Baltic Biophysics Conference

4th-5th October, 2018, Kaunas

ABSTRACT BOOK

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CONFERENCE PROGRAM:

Day 1 October 4, Vytautas Magnus University, Daukanto st. 28, Kaunas

13:00 – 14:00 Arrival and registration

14:00 – 14:15 Welcome address and introduction

Chairs: ***Saulius Šatkauskas, Daumantas Matulis***

14:15 – 15:00 ***Opening plenary lecture***. **Anthony Watts** (University of Oxford, Oxford, UK). *Peering into ligand binding sites of membrane bound targets: novel dynamics are functional determinants*

15:00 – 15:20 **Franz-Josef Meyer-Almes** (University of Applied Sciences, Darmstadt, Germany). *Why does drug-target binding kinetics matter?*

15:20 – 15:40 **Daumantas Matulis** (Vilnius University, Vilnius, Lithuania). *CA protein-ligand binding - Gibbs energies, enthalpies, entropies, and crystallographic structures for drug design*

15:40 – 16:00 **Mindaugas S. Venslauskas** (Vytautas Magnus University, Kaunas, Lithuania). *Fields, driving forces, cell poration and bioactive molecules delivery. A biophysical approach*

16:00 – 16:20 Coffee break

Chairs: ***Vytenis Arvydas Skeberdis, Janis Spigulis***

16:20 – 16:50 ***Plenary lecture***. **Gražvydas Lukinavičius** (Max Planck Institute for Biophysical Chemistry, Göttingen, Germany). *Biocompatible Probes for Imaging of Cellular Structures*

16:50 – 17:10 **Krzysztof Bryl** (Department of Physics and Biophysics, University of Warmia and Mazury, Olsztyn, Poland). *Can Purple membranes of Halobacterium Salinarum function as biosensors?*

17:10 – 17:30 **Rima Budvytytė** (Vilnius University, Vilnius, Lithuania). *Interaction of tethered bilayer membranes with beta-amyloid and s100a9 aggregates*

17:30 – 17:40 **Marijonas Tutkus** (State Research Institute Center for Physical Sciences and Technology, Vilnius, Lithuania). *Nanoscale platform for DNA - protein interaction studies at the single-molecule level*

17:40 – 19:20 Lithuanian Biophysical society member's meeting

19.30 Welcome reception and Social meeting

Day 2 October 5, Vytautas Magnus University, Daukanto st. 28, Kaunas

Chairs: ***Aidas Alaburda, Donatas Zigmantas***

9:00 – 9:30 ***Plenary lecture***. **Jorn Hounsgaard** (Department of Neuroscience, University of Copenhagen, Copenhagen, Denmark). *Making sense of Motoneurons*

09:30 – 09:50 **Armuntas Baginskas** (Lithuanian University of Health Sciences, Kaunas, Lithuania). *Muscarinic delayed inhibition of the recurrent excitation of the frog tectum column*

09:50 – 10:10 **Rapolas Žilionis** (Vilnius University, Vilnius, Lithuania). *Revisiting the composition of airway epithelium by single cell RNA sequencing*

10:10 – 10:30 **Visvaldas Kairys** (Vilnius University, Vilnius, Lithuania). *Analysis of the putative ligand binding to CARF domain of Csx1 protein using molecular docking and Molecular Dynamics simulations*

10:30 – 10:50 **Vytenis Arvydas Skeberdis** (Lithuanian University of Health Sciences, Kaunas, Lithuania). *Putative mechanism of Cx36 gap junction channel potentiation by short carbon chain alcohols*

10:50 – 11:00 **Lukas Gudaitis** (Lithuanian University of Health Sciences, Kaunas, Lithuania). *Influence of N-terminus amino acids on sensitivity of connexin-36 gap junctions to voltage gating*

11:00 – 11:20 Coffee break

Chairs: ***Artūras Žiemys, Gražvydas Lukinavičius***

11:20 – 11:50 ***Plenary lecture***. **Donatas Zigmantas** (Chemical Physics, Lund University, Lund, Sweden). *Light-harvesting processes in photosynthetic bacteria*

11:50 – 12:10 **Janis Spigulis** (University of Latvia, Riga, Latvia) In-vivo skin imaging techniques

12:10 – 12:30 **Julita Kulbacka** (Department of Molecular and Cellular Biology, Wroclaw Medical University, Wroclaw, Poland). *Enhancement methods in photodynamic therapy in vitro*

12:30 – 12:50 **Vida Mildažienė** (Vytautas Magnus University, Kaunas, Lithuania). *Pre-sowing treatment with cold plasma efficiently breaks seed dormancy*

12:50 – 13:00 **Tomas Drevinskas** (Vytautas Magnus University, Kaunas, Lithuania). *Instrumentation and Computations in Bio and Related Analytics*

13:00 – 14:00 Lunch

14:00 – 15:00 Poster Session and companies' exhibition

Chairs: ***Mindugas S. Venslauskas, Julita Kulbacka***

15:00 – 15:30 ***Plenary lecture***. **Artūras Žiemys** (Houston Methodist Research Institute, Houston, USA). *Progression-dependent transport heterogeneity in liver metastases*

15:30 – 15:50 **Vitaliy Khutoryanskiy** (University of Reading, Reading, UK). *Mucoadhesive interactions of polymers: from biophysics to pharmaceutical and food applications*

15:50 – 16:10 **Stine Krog Frandsen** (Center for Experimental Drug and Gene Electrotransfer (C*EDGE), Department of Clinical Oncology and Palliative Care, Zealand University Hospital, Roskilde, Denmark). *Calcium electroporation – a novel, low-cost anti-cancer treatment*

16:10 – 16:30 **Vitalij Novickij** (Vilnius Gediminas Technical University, Vilnius, Lithuania). *Feasibility of nanosecond electric fields for eradication of bacterial skin infections: in vivo*

16:30 – 16:50 **Arieh Ben-Naim** (The Hebrew University of Jerusalem, Jerusalem, Israel). *Hydrophobic-hydrophilic interactions in protein folding, protein-protein association and molecular recognition*

16:50 – 17:00 **Dominyka Dapkutė** (Biomedical Physics Laboratory, National Cancer Institute, Vilnius, Lithuania). *Stem cell-based delivery of theranostic nanoparticles to cancer cells*

17:00 – 17:20 ***Closing lecture***. **Dobilas Kirvelis** (Lithuanian Scientific Society, Vilnius, Lithuania). *Biophysics is becoming Bioengineering (in the 21st century)*

17:20 – 17:30 Closing and Award Ceremony

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ORAL PRESENTATIONS

PEERING INTO LIGAND BINDING SITES OF MEMBRANE BOUND TARGETS: NOVEL DYNAMICS ARE FUNCTIONAL DETERMINANTS

Anthony Watts
University of Oxford, Oxford, UK

It is now possible to resolve ligand (or drug) conformation, binding site environment and local dynamics within a membrane bound target at near physiological conditions in functionally competent natural membranes to inform design and mode of action, using solid state NMR approaches [1, 2]. This information is obtained by (non-perturbingly) isotopically (2H, 13C, 19F, 15N, 17O) labeling selective parts of either a ligand or the protein understudy, and observing the nucleus in non-crystalline, macromolecular complexes [3,4].

Conformational informational details come from precise (sub-Å, at <±0.5Å) distance measurements between defined sites within the bound ligand. Ligands with complex structure have differential mobility at their binding sites, which has implications for efficacy and (ant)agonist action. Substituted imidazole pyridines, for example, which inhibit the H+/K+-ATPase and have therapeutic use, are constrained in the imidazole moiety, but shows significant flexibility at the pyridine group [5] (see figure). It is this group which has a direct interaction with an aromatic (Phe198) residue, with the potential for □-electron sharing [6]. Similarly, the steroid moiety of ouabain undergoes motions which are similar to those of the target protein, the Na+/K+-ATPase, but the rhamnose undergoes a high degree of flexibility at fast rates of motions whilst interacting with Tyr198 [7]. For acetyl choline when bound in the nicotinic acetyl choline receptor (nAChR), the quaternary ammonium group undergoes fast rotation at an aromatic binding site, which is driven by thermal fluctuations which may be functionally significant [8]. Our current focus is on GPCRs, specifically the brain neurotensin receptor (NTS1) for which the structure (by single molecule cryo-EM) and ligand binding interactions are being studied [9 - 13].

References:

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WHY DOES DRUG-TARGET BINDING KINETICS MATTER?

Franz-Josef Meyer-Almes
University of Applied Sciences, Darmstadt, Germany

The importance of binding kinetics in terms of residence time and on-rate in drug discovery has been broadly accepted in the past few years. Furthermore, evidence has accumulated that the optimal binding mechanism of a drug to its target molecule is related to physiological efficacy as well as selectivity and thus drug safety. Homogeneous fluorescence-based binding assays have been shown to enable high throughput kinetics requiring only small amounts of protein. These assays can be used to elucidate even complex mechanisms of molecular recognition. A generalized approach is proposed that combines high quality kinetic and equilibrium data in an Integrated Global Fit analysis yielding the most probable binding mechanism. Arguments will be provided for the thesis that the relationship between quantitative kinetic and mechanistic information and chemical structures of active substances will serve as a valuable tool for drug optimization.

CA PROTEIN-LIGAND BINDING - GIBBS ENERGIES, ENTHALPIES, ENTROPIES, AND CRYSTALLOGRAPHIC STRUCTURES FOR DRUG DESIGN

Vaida Linkuvienė, Asta Zubrienė, Edita Čapkauskaitė, Vaida Paketurytė, Alexey Smirnov, Vytautas Petrauskas, Daumantas Matulis
Vilnius University, Vilnius, Lithuania

Target-based drug design is centered on the discovery of a most-strongly binding compound to a disease-causing protein. However, the binding affinity and the binding mechanism is usually interplay of highly compensating enthalpic and entropic contributions. Even homologous compounds that exhibit similar affinities often have significantly different enthalpies and entropies of binding. When high-resolution crystallographic structures are available for all compound complexes with the target protein, sometimes it is possible to assign these significant enthalpy and entropy differences to the behavior of the water molecules located at the compound-protein binding interface.

We have designed, synthesized and determined the binding thermodynamics of over 700 aromatic sulfonamides to the family of 12 human carbonic anhydrase (CA) isoforms. The proteins were cloned and expressed in bacterial and human cell cultures and affinity-purified in large quantity sufficient for isothermal titration calorimetry (ITC) and crystallography. The binding affinities were also determined by the thermal shift assay (FTSA, also termed ThermoFluor or differential scanning fluorimetry, DSF), a high-throughput method. The enthalpies and entropies of binding were determined by ITC, a medium throughput method, for a selection of compounds and CA isoforms. A correlation map between the compound chemical structures and the binding □G and □H was drawn. The map showed which structural features of the compounds generated the highest increments in exergonicity and exothermicity of compound binding. Furthermore, only some structural features were most useful in generating compounds that would

selectively bind to cancer-expressing CA isoforms, but would not bind to essential for life human CA isoforms. Over 60 X-ray crystal structures showed the position of compounds bound in the enzyme active center. ITC was essential technique that enabled the dissection of unknown contributions from linked reactions such as buffer protonation to the binding reaction. Only after the subtraction of pH-dependent buffer contribution to the enthalpy of binding, the intrinsic Gibbs energies and enthalpies of binding were obtained. All methods that determine the binding reaction, such as FTSA, ITC, SPR, thermophoresis, and enzymatic inhibition methods would provide only the observed thermodynamics of binding that is pH and buffer-dependent. It was important to calculate the true (intrinsic) parameters and use them in the structure-thermodynamics correlation maps.

FIELDS, DRIVING FORCES, CELL PORATION AND BIOACTIVE MOLECULES DELIVERY. A BIOPHYSICAL APPROACH

Mindaugas S. Venslauskas
Vytautas Magnus University, Kaunas, Lithuania

This study is aiming to discuss biophysical mechanisms of membrane electroporation (EP) evoked mainly by application conventional as well nanosecond supra electric fields (supra EP). Here, analysis of mechanisms of membrane electroporation is presented at the three quantitative modeling levels: i) planar bilayer lipid membrane (BLM), ii) monolayer and bilayer vesicles as well as iii) models of cell plasma membrane (PM) poration. Comparison of EP mechanisms similarity and differences in the above models argue that crucial issue in bioactive molecules (drugs, genes) effective delivery into the cells is the external field, E_{ext}, quantification in time that defines the following cell response appearing as field quantification in space expressed as a pattern of pores distribution in electroporated membrane. The assay also springs up two questions: i) what new unnoticed bioeffects are possible in between conventional and nanoseconds supra EP modes? ii) because in many cases in line with PM electroporation the endocytosis of penetrating molecules take place, a point naturally arises: what E_{ext}, quantification patterns really stipulate the start of endocytosis?

BIOCOMPATIBLE PROBES FOR IMAGING OF CELLULAR STRUCTURES

Gražvydas Lukinavičius
Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

The ideal fluorescent probe for bioimaging is bright, absorbs light at long wavelengths (> 600 nm) and can be flexibly implemented in living cells and in vivo. Typically, such probe consists of a fluorophore connected via a linker to a targeting moiety. The availability of targeting ligands is assured by a large number of studies aiming at the development of inhibitors for a wide range of biomolecules. However, the design of synthetic, highly biocompatible fluorophores has proven to be extremely difficult and is lagging behind. Recently, silicon-rhodamine was identified as a far red dye that can be specifically coupled to proteins,

lipids and nucleic acids using different techniques. Importantly, its high permeability and fluorogenic character permit imaging of proteins in living cells and tissues, while its brightness and photostability make it ideally suited for live-cell super-resolution microscopy. Further investigations resulted in identification of cell-permeable fluorophores spanning the whole visible spectrum.
One of the most intriguing and challenging structures to image in the cell is chromatin. This biopolymer, composed of DNA and proteins, contains all information of the functional cell. I foresee the creation of probes highlighting chromatin by combining newly developed fluorophores and available chromatin-interacting small molecules. Super-resolution fluorescence microscopy has sufficient resolving power to provide information about spatial chromatin organization in living cells. Living cells imaging has the potential to reveal how the protein-DNA complex is organized during various cellular processes like cell division, stress or electrical stimulation.

CAN PURPLE MEMBRANES OF HALOBACTERIUM SALINARUM FUNCTION AS BIOSENSORS?

Krzysztof Bryl
Department of Physics and Biophysics, University of Warmia and Mazury, Olsztyn, Poland

The membrane electrical changes in the purple membrane having different compositions in response to different alcohols were measured with the photoelectric method. Chemical modification of retinal (for example, proton substitution with fluorine or chloride atom) and modification of protein (aspartic acid substituted with asparagine) enhanced the responses of the purple membrane to chemicals examined. The experiments were performed under extreme environment conditions (harmful for conventional enzymatic biosensors) such as high or low pH, high temperature, and high salt concentration. The construction of biosensor with purple membranes as sensing elements that can be applied in biotechnological processes for alcohol detection will be also presented.

INTERACTION OF TETHERED BILAYER MEMBRANES WITH BETA-AMYLOID AND S100A9 AGGREGATES

Budvytytė Rima, Smirnovas Vytautas, Lösche Mathias, Valinčius Gintaras
Vilnius University, Life Sciences Center, Vilnius, Lithuania

A central event in pathogenesis of Alzheimer's diseases are thought to be intracellular and extracellular accumulation, aggregation and misfolding of low molecular mass peptides such as β-amyloid (Aβ1-42), tau protein (Tau) and s100A9. Small size aggregates-oligomers were found to be extremely neurotoxic in vitro and in vivo with the ability to disrupt the major neuron membranes and lead to synaptic dysfunction, mithochondrial dysfunction, neuronal apoptosis and brain damage. In this work different sizes of soluble recombinant Aβ1-42 and S100A9 aggregates were used to investigate their interaction with tethered phospholipid membranes (tBLM). Aβ1-42 aggregates were obtained without fluorinated compounds derivatives or solvents, e.g., dimethylsulfoxide, HFIP. The morphology and size of misfolded

protein aggregates (Aβ1-42 and S100A9) were monitored by dynamic light scattering (DLS) and atomic force microscopy (AFM). These protein aggregates exhibited the membrane damaging properties as probed by the electrochemical impedance spectroscopy (EIS). The function and morphology of misfolded proteins was depending on different oligomerisation conditions. Amyloid induced membrane conductance exhibited relatively weak temperature dependence. The effective activation energy of the ion transport through the membrane (which contains sphingomyelin) defects exhibited values below 10 kJ/mol, which is consistent with physical picture of the water-filled pores formed by pore forming toxins. Membrane composition was found to be one of the important factors affecting the interaction of the Aβ1-42 and s100A9 oligomers to phospholipid membranes.

NANOSCALE PLATFORM FOR DNA - PROTEIN INTERACTION STUDIES AT THE SINGLE-MOLECULE LEVEL

Marijonas Tutkus, Tomas Rakickas, Aurimas Kopūstas, Šarūnė Ivanovaitė, Oskaras Venckus, Vytautas Navikas, Mindaugas Zaremba, Elena Manakova, Ramūnas Valiokas
State Research Institute Center for Physical Sciences and Technology, Vilnius, Lithuania

Most of the single-molecule microscopy techniques require immobilization of molecules of interest to study them for an extended period. Therefore, it is essential to employ such immobilization strategies that would ensure the unperturbed functionality of the studied molecules and minimize nonspecific interactions. In addition to that, some immobilization strategies can provide extra information because of employed surface structuration strategies or possibility to manipulate the immobilized molecules mechanically. DNA curtains assay is a recently developed method allowing anchoring of DNA fragments on the supported lipid bilayer and extending them horizontally, using the using the buffer flow, along with mechanical barriers arranged perpendicularly to the flow. Here we present a new strategy for immobilization of biotinylated DNA on nano-fabricated streptavidin structures on the PEGylated glass surface, that allows monitoring of hundreds of single DNA molecules extended along the buffer flow. Streptavidin surface structures were produced using liftoff printing with a structured Si template (structures were inscribed by nano-grafting with an atomic force microscope). This method provides vast versatility because it can produce any shape and dimensions pattern of streptavidin structures and also it is relatively cheap and fast. Reliable surface passivation against nonspecific interactions is ensured by glass surface modification with a PEG monolayer containing methoxy-PEG and biotin-PEG derivatives. Difference between previously developed DNA curtains assay and currently presented is several folds: A) DNA molecules are immobilized on a stable streptavidin structures, which are more biologically friendly than the chromium barriers, B) without the buffer flow the immobilized molecules remains in a constant position, C) possibility to employ

wide variety of chemical modification strategies previously developed for self-assembling monolayers. We believe that methods to fabricate the nanoscale platform presented here may be widely applicable in any single-molecule fluorescence laboratories.

MAKING SENSE OF MOTONEURONS

Jorn Hounsgaard
Department of Neuroscience, University of Copenhagen, Copenhagen, Denmark

The transduction of synaptic input into impulse activity is the physical substrate for information processing in nerve cells. For this reason synaptic integration in the intercalated dendrites between synapses and the axon is at the center in any attempt to understand the cellular basis of brain function. In recent years many diverse electrical and chemical signaling processes activated by synaptic activity have been analyzed in great detail in isolated preparations (slices, cultures etc). However, during functional network activity thousands of synaptic terminals distributed over the dendrites of a neuron are active in parallel. Since synaptic signals interact it is not possible to predict the outcome of integration from their properties in isolation. This situation has left a set of basic questions unanswered. I have used the spinal motoneuron as a model for experimental inquiries.

MUSCARINIC DELAYED INHIBITION OF THE RECURRENT EXCITATION OF THE FROG TECTUM COLUMN

Armuntas Baginskas, Antanas Kuras, Artūras Grigaliūnas
Lithuanian University of Health Sciences, Kaunas, Lithuania

We are presenting results of investigation of cholinergic modulation of recurrent excitatory synaptic transmission in the frog tectum. Experiments were done in vivo on common grass frogs *Rana temporaria*.

Tectum is a main centre of the frog brain for processing of the optical information. Ganglion cells of the eye's retina send their axons to the tectum, where they make up synaptic connections with the tectum neurons. Axon of a single ganglion cell (also called an individual retinotectal or optic fiber) makes up connections with a group of tectum neurons, which comprise a functional unit of the tectum called tectum column. The tectum column consists of five recurrent pear-shaped neurons, one efferent pyramidal neuron, and two inhibitory interneurons. The experimental procedure lets to stimulate a single retina ganglion cell or its axon and, consequently, record an activity of the tectum column.

The pear-shaped neurons of the tectum column form a positive feedback by making up axo-dendritic and/or dendro-dedritic excitatory synapses with the efferent pyramidal neuron and the other pear-shaped neurons of the tectum column. Transmission at these synapses causes recurrent excitation of the tectum column (the positive feedback).

Results of the experiments have demonstrated that the recurrent excitatory synaptic transmission is modulated by the endogyneous acetylcholine. The acetylcholine, co-released during a burst of the retinotectal fiber, activates muscarinic

receptors located (presynaptically and/or postsynaptically) in the recurrent synapses made up by the pear-shaped neurones. The activation of muscarinic acetylcholine receptors leads to a depression of recurrent excitatory potentials more than 2 times (2.21 ± 0.07 times, n = 25). This muscarinic inhibition of the recurrent excitation occurs with a delay of ~80 ms (79 ± 1 ms, n = 25)

An application of the muscarinic receptor antagonist atropine has cancelled delayed muscarinic inhibition of the recurrent excitation and led to an increase of the intrinsic activity of the tectum column. During a perfusion with 50 μM solution of the atropine, the magnitude and duration of a slow negative wave, which is indicative of the intrinsic activity of the tectum column, increased from 82 ± 4.9 μV and 141 ± 2 ms to 98.6 ± 5.2 μV and 191 ± 11 ms, n = 7. Thus, the delayed inhibition of the recurrent excitation takes part in controlling of the activity of the tectum column by relieving its return to a resting state.

REVISITING THE COMPOSITION OF AIRWAY EPITHELIUM BY SINGLE CELL RNA SEQUENCING

Rapolas Žilionis
Institute of Biotechnology, Vilnius University, Vilnius, Lithuania

Single cell RNA sequencing (scRNAseq) has now established itself as a powerful analytical tool providing rich and unbiased molecular descriptions of individual cells, and inviting scientists to better appreciate the composition of heterogeneous cellular systems. In my talk I will discuss inDrops, a droplet-based high-throughput scRNAseq method [1,2], and how its application to the airway epithelium let to the discovery of an intriguing new cell type with potential relevance to cystic fibrosis [3].

References

[1] Cell 161(5),1187-1201 (2015).
[2] Nat. Protoc. 12(1), 44-73 (2017).
[3] Nature 560(7718), 377–381 (2018).

ANALYSIS OF THE PUTATIVE LIGAND BINDING TO CARF DOMAIN OF CSX1 PROTEIN USING MOLECULAR DOCKING AND MOLECULAR DYNAMICS SIMULATIONS

Visvaldas Kairys, Česlovas Venclovas
Institute of Biotechnology, Vilnius University, Vilnius, Lithuania

Csx1 and Csm6 proteins are encoded by the genes linked to the type III CRISPR-Cas systems. Cyclic oligonucleotides were shown to be activators of Csx1 ribonuclease activity in Csx1 and related Csm6 proteins [1,2], but the binding mode and the mechanism of the allosteric action of the ligand binding is not known. We explored possible locations of the cyclic (AMP)4 ligand binding on CARF domain surface by docking of ligand fragments to the experimentally solved structures. The best fit between the putative c-(AMP)4 ligand and the protein was found for the Csx1 proteins. After the geometry of the ligand bound to the Csx1 protein was constructed, the resulting structure was subjected to 200 ns GROMACS [3] Molecular Dynamics (MD) simulations. Analogous simulations were also performed without the bound ligand. The liganded and unliganded Csx1 MD simulation trajectories were compared using Bio3D analysis tools [4], revealing striking differences in the protein dynamics.

References:
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PUTATIVE MECHANISM OF CX36 GAP JUNCTION CHANNEL POTENTIATION BY SHORT CARBON CHAIN ALCOHOLS

Vytenis Arvydas Skeberdis
Lithuanian University of Health Sciences, Kaunas, Lithuania

Short carbon chain n-alcohols (up to octanol) stimulate while long carbon chain n-alcohols inhibit the conductance of connexin 36 (Cx36) gap junction (GJ) channels. In contrast, GJ channels composed of other types of Cxs all are inhibited by n-alcohols independently on their carbon chain length. To identify the putative structural domains of Cx36, responsible for the dual effect of n-alcohols, we performed structural modeling of Cx36 protein docking with hexanol and isoflurane that stimulated as well as nonanol and carbenoxolone that inhibited the conductance of Cx36 GJs and revealed their multiple common docking sites and a single pocket accessible only to hexanol and isoflurane. The pocket is located in the vicinity of three unique cysteine residues, namely C264 in the fourth, and C92 and C87 in the second transmembrane domain of the neighboring Cx36 subunits. To examine the hypothesis that disulfide bonding might be involved in the stimulatory effect of hexanol and isoflurane, we generated cysteine substitutions in Cx36 and demonstrated by a dual whole-cell patch-clamp technique that in HeLa and N2A cells either of these mutations reversed the stimulatory effect of hexanol and isoflurane to inhibitory one, typical of other Cxs that lack respective cysteines and a specific docking pocket for these compounds. Our findings suggest that the stimulatory effect of hexanol and isoflurane on Cx36 GJ conductance could be achieved by re-shuffling of the inter-subunit disulfide bond between C264 and C92 to the intra-subunit one between C264 and C87.

INFLUENCE OF N-TERMINUS AMINO ACIDS ON SENSITIVITY OF CONNEXIN-36 GAP JUNCTIONS TO VOLTAGE GATING

Lukas Gudaitis[1], Tadas Kraujalis[1,2], Lina Rimkutė[1], Kęstutis Maciūnas[1], Mindaugas Šnipas [1,3], Vytautas K. Verselis[1,4]
[1]Institute of Cardiology, Lithuanian University of Health Sciences, [2]Department of Applied Informatics, Kaunas University of Technology, [3]Department of Mathematical Modelling, Kaunas University of Technology, [4]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine

Connexin-36 (Cx36) is a protein, which mainly forms gap junctions (GJs) in neurons and in pancreatic beta cells. GJs provide electrical cell-to-cell communication as well as exchange of metabolites between cells. Electrical synapses in neurons formed by Cx36 are important for synchronization of neuronal networks and memory formation. In pancreas Cx36 plays significant role in secretion of insulin. GJs are sensitive to junctional voltage (Vj), therefore it

is important to understand mechanisms of GJs regulation by Vj in excitable cells. It is well documented that N-terminus amino acids of different Cxs contribute to Vj-sensitivity. Our earlier studies revealed that sensitivity of Cx36 GJs to Vj is highly dependent on intracellular magnesium concentrations ([Mg2+]i). Moreover, several studies suggest that N-terminus might be involved in regulation of GJs by intracellular pH (pHi), which could also affect the sensitivity to Vj.
In order to evaluate contribution of N-terminus amino acids to Vj-sensitivity of Cx36 GJs, we electrophysiologicaly measured dependence of GJ channel conductance (gj) on Vj in pancreas β cell insulinoma cell line, stably transfected with wild type (WT) Cx36 and its mutants. Additionaly, we assessed influence of different [Mg2+]i and pHi conditions on Vj-sensitivity. Results showed that the most prominent changes of Vj-sensitivity were determined by substitution of alanine-13 and histidine-18 to positive lysine (A13K and H18K, respectively), and deletion of H18 (delH18). A13K reduced sensitivity to Vj and highly reduced influence of [Mg2+]i on Vj-sensitivity as compared to WT Cx36. H18K increased sensitivity to Vj, which remained dependent on [Mg2+]i as compared to WT Cx36. DelH18 reduced Vj-sensitivity at low [Mg2+]i, however, it highly increased sensitivity to Vj at high [Mg2+]i and gained the steady state of gj at higher Vj values. The latter property is common among most of Cxs, but absent in WT Cx36. Substitution of glutamate-3 to glutamine (E3Q), deletion of glutamine-17 (delQ17) and delH18 were exclusive mutations, which reduced Vj-sensitivity at low pHi and high [Mg2+]i.
Our results suggest that introduction of positive charge at the 13th position of Cx36 may impede the access of Mg2+ ions to their binding site. Also, we conclude that amino acid at 18th position could be important for [Mg2+]i–dependent Vj-sensitivity. Measurements of Vj-sensitivity at acidic pHi revealed that amino acids at 3rd and 17th-18th positions possibly play an important role in regulation mechanisms by both [Mg2+]i and pHi.

LIGHT-HARVESTING PROCESSES IN PHOTOSYNTHETIC BACTERIA

Jakub Dostál, Erling Thyrhaug, Karel Žídek, Marcelo Alcocer, Kil Suk Kim, David Bína, Heiko Lokstein, Jakub Pšenčík, Donatas Zigmantas
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Green sulfur bacteria are photosynthetic organisms that can be found 100 meters below the sea level, where the number of photons available for light harvesting and utilization is extremely limited [1]. Thus, it is not surprising that these bacteria developed sophisticated light-harvesting structures. The photosynthetic unit in green sulphur bacteria consists of a gigantic light-harvesting antenna chlorosome, Fenna-Matthews-Olson (FMO) complexes and reaction centers.
To explore bacterial light harvesting we use two-dimensional electronic spectroscopy (2DES), a femtosecond spectroscopy technique, which allows for mapping energy transfer pathways through the energy landscape of any multichromophore system, including

bacterial photosynthetic complexes or even the whole photosynthetic units. Characterizing the energy level structure, as well as energy transfer pathways and rates lends to understanding of the remarkable energy transfer efficiency and robustness in photosynthetic organisms.
In the course of our studies we have determined the excitonic structure and energy transfer rates in chlorosome baseplate and showed that there are at least four excitonically coupled bacteriochlorophyll molecules [2]. By using polarization-controlled 2DES we resolved all excitonic level energies in the FMO complex, including elusive eight bacteriochlorophyll, and unraveled all the energy transfer pathways and rates [3]. In addition, we measured all major energy transfer pathways and determined functional connectivity of the photosynthetic unit of intact green sulfur bacteria cells [4]. Comparing the energy levels and energy transfer rates in the baseplate, FMO complex and reaction center raises an intriguing question regarding the light-harvesting strategies employed by photosynthetic bacteria.
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IN-VIVO SKIN IMAGING TECHNIQUES

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Three In-vivo skin imaging techniques will be briefly discussed:
- Multispectral imaging for remote skin chromophore distribution mapping;
- Fluorescence intensity, lifetime and photobleaching rate imaging for remote mapping of skin fluorophores;
- Photoplethysmography imaging for remote mapping of skin blood perfusion and monitoring of anaesthesia efficiency.

Methodology and prototype devices implementing the above-listed techniques have been developed in the Biophotonics laboratory, Institute of Atomic Physics and Spectroscopy and validated in clinical environment. Designs of the prototypes and the main clinical results will be presented.
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ENHANCEMENT METHODS IN PHOTODYNAMIC THERAPY IN VITRO

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Initiation of the photodynamic reaction (PDR) requires the application of a photosensitizer and its activation by laser light due to generation of free oxygen radicals. The radicals induce DNA destruction and, finally, the programmed cell death of the target tumor cells. However, high doses of the photosensitizer can cause uncontrolled photodamage, involving an uncontrolled release of biomolecules from the non-programed cell death into the extracellular space, initiating an inflammatory response in the surrounding tissue [1].

Our study concerns photodynamic reaction applied photosensitizers encapsulated in various types of polymeric micelles and solid lipid nanoparticles. Drug encapsulation reduces the effective concentrations of the photosensitizer by increasing its uptake by the cancer cells and finally decreasing the drug side effects. Moreover, larger nanocarriers delivery can be supported by electroporation (EP) method, if applied at optimal conditions. EP induces temporary cell membrane permeabilization and enhance a drug transport across the cell membrane. EP is highly selective approach because the permeabilization occurs only around the electrodes. Moreover, the electrodes provide minimally or non-invasive cancer treatment [2]. Additionally, photosensitizers encapsulation significantly enhances the drug uptake and the photodynamic effect. We show that photosensitizers may be combined and encapsulated with other bioactive substances, from the flavonoids group, and more efficiently delivered into the cancer cells with the electroporation process.

Concluding our study, the photodynamic approach still requires development to reduce its side-effects. The application of nanotechnology and biophysical methods to support the selective and faster photosensitizer uptake by a variety of cancer cells may significantly support any photodynamic procedures.

Acknowledgements

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PRE-SOWING TREATMENT WITH COLD PLASMA EFFICIENTLY BREAKS SEED DORMANCY

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The aim of this study was to evaluate effects of seed treatment with atmospheric dielectric barrier discharge cold plasma (DBD-CP) and capacitively coupled cold plasma (CC-CP) on phytohormone content in dry seeds and germination kinetics. The experiments were performed on common sunflower (Helianthus annuus), radish (Raphanus sativus) and red clover (Trifolium pratense) seeds. Germination and content of the main phytohormones controlling germination – abscisic acid (ABA) and gibberelins (GA7 and GA3) was estimated in dry seeds subjected to different durations of both treatments. Indices of germination dynamics – the final germination percentage Vi (%) and the median germination time Me (h), were determined using Richards plots. Morphometric seedling analysis was performed 4 or 12 days after sowing.

DBD-CP treatments strongly stimulated germination of Raphanus sativus seeds, but were less effective for H. annuus and T. pretense,

however, germination of T. pretense seeds was efficiently stimulated by CC-CP. Stimulating effects on germination were related to rapid and strong changes in the content of seed phytohormones, involved in the control of germination – the content of germination inhibitor abscisic acid (ABA) was reduced while the content of gibberellins (GA) was increased. ABA and GA amount changes are observed immediately after seed irradiation, however ABA/GA changes progressively developed with time. Stimulation of germination by both DBD-CP and CC-CP treatments on the molecular level can be explained by the induced changes in the ratio of the main phytohormones (ABA and GAs) involved in the control of seed dormancy and germination, i.e., plasma treatment is efficient dormancy breaking agent.

INSTRUMENTATION AND COMPUTATIONS IN BIO AND RELATED ANALYTICS

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Analytics in life- and bio-sciences is the main part of a scientific investigation process. High performance analysis can be achieved with the use of the edge-cutting instrumentation, or novel computation algorithms. The combination of both can even be used for reaching outstanding aims. One of the current trends in analytical life- and bio-sciences is autonomization. Autonomous instrumentation provides numerous advantages over classical laboratory bench-top hardware. On the other hand, autonomous and remote instrumentation have different problems that have to be solved. In this group of research works, multiple hardware and software improvements will be discussed.

Acknowledgements
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PROGRESSION-DEPENDENT TRANSPORT HETEROGENEITY IN LIVER METASTASES

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Metastatic disease is the major cause of mortality in most cancer patients. While many drug delivery strategies for anti-cancer therapeutics have been developed in pre-clinical studies using tumors in the primary site, the drug delivery properties of metastatic tumors have not been well investigated. Furthermore, therapeutic efficacy may hinge on efficient drug permeation into the tumor microenvironment, known to be heterogeneous, and thus potentially making drug permeation heterogeneous also. We have studied the effects of progression on local perfusion of metastases as well as local drug permeability of drugs by suing in vitro, in vivo, and in silico techniques. The results revealed the unfavorable and heterogeneous transport properties of 4T1 liver metastases.

Metastases perfusion analysis indicated that tumor progression can modulate drug vector biodistribution in tumor-associated capillaries.

Particles (drug vectors) shared 80-90% common flow volume, however different size particles have specific flow volume. Interestingly, the effects of hematocrit on specific circulation volume was opposite for small and large particles. The dysfunctional capillaries with no flow, appearing due to tumor progression, limited access to all particles, and diffusion was shown to be the only prevailing transport mechanism. In view of drug vector biodistribution inside tumors, with discarding formulation and other pharmacokinetic aspects, our results suggest that the evolution of tumor vasculature during tumor progression may influence efficiency of drug delivery.

Drug extravasation results in metastases are distinct from analogous studies with 4T1 tumors growing in the primary site. A probabilistic tumor population model was developed to estimate drug permeation efficiency and kinetics of liver metastases by integrating transport and structural properties of tumors and used drugs. The results demonstrate significant heterogeneity in metastases in terms of transport properties and doxorubicin delivery in the same animal model and even in the same organ. The results also suggest that the level of heterogeneity depends on the stage of tumor progression and that differences in transport properties can define transport-based phenotypes of tumors, i.e. transport phenotypes. Therefore, therapeutics can permeate and eliminate a certain transport phenotype of metastases sparing tumors with more challenging transport properties that render these surviving tumors resistant to repeated treatments in view of drug delivery. We anticipate that these results may challenge current paradigms of drug delivery into metastases and highlight potential caveats for therapies that may alter tumor perfusion.

MUCOADHESIVE INTERACTIONS OF POLYMERS: FROM BIOPHYSICS TO PHARMACEUTICAL AND FOOD APPLICATIONS

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Mucoadhesion is a phenomenon that is related to attractive interactions of various materials with mucosal surfaces in a human body. Mucoadhesive materials have been widely used in the design of drug delivery systems for transmucosal administration [1, 2]. Recently mucoadhesion was also recognised to have an effect on taste perception of certain food products and beverages [3]. This lecture will describe biophysical studies of interactions between various cationic and anionic polymers and mucins using isothermal titration calorimetry, turbidimetric titration, dynamic light scattering, transmission electron microscopy and zeta-potential measurements [4-6]. These studies facilitated rational design of various dosage forms for drug delivery [7-9]. Several biophysical in vitro and in vivo methods were developed and optimised to test the adhesion of various dosage forms (tablets, films, semi-solid and liquid formulations) on mucosal surfaces [9-12]. Application of these methods also provided understanding of the effects of mucoadhesive polymers on taste perception of certain food products [13, 14].

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CALCIUM ELECTROPORATION – A NOVEL, LOW-COST ANTI-CANCER TREATMENT

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Calcium electroporation is a potential novel anti-cancer treatment where supraphysiological calcium concentrations are introduced into cells by electroporation, a method where short, high voltage pulses induce a transient permeabilisation of the plasma membrane allowing passage of ions and molecules into the cytosol.
Calcium electroporation has been proven efficient in inducing cell death in vitro in several different cell lines [1] and tumor necrosis in vivo [2]. The first clinical trial was recently published showing that calcium electroporation is efficient on cutaneous metastases [3]. The mechanism behind calcium electroporation is not fully elucidated but it has been shown to be associated with ATP depletion and normal cells are less affected by the treatment than malignant cells in vitro and in vivo [2]. This difference in sensitivity between normal and malignant cells might be due to differences in the expression of the plasma membrane calcium ATPase and sodium calcium exchanger as well as differences in modulating the cytoskeleton organization [4].
Interestingly, it has recently been shown that calcium electroporation induces a systemic response in mice, where the mice were re-challenged with tumor cells 100 days after the calcium electroporation treatment and no tumors from the same cell line grew [5]. In the clinical trial with calcium electroporation, one malignant melanoma patient also had a systemic response with complete remission of untreated metastases [6]. These studies shows that calcium electroporation efficiently induce tumor cell death while sparing the normal surrounding tissue and studies indicate that this local treatment also induces a systemic immune response.
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FEASIBILITY OF NANOSECOND ELECTRIC FIELDS FOR ERADICATION OF BACTERIAL SKIN INFECTIONS: IN VIVO

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Skin infections by drug-resistant pathogens are frequent cause of morbidity and mortality. We present in vivo data of successful eradication of bacterial contamination (Pseudomonas aeruginosa) using high frequency nanosecond pulsed electric fields (PEF) in a murine model. The 15 kHz, 15 – 25 kV/cm 300 – 900 ns pulsing bursts were used separately and in combination with acetic acid (0.1–1%) to treat superficial bacterial contamination of the skin. The combination of PEF and acetic acid 1% treatment resulted in full eradication of the bacteria in the contaminated area. The presented methodology is useful for the development of new methods for treatment of extreme cases of wound infections, when the chemical treatment is no longer effective. Combination of the procedure with antimicrobials allows the treatment to become more effective and less painful and thus create a safety margin in energy control. Nanosecond range PEF pulses allow countering multiple limitations, which are experienced in conventional micro-millisecond range electroporation.

HYDROPHOBIC-HYDROPHILIC INTERACTIONS IN PROTEIN FOLDING, PROTEIN-PROTEIN ASSOCIATION AND MOLECULAR RECOGNITION

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Many biochemical processes such as protein folding or self-assembly of proteins are highly specific. Proteins fold spontaneously into a very specific 3-dimensional structure. Proteins also associate spontaneously to form multisubunit macromolecules. These processes occur in aqueous media. The question we pose is what is the role of water in these processes? Until recently it was believed that the hydrophobic effect, i.e. the tendency of hydrophobic groups to “escape” from the aqueous environment is the dominant driving force for these processes [1]. Analysis of all the solvent induced contributions to the thermodynamic driving forces for protein folding and protein-protein association reveals that, contrary to the commonly accepted paradigm, hydrophilic interactions might be more important than hydrophobic interactions [2]. This conclusion was reached after critically examining the data on the various contributions to the driving forces for protein folding, and protein-protein association. Examples on the role of hydrophilic interactions on solubility of proteins, protein folding, protein-protein association and molecular

recognition will be presented. Thus, hydrophilic interactions not only help in understanding the role of water in biochemical processes, but they can also be applies to design drugs that bind stronger to their targets [3,4].

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STEM CELL-BASED DELIVERY OF THERANOSTIC NANOPARTICLES TO CANCER CELLS

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Advances in nanotechnologies have provided the promising potential of cancer elimination. Owing to nanosize, nanomaterials gain exceptional physicochemical properties such as magnetic properties or bright luminescence, while large surface area enables conjugation of various diagnostic and therapeutic molecules. Such multifunctional nanoparticles gain increasing attention in oncology. However, nanoparticles themselves usually lack specificity to tumors. Therefore, cellular Trojan horses were proposed. Mesenchymal stem cells (MSCs) possess self-renewal and immunomodulatory properties and participate in wound healing, bone regeneration, angiogenesis, and homeostasis. Due to these characteristics, MSCs hold great promise in the treatment of wounds, degenerative diseases, and other pathologies. Moreover, MSCs have a tendency to migrate selectively toward tumor cells and thus could be used as nanoparticle delivery vehicles.

We were the first ones to investigate skin-derived MSCs in such theranostic delivery system. First, we tested tumor-tropic properties of quantum dot (QD)-loaded MSCs in both in vitro and in vivo, and proven MSC cancer-specific migration efficacy. To test the therapeutic potential, we have constructed a complex composed of QDs and a second-generation photosensitizer (PS) chlorin e6 (Ce6) to obtain multifunctional nanoparticles. QD-Ce6 complexes generate reactive oxygen species (ROS) without direct excitation of PS upon both linear and non-linear two-photon excitation, thus enabling excitation in optical tissue transparency window. The spectral properties, size, and zeta potential of QD-Ce6 complex were measured. The stability of the complex in cell culture media and the impact of serum proteins were evaluated. Finally, MSC and cancer cell response to QD-Ce6 complex were determined.

BIOPHYSICS IS BECOMING BIOENGINEERING (IN THE 21ST CENTURY)

Dobilas Kirvelis
Lietuvos Mokslininkų Sąjunga kb. LAISVIEJI

Today, in the 21st century, the science approach to the living world, life, and living organisms, especially in terms of the evolution of biological species, begin to see as to technology - organized systems of the technologies - from the positions of bio-cyber-engineering. The Living World is a continuous bioengineering creativity. Conceptually, biology is becoming technology, and physically – Vice Versa – technology is becoming biology (W. Brian Arthur, "The Nature of Technology", 2009). "Biology is the technology" announce Rober Carlson in the book (Harvard University Press, 2010). Because each biological species is a special bio-technological system to survive, i.e. successfully deal, effectively combat the entropy by bio-informatics technologies (genetics, hormones, and nerves) in certain ecological conditions. What, in the twentieth century, was called the System Biophysics, in the 21-st is recommended to call bioengineering, because engineering thinking is indispensable for both – in Synthetic Biology and in research for Analysis-by-Synthesis method.

Bioengineering is the creative application of biological sciences of technology, mathematical methods, and empirical evidence to the innovation, design, construction, operation and maintenance of structures, machines, materials, devices, systems, processes, and organizations. The discipline of engineering encompasses a broad range of more specialized fields of engineering, each with a more specific emphasis on particular areas of applied mathematics, applied science, and types of application. The term engineering is derived from the Latin ingenium, meaning „cleverness" and ingeniare, meaning "to contrive, devise".

Two decades ago the USA National Science Foundation (NSF) announced the an innovative Nano-Bio-Info-Cogno (NBIC) Convergence of Technologies program, which basically lies in the belief that future - Human enhancement technologies (HET) - must be developed and implemented in the field of living nano-technologies and principles. This NBIC2 program variation now is extended to 2030.

Decade ago in Harvard University came the HUB program - Bioengineering, Engineering Biology for the 21st Century, a Plan for Bioengineering at Harvard. Harvard has a unique opportunity to create a program that will define bioengineering for the 21st century. The Harvard University Bioengineering initiative to become a HUB and a worldwide focal point of pedagogy and collaborative and translational research of life scientists and engineers working together.

In 2012 the STOA of the European Parliamen discused program „ Making Perfect Life, European Governance Challenges in 21st Century Bio-engineering", have seen the emergence of a new engineering approach to life which is driven by an increasing convergence of the physical and the biological sciences in terms of two bio-engineering megatrends – „Biology is becoming Technology, and - Vice Versa - Technology is becoming Biology".

There is a need to look at life with the eyes of engineering – as to "machines" – therefore, the systems

biophysics is transforming into bioengineering, because the study of complex biosystems requires Analysis-by-Synthesis (Bionics and Systems Biology or Biophysics) methods. The „machine metaphor“ was proposed by René Descartes in the L'homme,1664. That’s has noticed that like any animal, so Man is a “machine” too. Therefore, there is a need to look at life with the eyes of engineering – as to “machines” - the systems biophysics is transforming into bioengineering, Descartes conceived of the universe as comprising three substances: res extensa (measurable things), res cogitans (thinking things), and God. Animals are automatons. The human bodies are like those of animals, but connected to a thinking thing (soul, after Descartes or information today) by way of the pineal gland. A healthy body is like a “well-made clock”. Thats has noticed that like any animal, so Man is a “machine”, made up of functionally purposeful rather than random components. The live “machines” requires a cybernetics or bioengineering approach.

It is worth pointing out, that in 2018 commemoration of the 250th anniversary prof. Vilnius University Jędrzej (Andrzej) Śniadecki, that, before ~ 215 years ago his Theory of Organic Beings and published books at the University of Vilnius with this title are the beginning of the Systems Biophysics not only in Lithuania. In 1904, Sniadecki claimed, that living organism are controlled by organic - organizing forces. Today, the organic forces, we call Gibbs free energy, and the organizing forces – as the information, as maintaining the system\'s operational order that is it control or management. Such a bio-cyber-physics, yet bionic-technological approach to Systems Biophysics at Vilnius University has been developing since 1962. Systems Biophysics as bioengineering is promoted in VILNIUS as the bioengineering Closed-Loop Coding-Decoding (CL-CD) concept. The cell as bioengineering CL-CD system (Synthetic Biology), as the first step to the biophys-engineering is also considered.

Today his Theory is trasforming to Bioengineering 21th century.

In recent years, the bioengineering approach has become particularly relevant after the Yuval Noah Harari 2015-16 bestseller “Homo Deus: A Brief History of Tomorrow”, which globally looks at the evolution of the living world and humanity and future transformations, seeing inevitably coming of the new era of the Earth - TRANSHUMANISM.

POSTER PRESENTATIONS

MULTISTAGE RESPONSE OF CELLS TO X-RAY EXPOSURE

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Ionizing radiation can cause cell damage indirectly by generating reactive oxygen species that interact with cell's DNA and other cellular macromolecular materials. In this study Chinese hamster ovary cells (CHO) were irradiated to the doses ranging from 0.5 to 10.0 Gy. 50μM/ml concentration of dichlorodihydrofluorescein diacetate (DCFH-DA) dye was used as a probe to monitor ROS generation inside the irradiated cells. COMET and Clonogenic assays were performed to assess DNA damage and cell viability dependence on irradiated dose. This paper covers results of evaluation and analysis of cellular DNA damage, post irradiative ROS production processes, assessment of relative mitotic arrest effect and cell viability after irradiation.

The linear dependence of DCF fluorescence intensity on delivered dose was evaluated one hour after irradiation. Cells irradiated to 4 and 8 Gy produced higher additional DCF fluorescence 3 and 6 h after irradiation, thus indicating different radiation induced effects in irradiated cells:radiation induced ROS generation and additional generation of secondary ROS related to cell mitotic arrest. Based on the performed investigation a dose threshold of 2-4Gy was suggested which allows differentiation between different stages of cell response to irradiation. COMET and clonogenic assay results support this observation. Cell irradiation at 2, 4, 6, and 8 Gy increased DNA damage to 5.1±0.6%, 24.8±2.4%, 32.0±3.2%, 45.6±3.5% respectively. Cell viability decreased to 82±5% after 2 Gy and to 48±1.8% after 4 Gy irradiation. That is the largest decrease in viability compared with 6, 8 and 10 Gy that led to viability decrease to 24±3.4%, 12±0.7% and 9±0.7% respectively.

PROTEOMICS OF POST-MITOTIC MIDBODY IDENTIFIED FACTORS REGULATING CELL DIVISION

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Cytokinesis is a final stage of cell division, during which the mother cell divides, leaving daughter cells connected by a thin intercellular bridge (ICB). Residing between the two daughter cells during cytokinesis is a microtubule and protein rich structure, known as midbody (MB). The MB is situated within ICB, with abscission usually occurring only on one side of the ICB. The MB has been well-studied for its role in recruiting abscission-regulating proteins during cytokinesis (Schiel et al., 2012), but its post-mitotic roles remain poorly understood. Not so long ago, the MB was thought to be discarded after

division by either releasing it into an extracellular space or by autophagosomal degradation. However, recent studies have shown that MBs can be retained and accumulate in stem and cancer cells after mitosis (Crowell et al., 2014). Thus, it has been proposed that post-mitotic MBs function as signalling platforms that regulate cell "stemness", as well as aggressiveness of cancer cells (Dionne LK and et al. 2017). For this reason, the function and fate of these MBs remains to be elusive and is the focus of this study. For that purpose, intact MBs were purified from HeLa cells and proteomic analysis was completed. Proteomic analysis identified over 600 proteins, including many known MB resident proteins, as well as 18 post-Golgi Rabs. Some of the Rab GTPases are known to mediate actin dynamics and endosome targeting at the abscission site. To begin with, dominant-negative mutants (SN) were overexpressed in cells to clarify Rabs' function during abscission. Next, shRNA (knock-down (KD)) and CRISPR/Cas9 (knock-out (KO)) were used to down regulate Rab14, a protein that was previously shown to play a key role in cell division. Finally, we tested the involvement of Rab14-interacting proteins in mediating abscission.

The study of the MB identified Rab14 as a novel regulator of cell division, since the down regulation of Rab14 disrupted cytokinesis and inhibited cell division. Also, Rab14-KD and -KO prolonged the time that cells require to divide, with the most noticeable difference seen in cells going from anaphase to abscission. Another Rab GTPase known as Rab11 was also shown to play a key role in cell division and was even capable of compensating for the loss of Rab14 function.

Based on all our data, we propose that Rab14 is a novel regulator of cytokinesis that functions by regulating the targeting of endosomes to the ICB during late telophase, and consequently, affecting the abscission of daughter cells.

EFFECT OF CELL PASSAGE TIME ON THE ELECTROTRANSFECTION EFFICIENCY

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An efficient gene transfer technique leads to subsequently improve the gene therapy protocols. Since last few decades, an electroporation has been a better alternative compared to the viral methods to be employed in in vitro and in vivo protocols. The mechanism is based on the formation of reversible pores in the cell by applying the short duration electric pulses in order to ease the injection of molecules. However, an optimal transfection efficiency without losing a significant number of cells is still a big concern for the researchers. Therefore, most of the research work has been based on the improvement of transfection efficiency.

In this work, the effect of cell passage time on the transfection efficiency of electroporation has been analyzed by considering the different plasmid concentrations. We observed the significant increase in the transfection efficiency for all plasmid

concentrations with 24 h cell passage time in comparison to 48 h cell passage time without a significant change in cell viability. These results are very useful in order to conduct future research for the improvement of elctrotransfection efficiency.

DEVELOPMENT OF MINIATURIZED CAPILLARY ZONE ELECTROPHORESIS EQUIPMENT FOR ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS DEGRADING ENZYMES

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In modern scientific research a lot of attention is devoted to miniaturization of chemical analysis equipment. Also in environmental protection research field more and more attention is devoted to development of new bio-remediation methods and technologies. One of emerging problems in environmental protection field is accumulation of out of use wooden railway sleepers. To protect wood form biological degradation, wooden railway sleepers are impregnated with creosote. Polycyclic aromatic hydrocarbons (PAH\'s) form a high proportion of creosote mass. A large number of PAH\'s is potential are carcinogenic compounds. The bio - remediation technology is based upon secretion of non - specific enzymes which are capable of degrading PAH\'s, produced by white root fungi. The aim of this research is to develop fully automated capillary zone electrophoresis equipment and optimize analysis conditions for effective analysis and identification of PAH's degrading enzymes. First step in this research is develop and manufacture fully automated capillary zone electrophoresis equipment coupled with contactless conductivity detector.

EXCITED STATE DYNAMICS OF PHOTOCHROMIC DIMETHYLDIHYDROPYRENE DERIVATIVES

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Molecular switches can be commonly applied to control different functions and properties of materials which can be used in organic electronics (for example in new memory elements based on single molecule) or in biology to manipulate biological systems. Photochromism of dimethyldihydropyrene (DHP) derivatives is a reversible transformation under UV and visible light irradiation between two, open-ring hexatriene and closed-ring cyclohexadiene, isomers with different spectroscopic properties.
New dimethyldihydropyrene derivatives were synthesized, and their optical properties as well as excited state dynamics were investigated in the solutions (ethanol, hexanol, octanol) using transient absorbtion experiment. We focus on the emissive properties of dimethyldihydropyrene derivatives with the aim to get more knowledge about the processes which appear during photo-chemical reaction.

STUDIES OF PLANT TRANSMEMBRANE LIGHT-HARVESTING PROTEIN COMPLEXES IN ARTIFICIAL AND NATURAL-LIKE ENVIRONMENT USING SINGLE MOLECULE FLUORESCENCE MICROSCOPY

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Photosynthetic organisms have developed extremely efficient systems for capturing light energy and directing it to reaction centres. But with powerful machinery comes great responsibility — single unmanaged excitations conjure reactive oxygen species, which cause costly damage to photosystem. Unpredictable fluctuations in light level require excellent control systems. To survive, photosynthetic organisms must collect only as much energy as possible to utilize. Leaves of plants are shown to respond to high illumination levels by initiating highly reversible non-photochemical quenching (NPQ). Currently the most supported explanation of NPQ is Horton aggregation model, according to which, composition of dissolved xanthophyl and proton gradient in thylakoid membrane induce various aggregation levels of light-harvesting comlexes (LHCII). Increased aggregation lengthen mean travel distance of excitation and due to its short life-time, reduce the probability of its arrival to reaction centre.
In this study Total Internal Reflection Fluorescence Microscopy (TIRFM) was employed to monitor single immobilized LHCII molecules in detergent solution, or in thylakoid mixture liposomes, resembling natural environment for this transmembrane protein.

ELECTROANALYTICAL METHODS FOR EVALUATION OF ELECTRIC FIELD EFFECTS ON YEAST CELLS

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For several decades, the budding yeasts (Saccharomyces cerevisiae) have been used in studies of basic phenomena of eukaryotic life and some of their fundamental properties. Yeast cells could also be used for whole-cell bioprocesses such as biocatalysis and recombinant protein fermentation, but natural barrier functions of the cell wall and cell membrane (envelope) often retards entry of substrates and release of products.
One of the possible techniques which could be used to improve permeability for target molecules is pulsed electric field (PEF), yet there is still a lack of sufficient data related to the effects of PEF on yeast cells. Here we employed yeast itself as an amperometric whole cell sensor for the investigation of pulsed electric fields effects on yeast cells and detection of lactic acid. For the analysis, PEF-treated cells were immobilized on carbon paste electrodes which were then immersed into solution with potassium ferricyanide acting as mediator and producing measurable currents through oxidizing electrode surface. Since mediator have positive charge, membranes of viable yeast cells are

only weakly permeable to them, thus, only small currents were detected after addition of lactic acid into solution. We varied electric field strength (E) of single pulses with duration of 300 μs. Currents obtained from amperometric whole cell sensor with treated cells were dependent on E and increased from 11 ± 2 nA (E = 0 kV/cm) up to 90 ± 10 nA (E = 15 ± 0.5 kV/cm). PEF effects on yeast cell wall were also evaluated by employing ion selective microelectrode sensitive to concentration of tetraphenylphosphonium ions (TPP+). Study of high power electrical pulses with nanosecond duration effects on yeast cells had demonstrated the following features: (i) potentiometry can be used to detect absorption rate of TPP+ ions and is an effective method for evaluating electric field effects on yeast cell wall; (ii) shortening of the electric pulse duration makes it possible to achieve more homogeneous electrical treatment of yeast cell clusters; (iii) exposure to PEF with nanosecond duration can enhance absorbtion rate of TPP+ ions (up to 65 times) without any influence on the vitality of the yeast cells.

We conclude that electroanalytical tools can be effectively used as a useful tool for investigation of various cellular responses after PEF treatment. After exposure to PEF, permeability of membranes as well as of cell walls was improved thus suggesting PEF as a tool for biocatalysis enhancement.

EFFECTS OF PULSED ELECTRIC FIELD ON MICROORGANISMS ISOLATED FROM HELIANTHUS TUBEROSUS L. TUBER

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Jerusalem artichoke (Helianthus tuberosus L.) is widely known crop plant with high nutritional value. In order to improve a shelf life, microbiological safety and reduce production costs, different preservation methods are being studied. In this study we investigate the effects of pulsed electric field (PEF) on bacterial cells isolated from Jerusalem artichoke tuber tissue. Microorganisms were isolated on selective media specific for nitrogen fixing bacteria. Morphological characterization showed that cells are motile, rod-shaped, Gram negative. For characterization of isolates' genotype 16S rRNA gene was amplified and sequenced. The sequence was compared with known 16S rRNA gene sequences in the GenBank database by multiple sequence alignment.

Exposure to PEF can cause reversible or irreversible electroporation of bacteria cells. Irreversible electroporation results in cells inactivation which was evaluated by the log reduction (LR). LR=-log (N⁄N_0), where N and N0 are number of colony forming units in treated and untreated suspensions respectively.

In order to evaluate PEF effect on LR of isolates', we used multiple pulses ($pn \leq 100$) with various electric field strengths ($E \leq 18$ kV/cm) and pulse durations ($\tau \leq 50$ μs). When pn = 100

and E = 14 ± 0.33 kV/cm, LR increased significantly and reached LR = 0.61 ± 0.04, when τ = 3 μs; 0.54 ± 0.34, when τ = 10 μs; LR = 1.08 ± 0.01, when τ = 50 μs. We also showed, that regardless of the pulse number, longer pulses cause more effective log reduction than shorter pulses. After exposure to stronger pulses (E = 18 kV/cm) with various pulse durations (τ = 3 μs; 10 μs; 50 μs), 100 pulses increased LR up to 0.59 ± 0.03; 0.7 ± 0.05; 1.33 ± 0.09 respectively.
We conclude that Helianthus tuberosus L. Tuber contains intratissual microorganisms which could contribute in its shelf life reduction. Analysis showed that isolate belongs to Rhizobium genus and phylogenetically is closest to R. pusense NRCPB10 strain. We also showed that exposure to PEF reduces the viability of such microorganisms in suspension. Log reduction increased with raise in electric field strength, pulse duration as well as pulse number.

MIXED SELF-ASSEMBLED MONOLAYERS WITH TERMINAL DEUTERATED ANCHORS: CHARACTERIZATION AND PROBING OF MODEL LIPID MEMBRANE FORMATION

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Self-assembled monolayers (SAMs) on solid surfaces are often used as supports for tethered bilayer lipid membranes (tBLMs). Such a platform is advantageous from the application point of view as it allows controlling the composition and geometry of the biomimetic assembly on the molecular scale. We describe herein a series of self-assembled monolayers (SAMs) on gold designed for adjustable tethering of model lipid membrane phases. The SAMs consist of fully deuterated aliphatic anchors, HS(CH2)15CONH(CH2CH2O)6CH2 CONH-X, where X is either –(CD2)7CD3 or –(CD2)15CD3, dispersed in a stable matrix of protein-repellent molecules, HS(CH2)15CONHCH2CH2OH. The mixed SAMs with variable surface densities of the anchors are thoroughly characterized by means of ellipsometry, contact angle goniometry, and infrared reflection-absorption spectroscopy (IRRAS). In all cases, the bottom portions of the mixed SAMs (i.e. the h-alkyl thiol segments of the molecules) are arranged in a highly ordered all-trans conformation stabilized by a network of lateral hydrogen bonds. The terminal portion of the anchors (the oligo(ethylene glycol) spacer and deuterated alkyl segments, respectively), however, possess less ordered conformations in the mixed composition regime. Two different scenarios of spatial distributions of anchoring molecules were observed. The short anchoring molecules are randomly distributed over the mixed SAM surface and does not segregate even at high surface densities (up to 40mol %). Contrary, the long anchor partially phase segregate and selfassociate on the surface, even at low surface densities of few mol %. Molecular dynamics simulations reproduced the above mentioned experimental findings and led to novel

detailed insights into the molecular structure of the differently composed SAMs. Despite of the observed structural differences of the mixed SAMs, the quartz crystal microbalance kinetics indicate that small unilamelar vesicles efficiently rupture and form a tBLMs on both type of surfaces. The developed anchoring SAM with well-controlled structural characteristics and composition is suitable for obtaining tBLMs that can be employed for advanced studies and applications of different cell membrane-mimicking assemblies.

OPPEL-KUNDT ILLUSION DECREASE AT ISOLUMINANCE

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The present experimental study reports a contribution of the real and imaginary contours to the Oppel-Kundt illusion manifestation. The illusion is known as the difference in visual estimating equidistantly distorted and undistorted-empty extent. In psychophysical experiments, the Oppel-Kundt stimuli of green color and varying luminance (0 cd/m2 to 4 cd/m2) were presented on the monitor screen against the red background of the constant luminance (0,5 cd/m2). The left stimulus interval (the reference) was fixed in length at 198 arc min and comprised 2, 3, or 4 filling stripes (3.6 × 36 arc min. in size). Also, a solid filling was used for the reference. The right stimulus interval (the test) was empty and varied in length according to the observers command while adjusting the perceived length equality between the two stimulus parts. Due to expansion effect, the subjects showed the test somewhat longer than the reference, and the errors made were considered the illusion strength values. For the stimuli with filing stripes, the illusion strength remained about constant (at various levels within 15–50 arc min. interval for different observers), when the stripe luminance varied, but the strength decreased nearly twice, when isoluminance was established. Thus, the experimental curves resembled a straight line with a distinct dimple at positions of the individual isoluminance. Contrarily, the dimple was absent on the relatively flat curves obtained by the stimuli with the solid filling. One may assume that the contours of the solid filling could be easily perceived by the observers at the presence of either the luminance or color contrast or both in the stimulus. The illusory contours of the stripe sequences could be sensed, when the color stripes possessed the luminance contrast in addition. But neither real nor illusory contours could be induced perceptually by a pure chromatic contrast of the stripes. This was, probably, the reason why the illusion decreased in magnitude for the isoluminant stripe stimuli. To confirm the assumption, the Kanizsa triangle illusion was tested in experiments. The observers reported an imaginary triangle arising in the stimulus of two colors, when the luminance contrast was present. The illusory triangle contours became weak if any, when the luminance contrast was eliminated and the stimulus turned into an isoluminant pattern. The results obtained support the hypothesis that the Oppel-Kundt expansion effect is inevitably influenced by integrity of

excitations along the real or imaginary borders of the observational objects.

NON-HOMOGENEOUS DISTRIBUTION OF TRPM6 IN THE HUMAN HEART

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Two members of the transient receptor potential channels of the melastatin subfamily, TRPM6 and TRPM7, have been identified as magnesium transporters. Originally, TRPM6 was thought to be essential for systemic (whole-body) magnesium homeostasis with high levels of expression seen in the lung, colon and renal tubules. Whereas, TRPM7 appears to be ubiquitously expressed, with highest expression in tissues such as the heart, and is thought to be involved in cellular magnesium homeostasis. Notwithstanding, very recent genetic studies demonstrated co-expressing TRPM6 and TRPM7 in numerous cell types. However, due to some conflicting data in literature, it still remains to be determined whether TRPM6/7 is expressed in cardiomyocytes, and specifically of the human heart.

Recently, using electrophysiological and immunofluorescence methods, we have identified TRPM7 channels in both human atrial and ventricular cardiomyocytes of patients with ischaemic cardiomyopathy (ICM), and showed an elevated TRPM7 protein expression in cardiomyocytes derived from patients with history of myocardial infarction (MI).

Here, we demonstrate that in cardiomyocytes from ICM patients the TRPM6 protein is expressed also. By applying a highly specific TRPM6 polyclonal antibody, we detected the presence of TRPM6 proteins mainly on the surface membrane of atrial and ventricular cardiomyocytes. The quantitative analysis revealed that TRPM6 expression (in a.u.) was higher in both the atrial and ventricular cardiomyocytes from ICM patients (0.27±0.03 and 0.16±0.03, respectively, n=21-29), comparing with such data obtained from patients without ICM (0.20±0.08 and 0.07±0.02, respectively, n=3, p>0.05). In addition, TRPM6 expression in cardiomyocytes, similarly as with TRPM7, was greater from patients with history of MI than without such diagnosis (0.30±0.05 vs. 0.22±0.03 in atrial cells (n=12-17) and 0.20±0.04 vs. 0.13±0.03 in ventricular cells, respectively, n=6-12).

Our results provide first evidence that TRPM6 protein is present in atrial as well as in ventricular cardiomyocytes of the human heart, alongside with the TRPM7, known to be expressed in the heart. TRPM6 was found inhomogeneously distributed in the membrane and also a preferential localization in a perinuclear domain, and this suggests an interaction of the channel with certain cytoskeletal components. We suggest that TRPM6 co-localization with TRPM7 channels, possibly by forming heteromeres with TRPM7, could confer changes in membrane proteins of cardiomyocytes from patients with ICM. TRPM6 protein level was elevated markedly in cardiomyocytes from patients with history of MI, known to be related to myocardial fibrosis.

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TWO-WAVELENGHT EXCITED MULTILAYER LANTHANIDES-DOPED NANOPARTICLES FOR CANCER THERANOSTICS

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Recently properties of various nanoparticles and their application possibilities in medicine were investigated. Breakthrough in the field was made when rare earth doped upconverting nanoparticles were introduced. These nanoparticles can absorb and emit light in tissue optical transparency windows; also, they can emit ultraviolet (UV) light upon near-infrared (NIR) excitation (via process of upconversion), which can be used for activation of photo-drugs. Here, we show that rare earth metal ions doped nanoparticles can be used to create advanced tools for both cancer diagnostics and therapy – theranostic nanoparticles. These nanoparticles under 806 nm irradiation behave as high-contrast NIR-to-NIR optical markers and can be used to visualize the area of interest without any therapeutic effect. On the contrary, under 980 nm NIR irradiation, upconversion process is proceeded and UV/visible light is emitted, which can be used to excite photo-drug coupled to the nanoparticle and as a consequence initiate photochemical processes, causing damage to cancer cells.

The aim of our study was to evaluate possibilities of multilayer decoupled LiYF4:Tm3+,Yb3+@LiYF4@LiYF4:Nd3+ nanoparticles (dNPs) and photosensitizer chlorine e6 (Ce6) complex in early cancer diagnostics and photosensitized tumor therapy. In this work dNPs emission spectra were measured upon 806 nm and 980 nm excitation to prove that we have NIR downshifting emission under heating-free 806 nm excitation and UV and blue radiation when dNPs are excited by the therapeutic 980 nm light. Spectral changes in Ce6 absorption spectrum were detected after dNDs-Ce6 complex was formed as the environment of Ce6 molecules has changed. LDH toxicity assay revealed that dNPs and dNPs-Ce6 complex had no dark toxicity for MDA-MB-231 and MCF-7 breast cancer cells. Also accumulation of dNPs and dNPs-Ce6 complex in MDA-MB-231 and MCF-7 cells was observed. It was shown that after incubation with dNPs-Ce6 complex and irradiation with 980 nm laser the cells have died, whereas irradiation with 806 nm laser had no toxic effect. Experiments with MDA-MB-231 spheroid cell cultures revealed that dNPs and dNPs-Ce6 complex have accumulated only in the periphery of spheroid and have not penetrated into the central part of the spheroid. Only the dNPs-Ce6 complex under 980 nm light induced damage to MDA-MB-

231 spheroid, while none effect was observed when the same complex was excited by the therapy-independent 806 nm light.
Overall, these dNPs represent a new class of theranostic agents in which the therapy and diagnostics are not prompted simultaneously, but rather on-demand, hence potentially increasing the safety and versatility of such nanostructures in the future.

THIADIAZOLE-BASED HSP90 INHIBITORS AS ANTI-PROTOZOAN LEAD COMPOUNDS

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Parasitic protozoan diseases, such as malaria and leishmaniasis, cause severe morbidity and mortality across the globe. The death-count stays high due to lack of effective cures, emergence of resistance, and severe side-effects of the existing drugs. To develop novel anti-protozoan compounds, we selected protozoan Hsp90 as the druggable target. Hsp90 is a molecular chaperone required for correct folding and/or function of a number of essential cellular proteins. It is vital for protozoan infection and life cycles, so Hsp90 inhibition would lead to termination of the parasites. In order to select for suitable compounds, we are screening thiadiazole-based Hsp90 inhibitors against protozoan Hsp90 proteins using a thermodynamics approach.

WHY BENZENESULFONAMIDES BIND TO CARBONIC ANHYDRASES WITH SUCH A LARGE GIBBS ENERGY CHANGE?

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Some synthetic chemical compounds such as primary sulfonamides bind to their target carbonic anhydrase (CA) protein with an extraordinary Gibbs energy change of over 70 kJ/mol. Here we will discuss the contributions of sulfonamide and other groups to the energy change. Carbonic anhydrases are metalloenzymes, which catalyze reversible CO2 hydration into bicarbonate and hydrogen ion. There are 12 catalytically active isoforms of CA in human body. Overexpression of particular CA isoforms has linkage to certain disorders. For example, CA IX is usually found in cancer tissue where hypoxic environment is present [1]. Consequently, selective inhibition of CA IX isoform is thought to be able to reduce the progression of the disease. To this moment sulfonamides are the main class of CA inhibitors. However, their selectivity is usually not sufficient for the inhibition of a particular CA isoform. Therefore, it is important to understand the fundamentals why primary sulfonamides are great inhibitors by themselves and how their modification might be beneficial for the development of new, better pharmaceuticals. For this purpose, a series of secondary sulfonamides were synthesized in our laboratory, which were analogous to primary sulfonamides synthesized previously

[2,3]. Observed binding affinity was determined by fluorescent thermal shift assay and intrinsic binding affinity representing the binding of benzenesulfonamide anion to the Zn-bound water form of CA was calculated. The compound structure-intrinsic Gibbs energy correlation map showed the contribution of the coordination bond to the binding energetics. The high-resolution crystal structure of CA complexes with analogous secondary and primary sulfonamides revealed different orientations of the ligands in the active site of the protein providing some clues to our understanding of the large Gibbs energy change upon binding.

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MAGNETIC PARTICLES DECORATED WITH GOLD NANOCLUSTERS AS A POTENTIAL AGENT FOR CANCER THERANOSTICS

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For the last two decades magnetic nanoparticles have been in the spotlight of the great importance and have been widely used for various applications due to their specific characteristics and promising applications. Due to their multifunctional properties, magnetic nanoparticles decorated with gold nanoclusters can be implied and used in numerous applications such as biomedicine and cancer diagnostics and therapy (theranostics). In order to evaluate the possible implementations of these nanoparticles in nanomedicine a wide range of experiments have been conducted in this study. First, we assessed both optical and magnetic properties and tested colloidal stability before further experimentation in vitro. Uptake dynamics and biodistribution of magnetic nanoparticles decorated with gold nanoclusters at subcellular level were carried out using confocal fluorescence microscopy. Cytotoxicity assessment performed with various cell lines showed no cytotoxic effects. Thus, the results of this study highlights the promising potential of such, gold nanoclusters-decorated magnetic nanoparticles, for effective integration in bimodal diagnostics and therapy in cancer treatment.

STUDY OF THE INFLUENCE OF IRON IONS ON THE FLUORESCENCE OF CALCEIN AND CALCEIN BLUE

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The process of a temporal increase of the cell membrane permeability occurring due to the action of the pulses of strong electric field (up to 300 kV/cm) is called cell

electroporation. This process is widely used in cell biology, biotechnology, and medicine [1]. When high–voltage pulse is applied to the electrolyte solution the oxidation of metal ions of the anode occurs besides other electrolysis reactions. As a result of this, the dissolution of the anode takes place [2].
When metal ions released from the electrodes, they reacts with fluorescent molecules and reduce the intensity of their fluorescence [3, 4]. Stainless–steel is one of the most popular materials utilized for electrodes, which are used to electroporate the cells. In these conditions, iron ions (Fe2+ and Fe3+) are released from the anode under the action of high–voltage electric pulses.
In the present research, the impacts of iron ions on the fluorescence of calcein and calcein blue in three different media – distilled water, 0.9% NaCl and Dulbecco\'s Modified Eagle Medium (DMEM) – have been studied. The most suppressed fluorescence was found to be in 0.9% NaCl media and the least suppresed in DMEM media. For example, in 0.9% NaCl media only 0.1 mM of Fe2+/Fe3+ was required to fully suppress the fluorescence of calcein, while in the case of calcein blue, much higher concentration of iron ions - 1 mM - was required to get the same effect. The results of this reasearch can be useful when estimating the efficiency of cell electropermeabilization.
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UPCONVERTING NANOPARTICLES' COLLOIDAL STABILITY AND IMPACT OF THEIR SURFACE MODIFICATION ON THE CELLULAR ACCUMULATION

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Limitation of detection sensitivity of small cancer tumors along with harmful radiotracers usage in current bioimaging methods (e.g. magnetic resonance imaging) [1] gave rise to discovery of alternative nanoprobes termed as upconverting nanoparticles (UCNPs). UCNPs are novel multifunctional nanoparticles suitable for photodynamic therapy and optical imaging due to their ability to emit ultraviolet and visible light following near-infrared (NIR) excitation via process called upconversion. Also, they can emit light in the NIR region – coinciding with the biological optical transparency window (700-1200 nm). Additionally, UCNPs have excellent biocompatibility, high penetration depth of emitted light in biological tissues [2, 3]. Nonetheless, before UCNPs application to biological systems, fundamental investigation of their interaction with biological microenvironment and colloidal stability in different solutions is required [4, 5]. Nowadays there is a big knowledge gap on the investigation reports about how

biological environment affect nanoparticles and their behavior in cells [5].
The aim of this work was to investigate optical properties of bare and coated with citrate, SiO2 and phospholipids LiYF4:Yb3+, Tm3+ UCNPs as well as their biocompatibility, and accumulation in cells. Colloidal stability studies of four types of nanocrystals were performed in cell growth medium. High colloidal stability in medium supplemented with fetal bovine serum allowed to carry out further studies in human breast adenocarcinoma cell lines. Accumulation dynamics was investigated in cancer cells and results showed that citrate coated nanoparticles had higher accumulation rates and were colloidally stable in cell growth medium comparing to bare and aforementioned coated nanoparticles. Biocompatibility studies showed no toxicity of UCNPs towards cells. These results allow to conclude that coated UCNPs is better choice for future investigations in vivo than bare UCNPs.

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FILLED-SPACE ILLUSION CAUSED BY CONTINUOUS DISTRACTOR

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In the filled – space illusion, the filled part of stimuli seems longer in juxtaposition with the empty one. The aim of present study was to try to develop a quantitative model capable to account the illusory effects induced by stimuli with continuous and discrete distractors.
In present study, we examined the strength of the filled-space illusion as a function of degree of contextual continuous filling of the referential interval of the three-dot stimulus. In the first series of experiments, the length of the line segment (placed in the centre of the referential interval) varied from zero to complete filling of the interval. In the second one, the length of the segment was constant but the extension of the referential interval varied (the positions of the terminal spots of the referential interval moved aside from the line segment ends symmetrically, thus, forming the empty gaps and producing discontinuities in the filling). Subjects adjusted the length of the empty test interval to that of the reference, and the matching errors were considered as the illusion magnitude.
The data obtained showed regular overestimation of the referential interval with completeness of the interval filling. The dependencies established were used to develop a new quantitative model, which was successfully applied to fit the experimental results of the present study and those obtained earlier for conventional Oppel-Kundt stimuli.

It was demonstrated that the model calculations appropriately correspond to all variations of the illusion magnitude changes for stimuli with continuous and discrete distractors.

Influence of Iron Ions and Hydrogen Peroxide on the Fluorescence of Amplex Red Dye Quenching and CHO cells Viability

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To study cell membrane electropermeabilization, fluorescent probes is a convenient, sensitive and versatile tool [1]. During high–voltage electric pulses to cell suspension, cell membrane permeabilization and various electrochemical reactions occur at electrode–solution interfaces.These may include evolution of gases (H2, O2, Cl2), changes of pH, dissolution of electrodes (releasing metal ions), formation of hypochloride acid, free radicals, reactive oxygen species (ROS) [2]. Metal ions (iron, chromium, nickel, and manganese), released from the electrodes during a high-voltage pulse, and ROS such as hydrogen peroxide (H2O2) can react with the fluorescent dyes and quench their fluorescence [3, 4]. This may have an impact, when estimating the efficiency of cell electropermeabilization.

In this study, the influence of iron ions and hydrogen peroxide on the fluorescence of Amplex Red dye as well as on the viability of CHO cell was studied. With increasing the concentration of the iron ions in the solution the intensity of the fluorescence of Amplex Red decreased. For example, 1 mM of Fe3+ ions suppressed fluorescence of Amplex Red by 30–50%. Also the reduction of the viability of CHO cells by iron ions and hydrogen peroxide has been demonstrated. The results of this work can be useful for optimizing the electroporation methods used in biotechnology, medicine, and food industry.

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Investigation of plant electrical signaling: biophysical approaches

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Electrophysiological techniques offer unique biophysical means to reveal fundamental mechanisms and patterns of electrical signaling. Investigations of plant model systems, while not as numerous as research on animal cells, are rapidly advancing towards better understanding of plant responses to various stimuli. Single internodal Nitellopsis obtusa cells due to their gigantic size constitute an ideal model system for complex multi-parametrical

investigations of instantaneous extracellular effects of various biologically active chemicals and physical factors on the generation of plant bioelectrical signals in vivo. Among long-distance signals, electrical signals - action potentials (APs) exert fastest systemic signal propagation in palantae. APs utilize ion currents which form local electrical circuits and thus facilitate rapid non quenching signal. Nature and generation of APs can be observed using voltage clamp and current clamp techniques which allow measurement of the amount of ionic current crossing a membrane at any given voltage at a given time thus offering insights into real time processes in the intact cell. Duration of electrical signals in plants is relatively long thus dynamics of multiple parameters of APs, namely the excitation threshold, AP peak and duration, membrane potential at various voltages and dynamics of ion currents, can be observed. Excitability of tonoplast (vacuolar membrane) of N. obtusa cells can also be observed and activity of single ion channels can be investigated using patch clamp technique. This approach enables examination of dynamics of single channel currents in real time in near-physiological conditions. Examination of the effects of bioactive compounds on plant cells based on modern electrophysiological techniques can be supported by molecular biology methods. New discoveries are anticipated in the light of recently unraveled genome of another model organism of Characeae family – Chara braunii. Overall, electrophysiological investigations have been irreplaceable at enriching knowledge of function of plant signaling systems and dynamics of ionic currents as well as providing insights into bioelectrical processes in general.

WHY BENZENESULFONAMIDES BIND TO CARBONIC ANHYDRASES WITH SUCH A LARGE GIBBS ENERGY CHANGE?

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Some synthetic chemical compounds such as primary sulfonamides bind to their target carbonic anhydrase (CA) protein with an extraordinary Gibbs energy change of over 70 kJ/mol. Here we will discuss the contributions of sulfonamide and other groups to the energy change. Carbonic anhydrases are metalloenzymes, which catalyze reversible CO2 hydration into bicarbonate and hydrogen ion. There are 12 catalytically active isoforms of CA in human body. Overexpression of particular CA isoforms has linkage to certain disorders. For example, CA IX is usually found in cancer tissue where hypoxic environment is present [1]. Consequently, selective inhibition of CA IX isoform is thought to be able to reduce the progression of the disease. To this moment sulfonamides are the main class of CA inhibitors. However, their selectivity is usually not sufficient for the inhibition of a particular CA isoform. Therefore, it is important to understand the fundamentals why primary sulfonamides are great inhibitors by themselves and how their modification might be beneficial for the

development of new, better pharmaceuticals. For this purpose, a series of secondary sulfonamides were synthesized in our laboratory, which were analogous to primary sulfonamides synthesized previously [2,3]. Observed binding affinity was determined by fluorescent thermal shift assay and intrinsic binding affinity representing the binding of benzenesulfonamide anion to the Zn-bound water form of CA was calculated. The compound structure-intrinsic Gibbs energy correlation map showed the contribution of the coordination bond to the binding energetics. The high-resolution crystal structure of CA complexes with analogous secondary and primary sulfonamides revealed different orientations of the ligands in the active site of the protein providing some clues to our understanding of the large Gibbs energy change upon binding.

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OPTICAL MAPPING IN THE HEART WITH INDOCYANINE GREEN

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Indocyanine green (ICG) fluorescent dye has been approved by the FDA for use in medical diagnostics. Recently, we demonstrated that ICG dye has voltage-sensitive properties with a dual-component (fast and slow) response in the Langendorff-perfused rabbit heart [1]. Here, we extended our studies by showing the different spectral properties of both components for analysis of the fractional change in ICG fluorescence in response to voltage changes.

A standard glass microelectrode and optical mapping, using a near-infrared ICG fluorescent dye, were used to simultaneously record electrical action potential (AP) and optical signal (OS) in a Langendorff-perfused rabbit heart that was fully stopped.We used light from LEDs to obtain excitation; emission was measured using an EMCCD camera with band pass filters and a spectrometer. We applied a graphical model with Gaussian functions to construct and evaluate the individual emission curves and calculated the voltage-sensitive portion of each component of the ICG fluorescence in the rabbit heart.

The ICG OS has a dual-component (fast and slow) response to membrane potential changes that accurately tracks the time of electrical signal propagation but clearly differ in their kinetics and voltage-sensitive spectral properties. The voltage-sensitive fraction of ICG fluorescence was not high relative to the fluorescence of standard VSDs. However, after averaging, the good signal-to-noise ratio (> 20 dB) of ICG rendered its signal suitable for observing cardiac electrical activity. The results revealed that each isolated component (fast and slow) emanates from a unique ICG pool in a different environment within the cell membrane and that each component is also composed of two

constituents (ICG-monomeric and ICG-aggregated).
We propose the existence of different voltage-sensitive mechanisms for the two components: electrochromism and field induced reorientation for the fast component; and field induced dye squeezing that amplifies intermolecular interactions, resulting in self-quenching of the dye fluorescence, for the slow component. [2].
Acknowledgements:
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METABOLIC INHIBITION INDUCES TRANSIENT INCREASE OF ACTION POTENTIAL DURATION IN THE RABBIT HEART

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Myocardial ischaemia is associated with significant changes in action potential (AP) duration (APD). Studies on cardiac preparations showed that myocardial ischaemia or treatment with potent mitochondrial inhibitors induces a biphasic response in APD, i.e., the initial prolongation is accompanied by a subsequent shortening of the AP (Verkerk et al., 1996). Since both prolongation and shortening of AP could be distributed spatially and transmurally throughout the heart, it may lead to APD and refractory period dispersion, which could be one of the reasons for development of dangerous arrhythmias. Therefore, investigations of the mechanisms of the very first minutes of ischaemic stress could be of pathophysiological importance. Here we investigated the mechanism of initial AP prolongation in whole Langendorff-perfused rabbit heart. We used glass-microelectrodes to record APs transmurally. Simultaneously, optical AP, calcium transient (CaT), intracellular pH and magnesium concentration changes were recorded using fluorescent dyes. The fluorescence signals were recorded using an EMCCD camera equipped with emission filters; excitation was induced by LEDs. We demonstrated that metabolic inhibition by FCCP resulted in AP shortening preceded by an initial prolongation and that there were no important differences in such response throughout the wall of the heart and in the apical/basal direction. AP prolongation was reduced by blocking the ICaL and Ito with diltiazem and 4 aminopyridine, respectively. FCCP induced a reduction in CaTs and intracellular pH and increased intracellular Mg2+ concentration. Also, resting potential depolarisation was observed, which clearly indicates a decrease in the inward rectifier K+ current (IK1) that can retard AP repolarization. Thus, we suggest that the main currents responsible for AP prolongation during metabolic inhibition are the ICaL, Ito, and IK1, the activities of which are modulated mainly by changes in intracellular ATP, calcium, magnesium, and pH.
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SPATIOTEMPORAL PATTERN OF ACTION POTENTIAL ALTERNANS DURING ACUTE REGIONAL ISCHEMIA

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Beat-to-beat alternation of the action potential at increased heart rates is extensively used as an ECG-predictor of ventricular arrhythmias and sudden cardiac death. Multiple clinical studies have demonstrated upsurge in T-wave alternans and repolarization heterogeneity precede spontaneous initiation of ventricular tachyarrhythmias in humans. In this context, acute regional ischemia, which is known to cause rapid development of conduction abnormalities and arrhythmias, is a useful model for investigating the link between these abnormalities and alternans at short time scales. Our study was designed to investigate the inducibility and magnitude of action potential alternans during early stages of acute ischemia (<15 minutes). Experiments were carried out in isolated Langendorff-perfused rabbit heart stained with near-infrared dye di-4-ANBDQBS. Using a combination of optical mapping and glass microelectrode recordings we investigated the development of the spatiotemporal pattern of action potential alternans during acute regional ischemia. The alternans pattern significantly changed over time and had a biphasic character reaching maximum at 6–9 min after occlusion. Phase I (3–11 minutes of ischemia) is characterized by rapid increase in the alternans magnitude and expansion of the alternans territory. Phase I is followed by gradual decline of alternans (Phase II) in both magnitude and territory. During both phases we observed significant beat-to-beat variations of the optical action potential amplitude (OAPA) alternans. Simultaneous microelectrode recordings from subepicardial and subendocardial layers showed that OAPA alternans coincided with intramural 2:1 conduction blocks. Our findings are consistent with the modeling studies predicting that during acute regional ischemia alternans can be driven by 2:1 conduction blocks in the ischemic region.

IN VITRO EVALUATION OF DNA DAMAGE AFTER BLEOMYCIN ELECTROTRANSFER TO MX-1 AND CHO CELL LINES BY USING COMET ASSAY

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Electroporation is a technique that is used to increase the permeability of cell membrane by applying electric fields. This leads to the increased intracellular delivery of membrane impermeant anticancer drugs. Therefore, anticancer drugs can be locally transported into targeted tumour cells. By using anticancer drugs, such as bleomycin (BLM), and the process of electroporation, a novel cancer treatment technique has been successfully used in clinics. This

cancer treatment is termed electrochemotherapy (ECT). Bleomycin induce the cellular DNA damage that is causing cell death. To the best of our knowledge, there is no published research that is quantitively illustrating DNA damage after BLM electrotransfer, though the same effect is present in ECT. Moreover, it is not clear whether the BLM has the same effect in healthy or tumour cells. In this in vitro study, the evaluation of DNA damage after bleomycin electrotransfer to MX-1 and CHO cell lines was done by using comet assay (a single cell gel electrophoresis (SCGE)) technique.

Chinese Hamster Ovary (CHO) and human breast cancer (MX-1) cell lines were used for BLM electrotransfer experiments. The anticancer drug bleomycin was used for electrotransfer experiments in the concentrations ranging from 0.2 to 20000 ng/ml. Cells with BLM were suspended in electroporation medium (conductivity 0.1 S/m, osmolarity 270 mOsm, pH 7.1) and the electroporation was performed by using 1 electric pulse that induced electric fields at the amplitude of 1400 V/cm for the duration of 100 µs. Then, comet assay was performed to evaluate DNA damage. In addition, clonogenic assay was performed to evaluate cell viability.

Obtained results indicate a significant DNA damage caused by BLM electrotransfer in both used cell lines, as compared to incubation with BLM without effect by electric field. Notable, that significantly higher DNA damage as a result of BLM electrotransfer was done in MX-1 cell line when comparing to incubation with BLM without effect by electric field. Nevertheless, the tendency of dealt DNA damage is observed to be higher in CHO cell line as compared to MX-1, when using same BLM concentrations were used. A higher DNA damage observed in CHO cell line can also be proved with cell viability measurements, since CHO viability has a greater decrease after BLM electrotransfer in the range from 0.2 to 20 ng/ml. A later similarity of CHO viability change as compared to the MX-1 cell culture is explained with extremely low cell viability at higher BLM concentration then 20 ng/ml.

THE EFFECT OF NANOSECOND PULSED ELECTRIC FIELDS WITH CALCIUM IONS ON INTRACELLULAR PROCESSES IN HUMAN COLON ADENOCARCINOMA CELLS

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Nanosecond pulsed electric fields (nsPEFs) utilizing short pulses (few ns) can modulate intracellular processes affecting enzymatic activity, cell proliferation, cytoskeletal organization etc. Pore formation during nsPEF enables for the passage of small ions e.g. calcium, which is one of the most important factors in

cell functioning. The controlled regulation of calcium levels using electroporation can cause different effect on normal and cancer cells. The effectiveness of electroporation combined with Ca2+ ions were examined in two human colon adenocarcinoma cell lines: sensitive-LoVo and drug resistant-LoVo/DX. CHO-K1 cells were applied as normal control. The obtained results indicate that nsPEF supported by Ca2+ is cytotoxic in particular for resistant LoVo/DX cells. LoVo cells occurred to be less sensitive, and the least normal cells. Our results suggest that the nsPEF affects cellular morphology, which is dependent on stabilization by the actin and pan-Cadherin cytoskeleton. The subtle modulation induced by nsPEF might be potentially be important for therapeutic protocols of colon adenocarcinoma using calcium ions.

PICOMOLAR INHIBITORS OF CARBONIC ANHYDRASE: IMPORTANCE OF INHIBITION AND BINDING ASSAYS

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Human carbonic anhydrases (CAs) are targets for drug design due to their role in numerous diseases such as glaucoma, epilepsy, and cancer. Clinically used CA inhibitors–drugs are relatively weak and non-selective for human CA isoforms thus exhibiting toxic side effects. Further drug development should lead to compounds with picomolar affinities and significant selectivities. Currently, the Ki of CA inhibitors is usually determined by the stopped-flow CO2 hydration assay, the method that directly follows inhibition of CA enzymatic activity. However, the assay has limitations, such as largely unknown concentration of CO2 and the inability to determine the Ki below several nM. The widely used direct binding assay, isothermal titration calorimetry, also does not determine the Kd below several nM. In contrast, the thermal shift assay can accurately determine picomolar affinities.
The inhibitor dose-response curves were analyzed using Hill and Morrison equations demonstrating that only the Morrison model is applicable for the determination of tight-binding inhibitor Ki. The measurements of interactions between ten inhibitors and seven CA isoforms showed the limitations and advantages of all three techniques. Inhibitor 6 exhibited the Kd of 50 pM and was highly selective towards human CA IX, an isoform which is nearly absent in healthy human, but highly overexpressed in numerous cancers. Combination of inhibition and binding techniques is necessary for precise determination of CA–high-affinity inhibitor(such as 6) interactions and future drug design.
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ELECTRIC FIELD TRIGGERED CELL SIZE CHANGE INFLUENCE TO PROPIDIUM IODIDE AND BLEOMYCIN ELECTROTRANSFER

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Electroporation (EP) is a method that is used to induce a temporal increase of cell membrane permeability for

hydrophilic molecules. One of the applications of such method is a local cancer treatment that is termed electrochemotherapy (ECT). During ECT cell membrane becomes permeable for anticancer drugs such as Bleomycin (BLM). Moreover, other cellular properties change as a result of EP triggered cell membrane permeability deviations. Published data show, that cells increase in size after successful electroporation process. According to Schwan equation that is commonly used in modeling electric field to trigger EP the increased cell size has positive impact to subsequent EP of particular cell. Such phenomena is termed electrosensitization. However, opposite results are also published and termed - desensitization. These processes can influence permeability of membrane and are important for modeling and preparing for ECT. The purpose of this research was to examine influence of increased cell size as a result of electroporation, to the subsequent electroporation induced molecule (BLM or Propidium Iodide) electrotransfer.

CHO cells were subcultured a day before the experiment. All used high voltage (HV) electric pulses induced electric field with amplitude of 1400 V/cm for the duration of 100 µs. 2 or 8 HV pulses at 1 Hz were used and after 1, 5, 10 and 15 min time interval BLM (5 nM) or PI (40 µM) was put with an application of additional 1 HV pulse. Then, cells were plated for clonogenic assay or flow cytometry measurements were performed 10 min after the incubation procedure. Cell size measurements after 2 or 8 HV pulses at 1 Hz and incubation of 10 min were evaluated by taking photos of cells by using Kern OCO-255 inverted microscope. Sizes of the cells were measured by using open source ImageJ software.

Cells can decrease or increase in size depending on applied electric field. According to Schwan equation this deviation can change the induced transmembrane potential, thus altering the EP and subsequent process of molecule electrotransfer. We show that cell size changing HV pulse combination prior to electrotransfer of molecule of interest can trigger both sensitization and desensitization phenomenon can occur.

IN SILICO OF THE INFLUENCE OF WATER ON THE L-ISOLEUCINE MOLECULE FRAGMENTATION

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According to the data of the Radiation Protection Center [1], in 2017 the average resident of Lithuania received 3.37 mSv of ionizing radiation equivalent dose exposure from natural and artificial sources. In certain cases, e.g. during abdominal computer tomography, people get 10 mSv dose [1]. Low-energy electrons are produced due to ionizing radiation. These low-energy electrons cause damage of biomolecules, including amino acids, e.g. isoleucine. Most biophysical processes (also amino acids fragmentation) occur in water. Information on fragmentation of amino acids in water is insuficient, though they are relevant to the analysis of processes in organisms. The aim of this research is to determine whether the appearance energies, and fragment formation

processes are different in vacuum and water. L-isoleucine is needed for the formation of hemoglobin and it is also involved in blood clot formation. We used density functional theory (DFT) B3LYP method and cc-pVTZ basis set. The Polarized Continuum Model (PCM) method is used to evaluate the presence of water. We applied Gaussian 03 Rev D.01/Gaussian 09 Rev D.0 and Molden programs. Positively charged fragments for study were selected on the basis of the experimental data of mass spectrometry published in the NIST database. We determined that fragment with mass 86 a.m.u. is $C_5H_{12}N^+$, m=75 a.m.u.- $C_2H_5NO_2^+$, m=30 a.m.u.- CH_4N^+, m=57 a.m.u.- $C_2H_3NO^+$, m=74 a.m.u.- $C_2H_4NO_2^+$. The energy of appearance of these fragments is presented in Table 1.

Table 1. The energy of appearance obtained

Fragment	Energy of appearance, eV	
	Calculated without water influence	Calculated with water influence
$C_5H_{12}N^+$	8.81	9.76
$C_2H_5NO_2^+$	9.37	10.32
CH_4N^+	11.24	12.05
$C_2H_3NO^+$	10.69	11.46
$C_2H_4NO_2^+$	11.47	12.27

In summary, more energy is needed for the formation of same fragments in the water.

Acknowledgments

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BIPHASIC RESPONSE OF L-TYPE CALCIUM CURRENT TO UNCOUPLING AGENTS IN ATRIAL AND VENTRICULAR MYOCYTES

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Ischemic heart disease and heart failure are diseases accompanied by metabolic inhibition in heart cells and impairment of heart function. These conditions are still between the most common causes of death in the world. Recently, it was confirmed that metabolic inhibition induces biphasic changes of action potential in the early phase of ischemia and one of the players involved in this process are the voltage-gated L-type calcium channels (LTCCs). We have investigated the effect of uncoupling agents FCCP and DNP on the activity of LTCCs in atrial and ventricular cells derived from human and rat heart. The results showed that metabolic inhibition induced by FCCP and DNP evokes biphasic changes of LTCCs current (L-type calcium current) in cardiac myocytes: an initial increase of L-type calcium current in the early phase of metabolic inhibition, and further strong inhibition of L-type calcium

current. We studied the mechanism of the initial increase of L-type calcium current in cardiac myocytes during β-adrenergic stimulation. In rat cardiomyocytes, the initial increase of L-type calcium current was eliminated when the cells were initially perfused with thapsigargin or ryanodine, leading to the depletion of sarcoplasmic reticulum (SR) calcium store or blocking of RyRs, respectively. These data indicate that the increase of L-type calcium current during metabolic inhibition may be due to a reduced calcium dependent inactivation (CDI) of LTCCs. We also analyzed the effect of FCCP on time dependent inactivation of L-type calcium current in isoprenaline stimulated cardiac myocytes by bi-exponential fitting to the decay of current. It revealed that FCCP significantly increased the time constant of the fast component $\tau 1$ suggesting a diminished CDI of the channels.

This finding was confirmed in the experiments where calcium was replaced by barium ions, thus eliminating CDI of LTCCs, and no FCCP-induced initial increase of L-type calcium current were registered in such conditions.

We conclude that the initial increase in L-type calcium current observed during metabolic inhibition in human and rat cardiomyocytes is a consequence of acute suppression of SR calcium release, resulting in reduced CDI of LTCCs. This may affect the action potential duration during the early phase of ischemia and be one of the reasons of arrhythmias arising because of the early afterdepolarization.

THE INFLUENCE OF ELECTROPORATION MEDIUM CONDUCTIVITY AND THE CONCENTRATION OF MAGNESIUM IONS ON THE ELECTROPORATION EFFICIENCY

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Electroporation is a biophysical technique, in which high amplitude and short duration electric field pulses are used to transiently permeabilize cell membrane and thus allow the entry of small cell impermeant molecules to the cytoplasm or facilitate the transfer of exogenuos DNA into the cell. The latter process is called electrotransfection. There are many factors that have an impact on electrotransfection efficiency, including the parameters of electric pulses, targeted cells and electroporation medium. The electroporation medium conductivity is one of these parameters. Although the impact of electroporation medium conductivity on small molecule electrotransfer has been assessed in a few publications, the data about the effect of electroporation medium conductivity on cell electrotransfection is scarce. In this study, we attempted to investigate the effect electroporation medium conductivity has on luciferase coding plasmid and green fluorescent protein coding plasmid electrotransfer to Chinese Hamster Ovary cells. Our results show that increasing medium conductivity decreases the electrotransfection efficiency while cell viability is not adversely affected. Additionally, we tested the effect different magnesium ion

concentrations have on electrotransfer efficiency, using electroporation media of different conductivities (0,3 S/m and 0,8 S/m) that had different amounts of magnesium ions (1 mM, 2 mM or 5 mM of MgCl2). We found that the electrotransfection efficiency decreased with increasing magnesium ion content in both of the tested media. In conclusion, our results show that both the increase of the medium conductivity and the increase in magnesium ion concentration while the conductivity was constant caused a decrease in cell electrotransfection efficiency.

THE RELATION OF SONOPORATION EFFICIENCY TO CAVITATION METRICS

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Sonoporation is a biophysical technique, designed to achieve site-pecific and temporally controlled therapeutic agent delivery to cells and tissues. The mechanism behind sonoporation is directly associated to ultrasound (US) induced microbubble (MB) stable and/ or inertial cavitation. In order to achieve sufficient molecular delivery efficiency as well as diminish cell death, the extent of microbubble cavitation must be precisely quantified and controlled. This implies the necessity of a well defined sonoporation dosimetry model relating cavitation metrics to biological outcome.

In this study we have evaluated four main sonoporation elements: 1) MB concentration, 2) US attenuation 3) US scattering and 4) anticancer drug, bleomycin, sonotransfer into Chinese hamster ovary cells.

We have shown quantified dependencies between US attenuation, root mean square of scattered US signal, MB sonodestruction and bleomycin sonotransfer efficiency in both acoustic pressure and exposure duration scales. In addition to this, we proposed a method to optimize sonoporation efficiency in the temporal domain of the ultrasound field by monitoring either: US attenuation, US scattering or MB concentration decrease to signal background or complete MB destruction levels, respectively.

MB concentration dynamics were used to quantify MB sonodestruction rate, while MB cavitation signals, recorded using passive cavitation detection system, were used to quantify attenuation rate and inertial cavitation dose. These metrics have shown strong intercorrelation as well as correlation with bleomycin sonotransfer and cell viability. The latter results imply inertial cavitation to be the key mechanism ir producing efficient cell sonoporation.

For the first time, the metric, attenuation rate, was used for sonoporation efficiency and cell viability prognostication. It holds promising prospects for future applications for sonoporation dosimetry as it is indicated by overall strong correlation with cavitation induced bioeffects ($R^2 > 0.85$).

COMPOSITE CHITOSAN-GLUCAN EDIBLE FILMS CONSTRUCTION

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Mushrooms are found in Nature in big amounts, but still are not widely used in biotechnology. Mushrooms are known to contain vast amounts of glucans and chitin as known as a natural polysaccharide. Our chosen research object was *Gyromitra esculenta* and *Phallus impidicus* mushrooms. They were selected for the following reasons: wide availability, esthetical approach and its uninvestigated properties. Beta glucan has unique rheological properties and can be used as food additive or raw material for edible film production. Also, glucans are notable for having antioxidant, antitumor, immunostimulant, antimicrobial, cardioprotective and hepatoprotective properties. Chitosan is most common natural polysaccharide after cellulose found in nature. This component is nontoxic, biologically compatible, antimicrobial, antifungal and also, edible. These characteristics make it very perspective target for food industry, medicine and biotechnology. The aim of our research was to make chitosan-glucan composite edible films and evaluate their physicochemical properties.

By using chemical methods for chitin and sonication method for glucans, these natural polysaccharides were successfully extracted. Chitosan-glucan composite edible films were made and analyzed by mixing these ingredients in different amounts and with addition different amounts of glycerol (plasticizer). Optical and mechanical properties of edible films were investigated. The results of the optical properties study showed that the transparency of the films - the green-red (Δa) and the yellow-blue (Δb) shades changed by adding glucans. With glucan concentration decreasing, the edible films were more transparent. In the mechanical properties study, the following film properties were assessed: Young's modulus, elongation at break point and maximum tensile strength. The results of the maximum tensile strength study showed that the maximum tensile strength of chitosan-glucan films decreased with increasing glucan content in the films. Regarding the elongation at break, the addition of 1 to 15% of glucan presented a positive influence while higher values reduced the ductility of the films. The results of the Young's module study showed that with increasing glucan concentration, the thickness of the chitosan-glucan films decreases. To sum up, chitosan - glucan based edible films can be adapted for mass production to replace plastic food bags, or even for food products coating to keep them fresh for longer periods of time.