP), and generation of bursts of AP during the depolarizing phase of the oscillations. In the previous investigations we established that 15 min exposure to static magnetic field of 10 mT induction (SMF) decreases the duration of bursts of APs of Br neuron by increasing the activity of Na^+/K^+ pump. Conversely, inhibition of Na^+/K^+ pump by 10^{-4} M ouabain increases the duration of AP bursts and decreases the duration of quiescent intervals in the Br neuron. In this study we measured the effects of SMF and ouabain on the period of OMP of the Br neuron. The sharp electrode current clamp recordings from Br neuron showed that both, SMF and ouabain, significantly decreased the period of OMP of Br neuron. SMF changed the period of OMP by decreasing the burst interval duration for 9.61%. Ouabain changed the period of OMP primarily by decreasing the duration of quiescent interval by 21.67% in control Br neurons, and by 13.92% in SMF exposed Br neurons. Obtained data indicate that Na^+/K^+ pump is involved in SMF induced changes in both, oscillation of membrane potential and generation of bursts of APs.

P37.S3

Metabolism of phosphate compounds during oxygen deprivation in fungus Phycomyces blakesleeanus

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The effects of oxygen deprivation on phosphate metabolites in *P. blakesleeanus* were investigated by ^{31}P NMR spectroscopy. It caused a decrease in polyphosphate/inorganic phosphate (PPc/Pi) ratio for 83.7%, 54.5% and 40.5% after 1.5, 3 and 5 h of hypoxia, and in anoxia, it fell to 61.1%, 43.2% and 40%.This decrease turned out to be reversible, since PPc/Pi ratio increased after reoxygenation, and the increase was greater in specimen that was exposed to hypoxia. ATP content in hypoxia and anoxia decreased less than expected, probably due to ability of PolyP to sustain energy and phosphate homeostasis of the cell under stress conditions. Aplication of azide, a cyt c oxidase inhibitor, also decreased PPc/Pi ratio, but to a much lesser extent in oxygen deprived than control and reoxygenated specimens. This finding suggests that there could be a connection between this process and relative activity of mitochondrial respiratory enzyme alternative oxidase, since this enzyme takes over most of cellular respiration in conditions of oxygen deprivation.

P38.S3

Influence of cancerostatic perifosine on membrane fluidity of different cell lines

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Perifosine (OPP) belongs to the group of alkyl phospholipids (APLs), a new class of anticancer agents, targeting directly cell membrane but not DNA. They show a selective apoptotic response in tumor cells, sparing normal cells. Perifosine seems to be most promising for breast cancer therapy. For this type of tumor, an antitumor effect was found only for hormone receptor negative tumors *in vivo*, while no effect was found for hormone receptor positive tumors. The reason for this difference is not yet understood and requires further studies.

To contribute to the understanding of the mechanism of OPP action, in this work influence of OPP on the membrane structure of estrogen receptor positive (ER+) MCF7 breast cancer, estrogen receptor negative (ER-) MT-3 breast cancer, and normal mouse fibroblasts (L-929) cell lines was investigated by EPR, using spin labeled derivative of OPP (5P) as a spin probe. The results show that OPP increases membrane fluidity of all cell lines at concentrations higher than 50 μM. The influence is less pronounced for MCF7 and is the most pronounced for normal L-929 cell lines. This indicates that OPP either doesn't incorporate into the alkylphospholipid resistant, ER+ MCF7 cell membranes but incorporates into alkylphospholipid sensitive, ER- MT-3 cells, or it doesn't concentrate in plasma membrane of MCF7 (ER+) cells at such high concentrations as it does in MT-3 (ER-) and normal L-929 cells. It seems that hormone receptors in MCF-7 cells are correlated with the binding of OPP to the membrane.

P39.S3

Lipid modulation on structure and function abilities of two Cholesterol-Dependent Cytolysins

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Perfringolysin O (PFO) and Listeriolysin O (LLO) are two Cholesterol-Dependent Cytolysins (CDCs) produced by Gram-positive bacteria. Their main task is to facilitate the infection disrupting plasma or endosomal membranes. Even if CDCs have been well studied, their pore-forming mechanism is not clear yet. Two models are proposed: pre-pore to pore transition and the arc-shaped pore formation.

Based on this, we investigated the electrophysiological properties and structures of the two toxins pores in different membrane compositions (i.e. phospholipid acyl chains and cholesterol concentration) using Planar Lipid Membrane (PLM) technique and Atomic Force Microscopy (AFM). Our analysis identified two distinct pore-forming abilities. On POPC-CHO 50% membrane both proteins form well defined pores with conductance values mainly around 10-15 nS, visible by AFM as complete ring inserted into the membrane of about 30 nm diameter. On the other hand on DOPC-CHO 20% membrane PFO and LLO form pores with heterogeneous conductance (from pS to nS) but in most of the cases, pores are difficult to be sorted out both by PLM and AFM analyses. Collectively, our results support the idea that prevalence of cylindrical pores or arc-shaped oligomeric intermediates are promoted by different membrane lipid compositions.

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P40.S3

Modulation of Ca^{2+} transport and electrophysiological properties in degenerative processess in CNS.

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Intracellular Ca^{2+} has a substantial importance in the regulation of neurite outgrowth and length, synaptic transmission and neuronal survival. Dysregulation of Ca-signaling contributes to pathology and leads to loss of structure and function of neurons entering neurodegeneration. In such states of CNS a fibrotic scar is created and with cumulated growth inhibitors prevents further outgrowth and reparation of neurons. Inositol 1,4,5-trisphosphate receptors (IP3Rs) are intracellular calcium transporters that modulate Ca^{2+} level in the cell in physiological as well as pathological states. IP3Rs play important role also in regulation of cellular response to neurodegenerative damage of axon.

On *in vitro* model of fibrotic scar-like structure we investigated changes of mRNA expression of IP3R1 related to such injury in correlation with the average length of neurites extended and possible changes of neurophysiological parameters of affected neurons. Primary cultures of rat cerebelar granule neurons were affected by TGFβ1 – the key effector responsible for creation of fibrotic scar (1). Both mRNA expression of IP3R1 and neurite length were downregulated by TGFβ1. Resting potential of neurons affected by TGFβ1 significant decreased ($p<0,05$) compare to control neurons. We also observed a tendency to faster activation of the action potential in neurons treated with TGFβ1.

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1. Kimura-Kuroda J. et al. Mol Cell Neurosci. 2010; 43: 177-87.

P41.S3

Ion currents in trigeminal neurons of gerbils induced by IgGs isolated from ALS patients

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by dysfunction and death of motoneurons. Previous studies have shown that the disease ethiopathogenesis has a significant humoral immunity component. The aim of our study was to explore the effects of immunoglobulins G (IgG) isolated from ALS patients on trigeminal neurons in gerbil brainstem slices. Acute slices were prepared from P14-P16 gerbils and trigeminal neurons were examined by whole-cell patch-clamp. IgGs, 100 mg/ml were applied for 100 ms by pressure ejection using a picospritzer. Application of ALS IgGs at Vh= -60 mV ($[Cl^-]_{pipette}$= 24 mM) induced inward currents, however in 3/10 cells the current was outward (not related to the IgG sample origin). The inward currents were not dependent on external Ca^{2+}. The I/V curve of evoked peak currents revealed a reversal potential close to the Nernst prediction for chloride. While strychnine blocked the ALS IgG-induced current in 1/4 neurons, inhibitor of $GABA_A$ receptors SR95531 caused a reversible current inhibition in 3/4 cells. These preliminary data indicate that the effect of ALS IgGs was not channel specific and further studies are necessary to elucidate its main target mechanism.

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P42.S3

Ultrastructure and reorganizations in plants thylakoid membranes as revealed by SANS measurements

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Small-angle neutron scattering (SANS) is a non-invasive structure analysis technique with flexible sample environment for 1-100 nm size range. It can be used to determine the average repeat distances (RDs) in multilamellar membrane systems without freezing, staining or chemically fixing the sample; it also provides information on other characteristic parameters of the membrane structure. Different constituents of samples can be emphasized by adjusting the contrast - based on different scattering length densities of water and heavy water. The accuracy (<1 nm) and time resolution (~10 s) of SANS measurements, depending on the technical characteristics of the equipment, allow the recording of relatively small and rapid structural reorganizations in vivo or under physiologically relevant conditions. We have identified characteristic the scattering profiles of isolated granal thylakoid membranes and observed the effects of different physico-chemical environments. Also, for the first time, we have provided evidence that the RDs of different thylakoid membranes in living algal cells are capable of undergoing light-induced reversible changes (Nagy et al. Biochem. J. (2011) 436:225-230, Photosynth Res. (2012) 111(1-2):71-9). Recently, we have used SANS on intact leaves of different plant species to study the membrane reorganizations in relation to different physiological states and regulatory mechanisms.

P43.S3

EPR investigation of free radical formation in rustyback fern (Asplenium ceterach L.)

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Group of higher plants, known as desiccation tolerant or resurrection plants, possess uniquely effective mechanism to withstand extreme dehydration and rapid rehydration of vegetative tissues without cell damage. Resurrection plants survive the loss of most of their tissue water content until a quiescent stage is achieved. Upon watering, the plants revive and are restored to their former state within 24 h. In order to cope with desiccation, resurrection plants have to overcome a number of stresses, among them the most critical being oxidative stress. In our previous investigations, we have shown that during the rehydration process, $^{\cdot}$OH radicals are produced. In this study, we have tested the possibility of quantifying this production by measuring the EPR signal of the ascorbyl radical generated as a result of $^{\cdot}$OH induced oxidation of externally added ascorbate to the rehydration medium. This quantification, in parallel, was investigated by measuring the rate of the reduction of TEMPON free radical. The experimental model used in this work were young and old fronds of the rustyback fern (*Asplenium ceterach* L.), one of the rare resurrection species represented in the flora of Serbia. The results obtained from the investigated model show that the production of $^{\cdot}$OH radicals is greater in old compared to the young fronds and follows a general order: old dry fronds > old fresh fronds > young dry fronds > young fresh fronds.

P44.S3

Studying the interactions of the stereoisomeric forms of indolicidin with DPC and SDS micelles

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Indolicidin is a 13-residue-long, cationic antimicrobial peptide (AMP), showing a broad spectrum of antibacterial and antifungal activities, and it is well-known as a membrane-active molecule. This AMP possesses a remarkable primary structure (H-ILPWKWPWWPWRR-NH_2), with a high content of aromatic (*i.e.* Trp) and basic (*i.e.* Lys and Arg) residues. However, it contains three Pro amino acids too, and according to the *cis-trans* isomerism about the Xaa-Pro peptide bonds, eight different stereoisomers could be distinguished for this AMP. In the present study, molecular dynamics (MD) simulations were carried out, in order to investigate the interactions of the stereoisomeric forms of indolicidin with two types of micelles (*i.e.* DPC and SDS), as well as to characterize their micelle-bound conformations. In the case of all peptide-micelle systems, after an initial energy minimization, a 20-ns-long MD calculation was performed with fixed geometry, which was followed by a 120-ns-long simulation without restraints. Based on these MD trajectories, it could be concluded that characteristic differences could be observed between the stereoisomers of indolicidin, with regard to their insertion depths and micelle-bound conformations, as well as to their interactions with DPC and SDS micelles.

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P45.S3

Swimming motility of bacteria in microstructured environments

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Motility helps bacteria to explore the environment and find favorable habitats. One way to move in a liquid environment is swimming. We use microfabricated devices to study the swimming motility of *E. coli* bacteria.

This technology enables us to study phenomena that are otherwise difficult to look into. The topics include: the hydrodynamics of swimming bacteria near solid surfaces, correlated motion of high density bacterial cultures and chemotaxis in static chemical gradients. The work presented show that microtechnology is a powerful way to study fundamental processes in microbiology.

P46.S3

Ion channels in cytoplasmic droplets membrane from Phycomyces blakesleeanus

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Ion channels in membranes from filamentous fungi are poorly characterized since obtaining high quality membrane seals of hyphal cell membrane is improbable due to the difficulties in removal of the cell wall completely. We developed model system of *P. blakesleeanus* cytoplasmic droplets which rapidly form membranes *de novo*, corresponding to hyphal plasma membrane, thus circumference the problems of the cell wall residues.

This is the first report of a patch clamp recording in a whole cell configuration from P. blakesleeanus vesicles. Whole-cell recordings showed presence of both inward and outward currents in the presence of symmetrical KCl concentration. Addition of 1 mM CsCl partially blocked inward currents demonstrating presence of potassium channel activity while, preliminary data with 2 mM $ZnCl_2$ block both, inward and outward currents, suggesting presence of a CLC2-like channel.

Detailed single-channel investigation of the same vesicles in inside-out patch clamp showed that predominant channel types are: (1). small conductance (10 pS) anionic outward rectifier (ORAC), present in 40% patches; (2) large conductance (112 pS) weak inward rectifier, present in 68% patches when recorded in the presence of ATP; (3) cationic 40 pS conductance with three distinct types of channel activity, possibly representing several different ion channels (present in 88% patches).

P47.S3

The effects of chronic administration of fluoxetine or clozapine on liver of socially isolated rats

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Chronic exposure to psychological stress results in increased oxidative stress and tissue damage. Fluoxetine and clozapine are drugs used for the treatment of psychotic mood disorders whereby primary site of both drugs metabolism is the liver. Given that they can have some adverse effects on the liver, we investigated the effects of chronic administration of fluoxetine (15 mg/kg) or clozapine (20 mg/kg) on liver of male rats exposed to 3 weeks of chronic social isolation, as an animal model of depression. The aim of this study was to follow up serum activity of aspartate transaminases (AST) and alanine transaminases (ALT), hepatic malondialdehide (MDA) and carbonyl group levels, as well as, histopathological changes. Animal's body weight was measured every week. Both drugs induced abnormalities with respect to body weight changes. AST leaking from liver to serum in clozapine-treated control rats indicated drug ability to cause hepatotoxicity. Changes in hepatic MDA and carbonyl group levels were observed following chronic isolation stress. Chronic isolation did not significantly affect the spectrum of histopathological changes compared to controls while it was more pronounced under clozapine treatment. Fluoxetine in control or chronically isolated animals resulted in more or less normal hepatic architecture. Identified histopathological changes could be used as the basis for determining the appropriate dose of clozapine to reduce its hepatotoxic effects.

P48.S4

Raman spectrosocopy as a diagnostic tool for cancer

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Raman spectroscopy is a valuable technique widely used in life sciences. Because of its advantages, like fast spectra collection and distinctive spectral response of various biological tissues, this technique tends to become an inevitable medical diagnostic tool. Moreover, its use has been recently expanded to the field of cancer research. In the present work, micro-Raman spectroscopy combined with neural network algorithm software was used to analyze and classify Raman spectra of a number of different types of cancers and the surrounding healthy tissues. It was found that the characteristic wave numbers obtained from Raman spectra of malignant tissue samples may be used as biomarkers for fast and reliable diagnosis of different kinds of cancer. So far, a small database of Raman spectra obtained from different types of pulmonary cancers has been created. The use of this database supported by a neural network learning algorithm, allows the identification of a specific cancer from a target organ, based only on a single Raman spectrum. The aim of further studies is to build a larger database (including liver, kidney, colon, bone and other cancers), which will be used for fast and efficient diagnosis of different malignancies. Furthermore, the assignment of selected Raman spectral bands from vibrational modes in proteins may provide additional information to the standard histochemical and immuno-histological assays that can aid in more accurate cancer type identification.

P49.S4

Improvement of FDLD images throught application of Gabor filters

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Fluorescence detected linear dichroism (FDLD) imaging is advanced microscopy method which allows determination of the anisotropic molecular architecture of different microscopic objects beyond the classical fluorescence emission microscopy. The DP-LSM we used was developed for confocals in the Biological Research Centre, using high frequency modulation and a demodulation circuit to obtain the polarization properties with high precision and in real time.

Accurate measurements of FDLD values of the orientation of absorbance dipoles depend to the sample alignment along the axis of interest. However, it is often difficult to set the sample at perfect orientation. It becomes even more difficult if multiple objects in different orientations are present on the same image.

Gabor filters is advanced method used for image analysis for decades. It is improved technique for object detection, digits recognition, image segmentation and texture analysis, but it has not been applied for improvement of FDLD images.

The procedure consisted of two analytical steps. In the first step, orientation of the sample was determined by changing the angle of applied filters and maximizing the sum of brightness intensity on the image. In the second step, FDLD values were recalculated according to the angle which was previously determined.

After the described refinement procedure was applied it was possible to compare the level of the anisotropic organization for non-ideal oriented objects, as we demonstrate on maize images.

P50.S4

Combined Influence of Competitive Binding and Mass Transfer on the Affinity-based Biosensor Response

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Detection of target biomolecules in solutions is an important task in medicine and environmental protection, while investigation of biomolecular interactions is of great significance for fundamental biological and pharmaceutical research. One class of biosensors intended for such applications use surface-based detection methods, where highly specific binding of a target analyte to the capturing probe molecules immobilized on the sensing surface is converted to the sensor's output signal. In real situations biological samples often contain other molecular species (competitors) which can also bind to the same capturing probes with a certain affinity. In this paper we analyze the influence of both competitive binding and mass transfer on the biosensor's time response. The presented analysis enables a correct interpretation of the response of affinity-based biosensors, i.e. a more accurate determination of both the concentration of target molecules and the kinetics of bimolecular reactions in detection methods based on time domain measurements of the sensor's output signal. Apart from being useful in biomolecular affinity studies, the analysis provides the guidelines for the improvement of sensitivity, selectivity, and response rate of biosensors. It is also useful for development of methods for simultaneous detection of multiple analyte specimens.

P51.S4

Three-dimensional simulations of different cavity designs in computed tomography scan- based tooth model

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In recent years, the virtual three-dimensional (3D) models and simulations became a valuable means in researching mechanical properties of biomaterials and human tissues [1, 2]. The main advantage is saving time and money associated with laboratory and clinical research. The purpose of this study was to present the generation of a 3D tooth model based on computed tomography (CT) data and its use in simulation of cavities designs. An extracted human upper premolar was scanned using the multilayer spiral CT machine (SOMATOM Sensation 64 Cardiac CT Scanner, Siemens, Germany). All obtained slices were written in DICOM (Digital Image and Communications in Medicine) file format. In this paper, the slices parallel to the xy plane (along the vertical z axis) were used. Computation of the contours of a tooth structures (enamel, dentin, pulp), necessary for the model generation, was done in AMIRA software (Visage Imaging Inc.USA). The contours were transfered to SolidWorks software (Dassault Systèmes SolidWorks Corp, USA), as DXF (Drawing eXchange Format) file format, and the 3D tooth model was created using a loft technique. After that, different cavity designs were simulated. The 3D tooth model was successfully created for the simulation of different cavity designs, in order to predict biomechanically the most appropriate design for the further clinical research.

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P52.S4

Relationships between the Cole-Cole impedance model parameters of human skin

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Bioimpedance spectroscopy (BIS) is a promising tool for studying numerous physiological events as well as to diagnose a variety of pathological changes within tissue or in whole body. This study aims to evaluate the bioimpedance *in vivo* measurements of human skin as a method to shed light on differences of electrical characteristics of human skin related to gender, age and stratum corneum layers. BIS measurements with 2-electrode set-up were taken at frequencies ranged between 10 Hz to 100 kHz. Using Cole-Cole impedance model, measurements data of female and male human skin were analyzed and compared. Electrical properties of tissue are followed according to Cole-Cole equation as four parameters: low frequency resistance R_0; high frequency resistance R_∞; relaxation time t and a parameter. Individual human skin electrical properties were determined for 30 women and 30 men aged 12-97 years. Comparative analysis of relationships between Cole-Cole parameters within human population, revealed that: a parameter is strongly related to gender; a is statistically significant higher in women than in men, independent of skin layer and age; parameter R_∞ is less, but statistically significant related to gender. However, R_0 and t seem to be slightly dependent of gender and age. Their values are indicative and strongly related to stratum corneum layers.

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P53.S4

Hydrodynamic synchronisation of light driven microscopic rotors

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Organelles of microscopic biological systems often exhibit synchronized motion. It has been proposed that the origin of the synchronization is hydrodynamic interactions. However, there has been yet no direct experimental demonstration of the effect at the corresponding microscale. We used a functional experimental model system to demonstrate hydrodynamic synchronization.

We created micrometer sized propellers by two photon photo-polymerization. Such rotors were held and rotated close to each other in optical traps produced by holographic optical tweezers. The distance of the rotors and the rotating torque could be freely varied. Synchronization of the light induced rotation due to hydrodynamic interaction was demonstrated. The effect was characterized under a broad range of parameters. Based on the comprehensive data we built a model to describe the effect and tested it in detail.

P54.S4

Utilizing cluster analysis in modelling AQP-4 distribution in rat ALS model

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In the field of data mining, cluster analysis of large collections of data tends to group objects based on the information describing the objects or their relationships. In our study we tested several different approaches in modelling distribution of membrane proteins, namely aquaporin-4 in a complex glio-vascular system called the blood-brain barrier (BBB). We previously showed an overexpression of aquaporin-4 in the spinal cord in the end stage of amyotrophic lateral sclerosis (ALS) SOD1^{G93A} rat model. Moreover, electron microscopy (EM) study with immunogold labelling indicated different clustering patters of anti-AQP-4 gold particles around blood vessels. We have used density function estimators, hierarchical MIN clustering, analysis of inter-cluster distances with Kolmogorov-Smirnov test and modelling stochastic flow over graph by Markov Cluster Algorithm to fully characterize particle distribution. By pre-clustering the data and analysing distances between particles and groups they form, we gathered sufficient data for running random walk over graph, and then characterized distribution with post-analysis. Further optimization of the algorithm is still necessary, yet preliminary results have delivered a description of AQP-4 clustering. Identification of the clustering process should be important for understanding the mechanisms that govern water entry, which may be beneficial in preventing the disruption of BBB and in search for drugs that modulate clustering.

P55.S4

Mathematical modeling of regulation of glucose concentration in blood

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In last decades, different quantitative methods - among them we point out systems biology - have been introduced in various fields of biology and medicine for deeper understanding of physiological processes running in human organs and organism. For medicine a central aim is to understand patophysiological events and consequent use of proper therapies in curing processes. Moreover, the complex relations and interactions between the affected organ and the others of the organism and their interrelations on different levels of biological organization from molecular to tissue and organ levels are the master task to be resolved.

Here, we present mathematical modeling of glucose regulation in blood. We start with the simplest version in which only interrelation between glucose and insulin is taken into account. Adding the glucogen loop into glucose-insulin metabolic pathways leads to much more complex model with largely increased number of model parameters. Model developed to this level already reflects clinical observations and allows simulation of various clinical cases. For example, some metabolic disturbances in diabetic patients may lead to increased fat production and consequent obesity. This case will be analyzed in more details, in particular with respect to the various ways of food ingestion (slow versus fast) and various values of glycemic index of food ingested. We shall also analyze the time dependent behavior of the model after instantaneous injection of insulin and glucose.

P56.S4

Elemental analysis of bone tissues using Laser induced breakdown spectroscopy

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Laser induced breakdown spectroscopy (LIBS) is an atomic emission spectroscopy method where high temperature plasma is generated as the emitting source. This provides spectral information about the elemental composition of investigated sample. Our experimental set up contained Nd:YAG laser working on wavelength 532 nm, the Andor Mechelle spectrometer system coupled with an intensified CCD camera covering spectral range 200-950 nm.

LIBS has been recently used for qualitative elemental analyses in biomedical research. Some elements are accumulated in human or animal bodies and hard tissues are the best for long time deposits monitoring. We observed elemental composition of animal bones (chicken and wild boar). Our work went trough the quantitative and qualitative analysis of compact bone tissues by means of calibration free LIBS (CF-LIBS). This method avoided the problem of matrix effect in means of using calibration methods, but the self-absorption effect had to be considered. In the spectra were identified following elements C, Ca, H, K, Mg, N, Na, O, P, Sr, Zn. For calibration free quantification method just Ca, P, Mg, Na, Zn, K, Sr were considered because of the air contribution to the N, O and H elements. For chicken (*Galus domesticus*) bones the weight percentage of calcium was quantified as 59,14%, phosphorous 32.73%, potassium 4.32%, magnesium 2.68%, sodium 0.76%, zinc 0.28% and strontium 0.09%. For wild boar (*Sus scrofa*) bones the weight percentage of calcium was estimated as 67.30%, phosphorous 30.30%, magnesium 1.37%, sodium 0.80% and zinc 0.19%.

This work presents for the first time elemental quantification of animal bones using the calibration free LIBS analysis.

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P57.S4

Bioinformatical and mathematical comparative analysis of ClpP exons and protein sequence

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In past years cell stress response has been investigated with great focus on chaperons and protein degradation machinery that are the main actors of this response. Protein degradation is carried out by protein complexes such as proteasomes or proteases. One of those proteases is ClpP. This protease can be found in cytosol of prokaryotes and in mitochondria and chloroplast of eukaryotes. ClpP exon sequence from several species with the complete coding regions (CDS) and corresponding amino acids were analyzed, and the results were compared between species. All the data was obtained from GenBank. Aligning was done using the Clustal W program which is a part of BioEdit (version 7.0.5). We used DnaSP in order to analyze average number of nucleotide differences, haplotype diversity (H_d), the nucleotide diversity (p), synonymous and nonsynonimous nucleotide diversity, polymorphic site (S), parsimony informative sites (PIP) and singleton variable site (SP). Average number of nucleotide substitution per site between species was also calculated. Also, informational entropy was determined. The phylogenetic tree among species based on the ClpP was constructed using the unweighted pair group method with the arithmetic mean (UPGMA) implemented in Mega 3.1 software.

P58.S4

Reflection mathematical model of the radial artery blood pressure waveform

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Age-related changes in blood vessels affect the pulse wave propagation. These changes may cause an increase in wave reflection and lead to the amplification of the pulse pressure. The pulse pressure changes are associated with several vascular diseases. Here we present a mathematical model of blood flow and blood pressure for different age groups. The model is based on the transmission line theory and assumes that the pressure waveform is a superposition of the forward propagating wave and the backward waves from several reflection sites. This approach enables the identification and separation of the reflected waves that originate from two different sources: [1] arterial branching and [2] terminal resistance. The model is built on experimental data acquired by direct measurement of radial artery blood flow.

The results of the mathematical model demonstrate a clear difference in the pulse-pressure waveform shapes between the three examined age-related groups: young, middle-age and elderly. These results fit well with the relevant radial pressure waveform data known from the literature. Application of the model to the direct blood flow measurements data, with proper adjustments of the reflected coefficient value or terminal resistance, enables the real-time pressure waveform monitoring. Therefore, the mathematical prediction of the arterial pressure waveform is a novel tool which can improve the biophysical interpretation of arterial hemodynamics.

P59.S4

Marine diatom cells and extracellular polymers: nanostructure and nanomechanics

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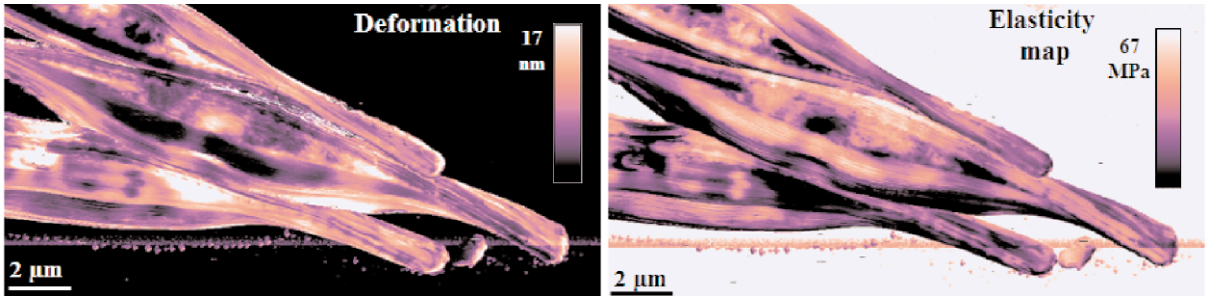
Atomic Force Microscopy (AFM) connects the nanometer and micrometer length scales utilizing a sharp probe tip that senses interatomic forces acting between the surface of a sample and the atoms at the apex of the tip. The physical basis behind AFM and its ability to „feel" the surface, make AFM a versatile tool in biophysics allowing high resolution imaging, nanomechanical characterization and measurements of inter and intramolecular forces in living and non-living structures. The potential of AFM as a tool in marine ecology will be presented. The nanomechanical properties (elasticity and deformation) of a weakly silicified marine diatom *Cylindrotheca closterium* were characterized using a novel AFM imaging technique, Peak Force Tapping [1]. The nanomechanical properties were measured over the entire surface of live cell in seawater and revealed nanostructure was related to the cell wall function. Diatom extracellular polymers were visualized as fine fibrils forming networks down to the molecular level using a high resolution imaging [2, 3]. The single molecule force spectroscopy was applied to probe the mechanical response of the polysaccharide fibrils and to quantify inter and intramolecular forces within the network. A preliminary data will be presented.



Quantitative nanomechanical mapping of marine diatom *Cylindrotheca closterium* in seawater using Peak Force Tapping AFM

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P60.S4

Modeling the time-delay between cortisol and ACTH in HPA axis under glucocorticoid perturbations

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The hypothalamic-pituitary-adrenal (HPA) axis is a neuroendocrine system involved in maintaining homeostasis under basal and various stressful conditions. Concentrations of HPA axis principal hormones, adrenocorticotropin (ACTH) and cortisol, exhibit complex nonlinear evolution, with ultradian oscillations superimposed on the circadian ones, but with a time-delay between the corresponding oscillation phases of the two hormones. The glucocorticoids, a type of anti-inflammatory and immunosuppressive cortisol-derived drugs, can significantly affect many dynamical variables of the HPA axis. The study of their influence can be notably supported by modeling HPA dynamics with stoichiometric reaction models. In that regard, we investigate in numerical simulations of the four-dimensional stoichiometric model of HPA axis activity the effects of cortisol pulse perturbations (simulate the glucocorticoid influence), focusing specifically on one of the HPA axis' dynamical variables, the time-delay between cortisol and ACTH. Our results suggest that glucocorticoids affect this time-lag in a nonlinear fashion, depending on their concentration and the oscillatory phase of cortisol at the moment of their application. These results might be indicative for setting further improvements of glucocorticoid therapy administration designs, where the combined effect of both drug dosage and administration timing relative to a patient's HPA axis' dynamical status should be acknowledged more cautiously.

P61.S4

Optically-driven microtools for microfluidic and biological applications

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The preparation of specialized tools in the micrometer size range for applications from physics to biology is a rapidly expanding field. One of the most effective and flexible method to make these structures is two-photon polymerization (TPP). With TPP practically any 3D structure can be made with even 100nm resolution and with the size of tens of micrometers. The transparency of the used materials enables the microtools to be trapped by optical tweezers thereby providing them with precise movement and positioning. The relative ease of the chemical modification of the TPP materials' surface broadens substantially their application spectrum. For instance, hydrodynamic synchronization of rotating micro objects in water such as bacterial flagella can be modeled with specially-shaped microstructures. With careful design they can be shaped into portable micro light-guides for fluorescence excitation. Coating their surface with proteins, practically of any kind, enables their association with biological surfaces, such as cell membranes. When coated with metal nanoparticles, localized metal enhanced fluorescence measurements can be performed with them on fluorescent surfaces.

P62.S4

Visual analysis of astorcytes in vitro using AFM

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Atomic force microscopy (AFM) is proven as an essential tool for the analysis of biological systems, due to its unique abilities. AFM lends itself to investigate the topological surfaces of biological objects, from whole cells to protein particulates, and can also be used to determine physical properties such as Young's modulus, stiffness, molecular bond strength, surface friction, and many more. When applied under appropriate operating conditions, AFM is less destructive to the biological specimens than the high-resolution electron microscopy. It allows operation in the liquid environment, as well as direct observation of *in vivo* dynamics of biological systems. In this work AFM technique operating in semi-contact mode has been employed for investigation of actrocyte membrane surface topography. Primary cultures of astrocytes were prepared from postnatal (2-day old) Sprague-Dawley rats (transgenic hSOD (G93A) and their non transgenic littermates. The presence of mutation was confirmed by PCR. Since the measurements were done in air, special preparation of the samples was needed. Astrocytes were fixed for 30 min. with 2.5% glutaraldehyde in Ca^{2+} and Mg^{2+} free Earle's balanced salt solution containing 10.6mM sodium citrate. After extensive washing, cells were dehydrated by addition of increasing concentrations of ethanol (50%, 70% and 95% each for 5 minutes) and air dried. Topography images of the cell surface and their corresponding phase images were obtained for both transgenic hSOD cultures of astrocytes and their non transgenic littermates. Preliminary results show no difference in the topography images of transgenic and non transgenic cultures, whilst a slight difference in phase images exists.

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P63.S5

Texture image analysis fallowed by multivariate analysis of textural parameters

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The differential diagnosis between benign and malignant causes of vertebral compression fracture is a common clinical problem. It has been reported that both malignant and acute benign fractures may give rise the pattern of to low T1 and high T2 signal on MRI. This is attributed to increased focal water content resulting from hemorrhage and edema. After the acute stage, hematoma and edema decrease, resulting in a low to intermediate signal intensity on T2-weighted images. However, in malignant fractures, the infiltrated abnormal tissues and associated reactive response continue to show the low T1 and high T2 signal patterns. These characteristic have been reported to be helpful in differentiating between benign and malignant fractures. Characterization and classification of fractures could be performed by well known Haralick texture analysis. Major improvement lays in further analysis of histogramized textural parameters based on multivariate techniques. Such an approach provides trustful classification applicable in clinical practice.

P64.S5

Human serum albumin as a biomarker for cancer. An EPR spin-labeling study revisited

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Human serum albumin (HSA) has a potential to be used as a biomarker for various medical conditions. Binding of certain metabolites to HSA, such as proteins secreted from cancer cells, results in the modification of its structure and function, which in turn leads to altered HSA binding capacity for fatty acids. These conformational changes can be studied by the EPR spin-labeling method. Current literature data suggests that the 16-doxyl stearic acid (16-DS) spin-label should be used for this purpose due to its structural congruence with HSA. However, other studies have shown that due to the position of the nitroxide group at the terminal part of the FA chain, 16-DS may not be the best choice. In order to resolve this discrepancy, this study evaluates the use of two spin labels, 5-DS and 16-DS, with nitroxide groups attached at different positions on the fatty acid chain, and consequently with different abilities to fit into hydrophobic pockets of HSA. The intensity of the EPR signal related to different HSA binding sites has been correlated with the HSA/spin-label concentration ratio, temperature and pH. Based on the results, it is possible to propose optimal experimental conditions in which the EPR spin-labeling method should be carried out. Furthermore, HSA from patients with locally advanced breast cancer (LABC) and healthy individuals was analyzed by this method. The results indicate that HSA may be a promising biomarker for LABC detection and prognosis.

P65.S5

LDL-dextran: a new delivery system for hydrophobic photosensitizers

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Low-density lipoproteins (LDL), a natural *in vivo* carrier of cholesterol in the vascular system, play a key role in the delivery of hydrophobic photosensitizer (pts) to tumor cells in PDT, including hypericin (Hyp), potent natural pts. To make this delivery system even more efficient, we have constructed a nano-delivery system by coating of LDL surface by dextran. Fluorescence spectroscopy, confocal fluorescence imaging, stopped- flow experiments and flow-cytometry were used to characterize redistribution of Hyp loaded in LDL/dextran complex to free LDL molecules as well as to monitor cellular uptake of Hyp by U87-MG cells. Dynamic light scattering was also used to characterize the size of the complex. It was shown than the redistribution process of Hyp between LDL molecules is significantly suppressed by dextran coating of LDL surface. The modification of LDL molecules by dextran does not inhibit their recognition by cellular LDL receptors and U-87 MG cellular uptake of Hyp loaded in LDL/dextran complex appears to be similar to that one observed for Hyp transported by unmodified LDL particles.

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P66.S5

Genetic polymorphisms of dopamine D2 receptors in Parkinson's disease

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Introduction: Genes encoding proteins involved in dopaminergic transmission have been of special interest during the evaluation of candidate genes for Parkinson's disease (PD). The dopamine D2 receptor (DRD2) gene has been proposed as a candidate gene underlying several psychiatric and neurologic disorders.

The aim of the study was to examine if selected polymorphisms in the DRD2 gene are associated with Parkinson's disease (PD). We determined the allelic frequencies for two polymorphisms located in the DRD2 gene in a sample of 51 patients with PD and 100 normal control subjects.

Methodology: DNA was extracted from whole blood using standard techniques. TaqIA and -141C Ins/Del genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), using known primers and conditions.

Results: No significant difference was observed in the allelic frequencies between patients with PD and control subjects with regard to the -141C Ins/Del variant. On the contrary, the A1 allele of the TaqIA polymorphism was more frequent in patients with PD than in control subjects (control subjects: TaqIA A1 = 10,6%, patients with PD: TaqIA A1 = 22,3%).

Conclusions: Genetic variation in the DRD2 gene may influence the risk of developing PD, thus confirming that the DRD2 gene is a susceptibility locus for PD.

P67.S5

Measurement of putamen volume in transsexual subjects using MRI anatomic images

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Physiological, morphometric and volumetric changes in brain structures of transsexual subjects has been an object of a number of studies. However, general consensus about their extent and localization has not been achieved yet. The aim of this work was to trace possible differences in volumes in putamen in male-to-female and female-to-male transsexuals compared to the volumes of the same structure of the brain in control subjects.

Study included 10 transsexual subjects and 10 age-matched controls. MR scans were performed at Siemens Avanto 1.5 T using MPRAGE and 3D-FLAIR sequences. Image analysis was performed using MIPAV software (NIH, Bethesda, USA). Manual delineation, segmentation of right and left putamen was done, followed by determination of corresponding volumes.

Significant difference was found between volumes of left and right putamen within group female-to-male subjects. Further, significant difference was found between same parameters determined for transsexuals and control subjects. Opposite to this, neither difference were found within group neither of male-to-female subjects nor in comparison with controls.

The results obtained in our study pinpoint to change in volume of putamen in female to male transsexual subjects, which is in agreement with assumed association of putamen with transsexual identity. The observed lack of significant differences for male to female subjects may be consequence of small number of subjects included in study.

P68.S5

Radioprotective effect of a novel thiol-based compound

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Numerous thiols have been tested in radiation protection; however majority of them were toxic and therefore excluded as candidates for clinical treatments. A newly synthesized thiol compound, GL2011, has been shown to be non-toxic to rodents and was tested here for its radioprotective capabilities. Male Wistar rats (around 200 g) were injected intra peritoneally (100 mg/kg) with GL2011 solution in phosphate buffer (PBS) at 30 min prior to and 3 and 7 hours after irradiation. Animals were irradiated using 60-Co gamma ray source at the dose of 6.7 Gy. Four groups of 30 animals were analyzed: two irradiated groups (GL2011 and PBS injected) and two non-irradiated groups (GL2011 and PBS injected). Survival was assessed at four weeks after irradiation and was 30% in the untreated group and 87% in the treated (protected) group. GL2011 did not have an effect on survival in the non-irradiated group. Cryptogenic assay of small intestines showed a significant decrease in the total number of villi in irradiated unprotected animals as compared to the control, while GL2011- protected animals retained total number of villi intact. Assay of the femoral bone marrow cells showed significant reduction of the number of nucleated cells in unprotected animals, while after GL2011 protection their number was similar to the controls. The same result was obtained with the colony-forming unit fibroblast assay. Based on all these data it appears that GL2011 is a potent radioprotector and further research on dosage schemes and mechanism of action are warranted.

P69.S5

N-gram analysis of prokaryotic genomes: characteristics and predictions of pathological islands

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Bacterial genomes were shown to contain parts of various lenght that differ in base composition (GC frequency, codon usage, signature profile) and gene content (novel genes that may contribute to bacterial adaptation), that are probably aquired by horizintal gene transfer and are designated as genome islands (GI). GIs taht contain a variety of virulence factors and contribute to bacterial pahtogenecity are designated as Pathogenic islands (PAIs). In order to predict and characterize PAIs more precisely, we introduced n-gram analisis in addition to other compositional features analysis. Results obtained include: binary sequence classification into PAI candidate and others, model based PAIs prediction. Our predictions were also compared with the results of others (as in PAI DB), and impruves relative precision significantly (up to 60% as compared to PAI DB). It was demonstarted that union of all compositional features results in maximum recall up to 37%. Thus, n-gram analysis significantly impruves PAI perdictons and may be used alongside exsisting and newly developed methods.

P70.S5

Gene delivery using novel nanocarrier based on comb-like copolymer of DMAEMA-VEP

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Gene delivery is widely used in molecular biology, as well as in biotechnology and medicine. The development of efficient and safe systems for gene transfer into target cells is of crucial importance. Although significant progress has been achieved in using nanosized systems for overcoming biological barriers at gene delivery, several drawbacks still exist. Novel comb-like copolymers of DMAEMA-VEP (BG-2) was synthesized by controlled radical co-polymerization. We demonstrated that such nanocomposite can bind DNA efficiently, and 0.01% BG-2 per 1μg DNA was defined to be optimal ratio. It was found that BG-2 protected DNA of degradation by the DNase I used in dose of 0.1 U per lμg DNA. BG-2 was not only capable of forming stable and compact complexes with DNA, but it was also efficient in crossing membrane barrier due to its high lipophilic activity. BG-2 demonstrated lower cytotoxicity comparing PEI and Lipofectamine, and it was not mutagenic. BG-2-DNA complexes were successfully delivered to human HEK-293T cells. It should be noted that in our experiments the transfection efficiency of BG-2 was 3-7 times higher than that of PEI and Lipofectamine. Besides, BG-2 provide 2-5 times higher transformation efficiency for yeasts *Hansenula polymorpha* and *P. pastoris* than the electroporation and 17-62 times higher than Li/Ac method. Thus, the results of our study showed that novel DMAEMA-VEP nanoscaled polymer BG-2 can be considered as a perspective non-viral gene delivery system.

P71.S5

An optical microscopy study of a thrombolytic efficiency of rt-PA and plasmin in an in vitro flow system

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In the past few decades, studies of thrombolytic therapy were focused mostly on plasminogen activators (PAs) among which recombinant tissue-type plasmin activator (rt-PA) is now in a routine clinical use. rt-PA is an efficient thrombolytic agent, however, its activity is not limited only to the site of a blood clot even if administered locally. Often, as a side effect, application of rt-PA may result in bleedings at sites of hemostatic plugs (1). Therefore, rt-PA may not be considered as a safe thrombolytic agent. In this study, rt-PA was compared to plasmin, i.e., a thrombolytic agent with only a local thrombolytic effect. Results of studies on animal models already confirmed that plasmin is much safer thrombolytic agent than rt-PA (2), because its thrombolytic activity is rapidly neutralized in the circulation system by plasmin inhibitors. For the same reason, plasmin has to be administered locally at the site of a blood clot.

Model blood clots were first exposed to a thrombolytic agent, either rt-PA or plasmin in equimolar concentrations, for 10 min. This was followed by a slow flow plasma perfusion causing removal of clot fragments. This process was dynamically imaged by a digital camera connected to an optical microscope. Acquired images were analyzed for a non-dissolved blood clot area as a function of time thus obtaining blood clot dissolution curves.

Results of the study showed that blood clots swell a little during the incubation period due to chemical reaction of the thrombolytic agent with the clot, while plasma perfusion initiates mechanical degradation of the clot. The degradation is most intense in the first two minutes and then slows in the case of plasmin reaching plateau, while the degradation continues with almost unreduced rate in the case of rt-PA (Fig. 1). A sudden reduction of plasmin thrombolytic activity indicates its neutralization by its inhibitors in plasma. No reduction of thrombolytic activity with rt-PA was observed due to uninterrupted supply of plasminogen and its activation to plasmin by rt-PA bound to fibrin of the clot.

The study provides an additional evidence for plasmin potential to combine thrombolytic efficiency with minimal bleeding side effects.

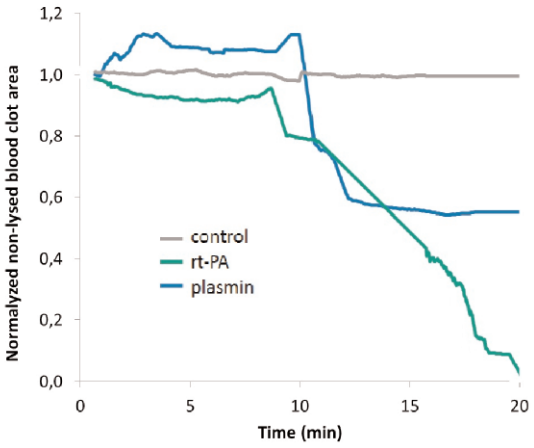

Figure 1: *Clot dissolution curves.*

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P72.S5

Mathematical modelling in aspirin induced asthma

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In 10-20% of asthmatic patients ingestion of aspirin and other non steroidal anti-inflammatory drugs (NSAIDs) induces bronchoconstricton, which is usually accompanied also with other clinical signs of inflammation of upper and lower airways. This is known as aspirin induced asthma or aspirin intolerance and represents a mayor clinical problem. The main event that lead to bronchoconstriction in aspirin-intolerant asthmatic patients seems to be disturbed balance between synthesis of cisteinil leukotriene C_4 (LTC_4) and anti-inflammatory prostaglandin E_2 (PGE_2) during the inhibitory action of NSAIDs on the enzymes prostaglandin H synthases 1 and 2 (PGHS1 and PGHS2) in arachidonic acid metabolism. The risk of bronchoconstriction is increased if concentration of LTC_4 is higher than concentration of PGE_2. It is hypothesized that such situations could occur due to the altered expressions of the enzymes PGHS1, PGHS2 and leukotriene C_4 synthase in aspirin-intolerant asthmatic patients. In this contribution, mathematical model of arachidonic acid metabolism is elaborated, which considers also the inhibiton of PGHS1 and PGHS2 by NSAIDs. The ratio between PGE_2 and LTC_4 concentrations is used as the criterion in predictions of bronchoconstriction. We predict the limiting doses of different NSAIDs that could induce bronchoconstriction for three populations of aspirin-intolerant patients. The strategy for safe managing of NSAIDs to aspirin-intolerant patients is also proposed and evaluated.

P73.S5

Diffusion weighted MR imaging of breast tumors: the effect of cellularity on diffusion in fibroadenomas

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Diffusion weighted MR imaging (DWI) has been suggested as a new technique which can improve the specificity in distinguishing malignant from benign tumors of the breast. It has been shown that DWI is better than standard MRI techniques; however values of apparent diffusion coefficient (ADC) still overlap between malignant and benign tumors especially in small ones. This implies that changes in cellularity during tumor growth might be responsible for corresponding changes in ADC. The purpose of this work is to examine the correlation between ADC values in fibroadenomas of various sizes and their histology. Thirty-two cases of breast fibroadenomas were examined on a Siemens Avanto 1.5 T scanner using a single-shot echo-planar imaging with 7 different gradient strenght (*b*) values. Internal structure of tumors were analysed on eosin hematoxylin stained slides, scanned with LCD-35 digital microscope and then analized with ImageJ software. ADC values increased from 1.15 (typical for malignant) to 1.85 mm^2/s (typical for benign) with increasing tumor volume (0.5 – 31 cm^3) for b = 1300, while they were in the range 1.85 – 2.0 mm^2/s for b = 200. Based on histology, a mathematical model where cell membranes represent a barrier for unhindered movement of water molecules was developed and results fitted nicely measured ADC values. The shape and size of internal barriers for water diffusion are related to the tumor size and this has to be taken into account when interpreting DWI results.

P74.S6

Mathematical model of Raspberry drying

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The drying kinetics of raspberry in a laboratory dryer was studied. The logarithmic model was found to be the best model for describing the characteristics of raspberry, regarding correlation coefficient (r^2). The changes of color on raspberry's surface were determined by digital camera. A mathematical model to predict the shrinking of geometrical bodies was proposed, assuming unidirectional drying and two-dimensional shrinkage. The model was numerically solved by finite differences, taking into account a convective term in the mass balance equation, which appears as a consequence of non-unidirectional shrinkage. Thermal analysis, by means of differential scanning calorimetry (DSC) and thermogravimetry (TGA) of fresh and dried raspberries have been performed. DSC scans were conducted in temperature range from -90°C to 400°C, with heating rate Hr=5C°/min, and TGA scans were performed in temperature range of 25°C to 900°C with heating rate Hr=5C°/min. From obtained results differences in thermal stability of fresh and dried raspberries were shown.

Keywords: Raspberry; Convective drying; Mathematical model; Digital image analysis; Shrinking; Thermal analysis

P75.S6

Pork meat osmotic dehydratation process-artificial neural network model

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Mass transfer of pork meat *(M. triceps brachii)* cubes, shaped as 1x1x1 cm, during osmotic dehydration (OD), and under atmospheric pressure was investigated in this paper. The effects of different parameters, such as concentration of sugar beet molasses (60–80%, w/w), temperature (20–50°C), and captivation time (1-5 hours) in terms of water loss (*WL*), solid gain (*SG*), final dry matter content *(DM)* and water activity *(a_W)* were investigated, using experimental results. An artificial neural network (ANN) model was developed for the prediction of *WL*, *SG*, *DM* and a_W in OD of pork meat cubes. These models were able to predict process outputs with r^2 of 0.952 for *SG* and r^2 0.980 for *WL*. The wide range of processing variables considered for the formulation of these models, and their easy implementation in a spreadsheet calculus make it very useful and practical for process design and control.

Key words: Mass transfer, Osmotic dehydration, Pork meat, Sugar beet molasses, Neural network

P76.S6

Different osmotic solutions efficiensy for pork meat osmotic dehydration

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Pork meat (*M. triceps brachii*) was dehydrated in three different osmotic solutions (sugar beet molasses, ternary solution and combination of these solutions in a 1:1 ratio) under atmospheric pressure, at room temperature T (20°C), with manual stirring every 15 minutes, to analyze the efficiency of mass transfer kinetics during osmotic dehydration (OD). The most significant changes of observed kinetic parameters were: water loss (WL), solid gain (SG), weight reduction (WR), and also rate of water loss (RWL), rate of solid gain (RSG), rate of weight reduction (RWR) and dehydration efficiency index (EI). The values of these parameters were determined after 1, 3 or 5 hours of OD. Also, final dry matter content (DM) and water activity (a_W) were measured. The optimum osmotic solution seems to be sugar beet molasses, and the OD conditions were determined by response surface method (RSM), by superimposing the contour plots of each process variable.

Key words: Osmotic dehydration, pork meat, mass transfer kinetic, sugar beet molasses, ternary osmotic solution

P77.S6

Antimicrobial properties of mushroom juice

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The antibacterial activity in some kinds of mushrooms is thought to be due to the high content of beta-glucans. The aim of this work was to obtain the shiitake mushroom juice and to show its long stability due to absence of microorganisms. The basic technological operation was just freezing and unfreezing at room temperature, with complete absence of heating, whereby a large quantity of native juice was formed and separated from pulp using filter press and centrifuge. Both the native juice and the pulp had a high content of antimicrobial components, which was confirmed by standard microbiological analysis. On agar plates with shiitake juice and pulp respectively, the absence of bacteria *Salmonellae, Coagulase positive staphylococcae, Sulphite reducing clostridia, Proteus specia* and *Escherichia coli* was confirmed. Further, there was no need to pasteurize or sterilize them to obtain a final product. As the microbiological stability of these products has been confirmed, they could be applied as a dietary product or a functional food component.

P78.S6

Positive effects of physical and chemical processes on the content of bioactive components in berry fruits

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The aim of this work was to introduce some physical processes during the fruit processing in order to obtain the higher content of bioactive compounds in the final products. As the final products, the fruit jellies were chosen, whereby their production was done on two different manners. The first type of jellies was produced with traditional procedure, which included high temperatures and long cooking, and the comparative products described in this paper were made on the contrary in the low temperatures regime and short time intervals. After the jellies were prepared, the polyphenols were extracted with methanol, their content was measured with Folin-Ciocalteau reagent and expressed as mg gallic acid equivalent (GAE) / 1 g of original sample. The obtained results for three kinds of fruits clearly showed the enhancement of total polyphenols content in the new developed products. The key contribution of the used methods is, among the other things, the positive effect of physical processes on enhancement of bioactive compounds (polyphenols) in final products due to the absence of their denaturation and degradation.

P79.S6

Thermal analysis of fresh and osmotically dehydrated pork muscle proteins

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The advantage of the DSC method is that it can be used in complex mixtures and at high concentrations of proteins, which is the situation occurring in muscle. A typical curve from thermal transitions found in a muscle is composed of three major transition zones. The first transition displays its maximum between 54°C and 58°C and has been attributed to myosin the second transition, which occurs between 65°C and 67°C, was assigned to collagen and to sarcoplasmic proteins. The third transition has been assigned to actin and is found between 80°C and 83°C. This work examines effects of sugar beat molasses used in the process of osmotic dehydration on meat protein stability. Thermal denaturation of the muscle proteins was studied by means of a TA Instruments DSC Q1000 Differential Scanning Calorimeter and weight loss by thermogravimetry TA Q500 termogravimetric analyzer (TA Instruments, USA) under N2 purge flow of 50ml/min and 60ml/min respectively.) DSC scans were conducted in temperature range from 3°C to150 °C, and from -80 °C to 180°C with heating rate Hr=5C°/min, and TGA scans were performed in temperature range of 25°C to 900°C with heating rate Hr=5C°/min. Decreased enthalpy (ΔH), and temperature maximum (T_m) of protein denaturation suggest that destabilization of meat proteins and conformational changes have been induced by process of osmotic dehydratation.

P80.S6

Thermal behavior of raspberry and balackberry seed flour followed by MDSC

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Thermal behavior of red raspberry (*Rubus ideaus*) and blackberry *(Rubus fruticosus)* seed flours have been followed by Modulated Differential Clorimetry (MDSC) and Termogravimetric analysis (TGA). MDSC allows the separation of the (total) heat flow signal into the heat capacity (reversible heat flow) and the kinetics components (non-reversible heat flow). MDSC and TGA of blackberry and raspberry seed flours have been performed on TA Instruments DSC Q 1000, differential scanning calorimeter and TGA measurements on TA Instruments TGA Q 500 thermogravimetric analyzer under N2 purge flow of 50ml/min and 60ml/min respectively. MDSC scans were conducted in temperature range from -90°C to150°C, with heating rate Hr=5C°/min with modulation of ±0.50 °C amplitude and 40s period of modulation. TGA scans were performed in temperature range of 25°C to 700°C with heating rate Hr=5C°/min. Total DSC curve is characterized with overlapping effect in low-temperature region caused by the freezing and unfreezing of large-amplitude motion. It was shown that thermal transitions, observed in the range of −80 to −10 °C were independent on water content, and they were mainly attributed to lipid melting transitions. Broad endothermic peak with T_m at about 93°C corresponds to protein denaturation. Using MDSC, the reversing and nonreversing curves, thermal events in the low temperature region of seed flour have been rougly separated, suggesting existence of two thermal processes.

P81.S6

Thermal analysis of actinidin

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The digestive properties of kiwifruit extract have generally been attributed to its cysteine protease actinidin. Therefore actinidin could potentially be utilized as a suplement for enhacement of food digestion. The aim of this study was to evaluate the thermal stability of actinidin. Actinidin was isolated from kiwifruit under native conditions. The differential scanning calorimetry of actinidin was carried out on a MicroCal MC-2 (Micro Cal Inc., USA) differential scanning calorimeter, using a standard DA – 2 software package for data acquisition and Origin software for DSC data analysis (non-two-state curve fitting model for estimating thermodynamic parameters of protein unfolding: temperature of transition maximum (T_m), calorimetric enthalpy (DH^{cal}), and van't Hoff enthalpy (DH^{VH}) were used). Thermal denaturation experiments showed that native actinidin represents a compact protein structure, with one transition maximum temperature (T_m) at 73.9°C. Due to its thermal stability actinidin can be considered for suplementation of certain food products.

P82.S6

Epigallocatechin-3-gallate binds via non-covalent interactions to several major food allergens and induces conformational changes

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Non-covalent interactions od dietary proteins and polyphenols may influence protein conformation and solubility, mask antioxidant activity of polyphenols and thus affect biological activities of both proteins and polyphenols.

We analyzed binding of a major green tea catechin, epigallocatechin-3-gallate, (EGCG) to several food proteins of cow's milk (ALA), peanut (2S conglutins) and eggs (OVA). All tested globular proteins also represent a health risk to allergic individuals, being major allergens of the foods selected in this study.

EGCG binding and the effects of EGCG–protein complexation on the food proteins secondary structure, was analyzed by fluorescence quenching method, CD spectroscopy, docking analysis and microcalorimetry. IgE binding to non-covalent complexes of EGCG-allergens was examined in the test of basophil activation.

EGCG binds to all tested globular proteins via non-covalent interections of low affinity (10-4 M-1). Binding of EGCG induces conformational change and resulted in secondary structure changes in all tested proteins. Computational analysis demonstrated that EGCG binds to the surface residues of the proteins. Non-covalent complexes of EGCG and food allergens retained biological activity and were able to degranulate basophils of food allergic individuals.

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Catechin-enriched green tea supplements interfere with IL-4/IL-4 receptor signaling in human peripheral blood mononuclear cells

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Green tea catechins and epigallocatechin-3-gallate (EGCG) in particular, have many beneficial effects in immune system disorders. EGCG can also generate reactive oxygen species (ROS). ROS are involved in many signal transduction pathways, including IL-4/IL-4 receptor signalization. Evaluation of the effects of dietary catechin-enriched green tea supplements (GTC) on ROS generation in peripheral blood mononuclear cells (PBMC) and its possible interference with IL-4/IL-4R signaling are important giving the increasing use of pharmaceutical and dietary supplements. In human PBMC, especially monocytes, GTC increased intracellular ROS levels in vitro, as measured by ROS-sensitive fluorescent dye. No further augmentation of ROS in PBMC occurred upon addition of IL-4. GTC and EGCG inhibited STAT6 (Y641) phosphorylation by IL-4 in PBMC and downregulated expression of CD14 receptor on monocytes. IL-4 and GTC/EGCG did not act synergistically on downregulating CD14 receptor in isolated human monocytes.

Catechin-enriched supplements of green tea, and its major component EGCG, interfere with STAT6 phosphorylation by IL-4 in PBMC and affect functionality of monocytes. These biological activities of catechins can contribute to beneficial effects of green tea in immune system disorders.

Acknowledgment: The authors acknowledge support of the GA No. 172024 of the Ministry of Education and Science of the Republic of Serbia, COST Action FA1005 and FP7 RegPot project FCUB ERA GA No. 256716. The EC does not share responsibility for the content of the article.

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