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Plenary lectures

MORPHOLOGY AND MOLECULAR ORGANIZATION OF AMPHOTERICIN B AND ITS COPPER COMPLEX IN MODEL MEMBRANE SYSTEM STUDIED BY BREWSTER ANGLE MICROSCOPY

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The polyene antibiotic amphotericin B (AmB) is currently the drug of choice in the treatment of fungal infections despite its adverse side effects. The AmB molecules tend to self-aggregate in both aqueous and lipid environments, which is related to their toxicity. Since AmB is not an ideal formulation, our group have synthesised an AmB-Cu(II) complex, which is a novel compound with different physicochemical properties [1] and altered biological activity, in comparison to the parent compound [2].

It was concluded that the water-soluble self-associated forms of AmB are mainly responsible for being toxic to human erythrocytes. Therefore, we have undertaken investigations focused on comparison of the effect of AmB and its copper complex on a model membrane resembling outer leaflet of erythrocyte membrane performed by means of the Langmuir monolayer technique. The surface activity of AmB predisposes it for formation of monolayers at the air/water interface [3]. The properties of the ternary lipid system in relation to the content of AmB and AmB-Cu(II) in the mixture were analysed with the parameters calculated directly from the π-A isotherms. The analysis of the interactions between components and morphology of mixed films were visualised by using Brewster angle Microscope (BAM).

The obtained results indicate that the addition of AmB into studied mixed films is thermodynamically unfavourable in comparison with AmB-Cu(II). The intermolecular interactions between AmB-Cu(II) and lipid components of the mixed monolayers were found to be stronger and the films were thermodynamically more stable, compared with the AmB-rich mixtures. The weak interactions and limited miscibility of AmB in erythrocyte model membrane manifested by the precipitation of crystalline structures at the antibiotic-rich membranes.

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SESSION "BIOLOGICAL MEMBRANES"

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Biophysical studies of membranes have long a strong tradition in the Polish biophysics. They were stimulated to a considerable extent by 13 international schools of biophysics membrane transport organized by Prof. Stanisław Przestalski and his team in the years 1974-1997. These schools with excellent lecturers constituted an important platform of international exchange of biophysical ideas.

Membrane biophysics covers a broad range of research areas starting from structure of model and cellular membranes and its modifications by additives, via behavior of various compounds in model and biological membranes, to transport across natural and model membranes and molecular modeling of membrane dynamics and transport. The width of the field of membrane biophysics is reflected in the titles of communications presented within the session.

Neunert et al. report the effect of cholesterol esters on the DPH fluorescence anisotropy of DPPC liposomes, confirming fluidization and rigidification of the lipid bilayer below and above the phase transition temperature, respectively, and lowering the transition temperature [1].

Matwijczuk et al. present data of organization of substituted 1,3,4-thiadiazoles in model solvents and DPPC vesicles as inferred from fluorescence and FTIR measurements[2].

Środa-Pomianek et al. reports the effects of newly synthesized piroxicames on the level of expression of multidrug resistance proteins in LoVo and LoVo/Dx colon cancer cells and stimulation of membrane lipid peroxidation by these modulators of multidrug resistance [3].

A new mode of action of an anticancer compound, 3-bromopyruvate, is reported by Sadowska-Bartosz et al. This inhibitor of glycolysis is demonstrated to inhibit also

glucose transport into the cells [4].

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INTEGRAL TRANSFORMS IN THE ANALYSIS OF SIGNALS REPRESENTING THE CONTRACTILE ACTIVITY OF THE UTERUS

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Integral transforms are a powerful tool for signal and image processing. From a mathematical point of view, the equations defining the below mentioned transforms are expressed in the same general relation. There is only one difference - in the kernel of the transform. The properties of the kernel determine the transform nature.

The Fourier transform is a description of how the signal is constructed from sinusoids of different frequencies. Power spectrum gives a measure of the contribution to the signal made by each of its sine wave component. Fourier transform gives no information about the localization of the individual frequency components. The question “Which frequencies are present in this signal?” for the Fourier analysis is replaced by “Which frequencies are present in this signal and when (or where) are they present” for wavelet analysis [1].

The time-frequency plot shows the distribution of energy. We can analyze the whole electromyographic (EMG) signal, the fragments of the signal (bunches of spikes) or separate spikes. The Hilbert transform allows the study of phase synchronization between simultaneously recorded time series. Changes in electrical potentials are associated with the mechanical activity i.e. contractions of uterine muscles. Uterine contraction may be detected by means of Hilbert envelope [2]. Spectral analysis of binary signals obtained from EMG signals may be performed by the Walsh-Hadamard transform [3].

In summary, not only Fourier transform may be used to the analysis of signals representing the contractile activity of the uterus.

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ELECTROMAGNETIC FIELDS GENERATED SYSTEM FOR APLICATION IN BIOLOGY AND MEDICINE.

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For studies related to the influence of magnetic field on the plant material was built field generator (solenoid) in the Department of Biophysics at the Faculty of Mathematics and Natural Sciences University of Rzeszow [1]. Combined with the power supply laboratory DC and AC generator was used in preliminary testing. This apparatus is able to produce a magnetic field between 0 and 22 mT. The results showed that germination rate index, vigour index and seed germination was increased after magnetic treatments except variable magnetic field in time of 12 min [2,3]. These studies will be continued with checking of plant genetic modification.

For studies related to the influence of magnetic field on human stem cells it was built cell culture incubator with CO_2 atmosphere with a built-in generator magnetic and electric fields in the Laboratory of Molecular Biology at the Faculty of Health Sciences of the University of Rzeszow. This equipment includes solenoid inside a cellular incubator. It is connected with a signal generator, which is driven by a lab view program (control system). This apparatus is able to produce a frequency from 0.01

Hz to 1 kHz, an magnetic field between 0 and 2 mT. Current knowledge shows the effects of EMFs on human adult stem cell biology, such as proliferation, the cell cycle, or differentiation [4]. The research is an early stage of development.

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FLUORESCENCE APPROACH TO INVESTIGATE INTERACTION OF A DANSYL-LABELED 5' mRNA CAP ANALOG WITH PROTEINS

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Decapping scavenger (DcpS) enzyme plays a crucial role in post-transcriptional control of gene expression as a critical determinant of mRNA turnover. Comparative studies of the newly syntesized dansyl-labeled 5' mRNA cap analog binding to DcpS enzymes from human, yeast, *C. elegans*, and *A. suum*, were performed by means of steady-state and time-resolved fluorescence. Fluorescence titration of the proteins with the DNS-cap turned out to be a convenient method to provide the suitable equilibrium association constants, consistent with those derived from analogous measurements of intrinsic protein fluorescence quenching.

A monoexponential, 4.77 ns decay of dansyl fluorescence showed an additional component of ca. 20 ns life-time upon specific binding to DcpS proteins. A similar, 14 ns second component, was also observed for the specific association to another cap-interacting protein human eukaryotic initiation factor 4E (eIF4E). Obtained values of fluorescence decay components are characteristic for dansyl probe buried in protein cavity, proving its utility as environment-sensitive dye.

Fluorescence resonance energy transfer (FRET) from the protein tryptophans to the dansyl acceptor reflects significant differences in the average conformations of the cap-bound DcpS enzymes from various spiecies. The fluorescence data enable a deeper insight into on the DcpS - cap binding specificity and dynamics of the complexes, verified theoretically by molecular dynamics simulations.

MAMMALIAN MONOMERIC PURINE NUCLEOSIDE PHOSPHORYLASE - FROM DESIGN TO PROPERTIES OF THE MUTANT

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Homotrimeric purine nucleoside phosphorylase (PNP) performs purine nucleosides catalytic breakdown and is a target for anti-cancer and immune system suppressing therapies [1]. Some previous reports suggest, that this enzyme exhibits a very strong negative cooperativity, so called one-third-of-the-sites-binding [2] but our studies have shown that although the enzyme exists in a trimeric form each subunit functions independently [3]. To answer the question why PNP forms a homotrimer we have prepared monomeric mutant of PNP (PNP M).

The main form of this mutant has sedimentation coefficient 2.9-3.2 S, consistent with the theoretically calculated value for the monomeric form. CD analysis revealed that PNP M has similar as WT PNP α-helix and β-strand content. But monomeric enzyme retains only residual catalytic activity towards natural substrates. In order to explain this phenomenon we have performed MD simulations, where structural differences between WT PNP and monomeric form were analyzed.

We conclude that the trimeric architecture is obligatory for the stability, solubility and biological activity of this enzyme.

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STREAK CAMERA MEASUREMENTS OF EXCITATION DYNAMICS IN PHOTOSYNTHETIC SYSTEMS

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In algae and higher plants the light-dependent phase of photosynthesis is carried out by two types of pigment-protein complexes: photosystem I (PSI) and photosystem II (PSII). Most of the pigments in PSI and PSII form antennas that capture sunlight energy. Photon absorption creates an electronic excited state which is transferred to the reaction center and utilized to drive the charge separation reaction.

Excitation dynamics in photosynthetic systems can be investigated by the time-resolved fluorescence spectroscopy. The use of streak camera as a detection method enables measurements with relatively good sensitivity and high temporal resolution (~3.5 ps). Another important advantage of streak cameras is the possibility to detect a fluorescence decays in a wide spectral range during a single measurement.

Various approaches are used to analyze spectrally and temporally resolved fluorescence signals. The most widely used approach is a global analysis, which aims to describe the observed signal as the sum of several components with an exponential time evolution identical to all detection wavelengths. We applied the global analysis for detailed description of excitation dynamics in algal PSI [1].

Another method, called target analysis, is based on fitting a certain physical model to the experimental data. It allows to obtain rate constants for transitions between individuals assumed in the model and spectra of these individuals. We used this method to describe the excitation transfer between spectrally different pools of chlorophylls in cyanobacterial PSI (in solution and immobilized on conducting glass).

In the case of more complex models, the Monte Carlo simulations can be performed to find the fit to the experimental data. We carried out the Monte Carlo simulations to obtain the average time of excitation hopping between polypeptides forming PSII supercomplex [2].

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ATOMISTIC SIMULATION OF SINGLE MOLECULE EXPERIMENTS: MOLECULAR MACHINES AND A DYNASOME PERSPECTIVE

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Proteins are biological nanomachines which operate at many length and time scales. We combined single molecule, x-ray crystallographic, and cryo-EM data with atomistic simulations to elucidate how these functions are performed at the molecular level. Examples include the mechanics of energy conversion in F-ATP synthase and tRNA translocation within the ribosome. We will show that tRNA translocation between A, P, and E sites is rate limiting, and identified dominant interactions. We also show that the so-called L1 stalk actively drives tRNA translocation, and that 'polygamic' interactions dominate the intersubunit interface, thus explaining the detailed interaction free energy balance required to maintain both controlled affinity and fast translation We will further demonstrate how atomistic simulations enable one to mimic, one-to-one, single molecule FRET distance measurements, and thereby to markedly enhance their resolution and accuracy. We will, finally, take a more global view on the 'universe' of protein dynamics motion patterns and demonstrate that a systematic coverage of this 'dynasome' allows one to predict protein function.

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QUEST FOR HIGHER SENSITIVITY IN FLUORESCENCE BASED DETECTION

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Background is a fundamental problem restricting sensitivity of biomedical assays and imaging of molecular processes in biological/physiological samples. The limiting factors are sample autofluorescence (fluorescence of inherent components of cells, tissue, and fixatives) and scattering of excitation that can produce secondary unwanted excitations or may simply leak into the detector. To increase detection sensitivity for a long time efforts have been focused on developing new brighter probes and novel detection approaches. Background from biological components decreases in the red spectral range and development of probes emitting in red and NIR range significantly improved detection limit. Long lifetime probes offer additional possibility for enhancing signal-to-background ratio by applying time-gated detection. We recently developed new red emitting probes based on azadioxatriangulenium structure (ADOT and DAOTA) that present very useful properties for assay development and cellular and tissue imaging. Recently we realized that detection can be highly improved when using multi-pulse excitation technology. Implementation of multi-pulse pumping and use of time-gated detection allows for high (few orders of magnitude) enhancement of the fluorescence signal of the probe over the autofluorescence. A long fluorescence lifetime of the probe also allow for new application to plasmonics based detection. We will discuss applications of pumping technology in combination with time-gating to increase sensitivity of biomedical assays, cellular imaging, and deep-tissue imaging.

FLUORESCENCE MICROSCOPY – NEW DEVELOPMENTS

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Over last two decades fluorescence based detection becomes a driving technology in cellular and molecular biology. Fluorescence microscopy offers high sensitivity and temporal resolution allowing many studies on cellular level. Forster Resonance Energy Transfer (FRET) and Florescence Lifetime Imaging Microscopy (FLIM) enabled studying molecular interactions on a cellular and sub-cellular levels. Development of new optical probes sensitive to environment (e.g. pH and viscosity) opens new ways for studying various cellular processes leading to cell malfunction and disease development. Development of bright red dyes made single molecule studies feasible bringing fundamental information on molecular functions. Most recently far-field optical imaging with a resolution significantly beyond diffraction limit has attracted tremendous attention allowing for high resolution imaging in living objects.

In this presentation we will introduce basics of fluorescence, microscopy, FRET, FLIM, and super-resolution methods. We will discuss our recent applications to imaging of cellular processes like exocytosis, single molecule detection, and new way to achieve super-resolution in far-field fluorescence imaging by the use of controllable (on-demand) bursts of pulses that can change the fluorescence signal of long-lived component over one order of magnitude.

MODELING OF TRANSPORT OF ELECTROLYTE SOLUTIONS THROUGH BACTERIAL CELLULOSE MEMBRANE IN CONDITIONS OF CONCENTRATION POLARIZATION

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The model of electrolyte transport through the membrane and evolution in time of concentration boundary layers (CBLs) at the membrane surfaces was developed. These layers significantly reduce the membrane transport. The model is based on the Kedem-Katchalsky equations of transport through the membrane and diffusion equation with migration for electrolyte solutions (KCl) in chambers. On the basis of diffusion equation the difference equations were elaborated in order to calculate formation and change in time of concentrations of solute which permeate through the membrane. Using obtained distribution of concentrations in near membrane areas the activity of K^+ ions and differences of potentials for the points located symmetrically on both sides of the membrane were calculated. The obtained results of distribution of concentration (activity) of K^+ ions permeating through bacterial cellulose membrane indicate that: 1. with lapse of time of transport through the membrane CBLs are expanding up to the distance of few mm from the surface of the membrane, 2. Within a few

minutes from the beginning of observation of evolution of CBLs the concentration difference on both sides of the membrane decreases to a value much smaller compared to the concentration difference at the initial moment, indicating a significant reduction in osmotic pressure on the membrane, 3. The potential differences calculated at selected points located symmetrically on both sides of the membrane, demonstrate the dependence of potential differences on positions of these points, and in addition, 4. the maximum of potential difference between electrodes located in points at both sides of membrane as a function of mechanical pressure difference on the membrane is observed. The position and amplitude of maximum depend on the initial value of osmotic pressure on the membrane. The results obtained from this model have been experimentally verified by means of the membrane system with bacterial cellulose membrane located in a horizontal plane in hydrodynamically stable configuration (solutions with higher concentrations of electrolyte were under the membrane). In this type of configuration of the membrane system, CBLs are rebuilding only by diffusion. The membrane system was connected to the system of stabilization of mechanical pressure difference on the membrane. Besides the potential differences between the electrodes placed directly in solutions symmetrically on both sides of the membrane were measured. Furthermore, the volume flux through the membrane, forced by mechanical pressure difference, causes the movement of solutions in chambers. Assuming that the diameter of the cross section of the chambers is equal to the diameter of the membrane the velocity of this movement is equal to value of volume flux. Good accordance of results from the model with the experimental results was obtained.

INSIGHT FROM PHOTOLUMINESCENCE INTO MECHANISM OF HEME AND IRON-SULFUR CLUSTER PHOTOREDUCTION MEDIATED BY CDTE QUANTUM DOTS

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Colloidal quantum dots (QD) are fluorescent nanoparticles, composed usually from semiconductor materials, e.g. CdTe or CdSe [1]. QDs are well known for their size-tunable emission maximum, broad excitation spectrum and high stability over bleaching. These properties encourage life sciences and medicine researchers to apply QDs, mostly as fluorescent labels. Fluorescence decay of QD is complex process and may be approximated by more than one component, being assigned to deep and shallow trap states, and to recombination of intrinsic core states [2,3]. Dynamics of fluorescence decay is generally reacting to the environment change. QDs may also serve as photoinduced electron donors. Based on that, we already successfully applied CdTe QDs as photoreductors of natural proteins, ferredoxin (Fd) and cytochrome c (Cyt c), with two different redox cofactors – iron-sulfur cluster and heme, respectively. Efficiency of photoreduction was related to QD diameter [4]. Understanding of this phenomenon has a great significance for future QDs application in e.g. in tuning redox state of the cell.

Here we are trying to explain a mechanism of QD-related photoreduction process by detailed analysis of fluorescence decays and reconstruction of time-resolved emission spectra (TRES). We used two types of CdTe QDs, of 2.6 nm and 2.9 nm diameter, with emission maximum at 550 nm and at 650 nm, respectively. First, we observed that for pure QDs solution, the fluorescence decay is well fitted by three components. The average fluorescence lifetimes, as well as separate τ length, depend on QD diameter. In presence of proteins, fluorescence decay is faster, although Cyt c has much greater impact than Fd. TRES experiment showed additionally that component of medium τ is dominant in pure QD solution, with maximum corresponding to steady-state spectrum maximum. Ferredoxin addition does not change this pattern, but presence of Cyt c promotes strongly the shortest τ. We will discuss these findings regarding to origin of individual decay components, using also complementary potassium iodide titration data to strengthen our hypothesis.

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INSIGHTS INTO THE IN VITRO MECHANISMS OF ANTICANCER ACTIVITY OF DIRUTHENIUM-1

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The anticancer activity of diruthenium-1 complex *in vitro* was evaluated against a panel of human cancer cell lines: HepG2 (liver hepatocellular carcinoma), MCF-7 (estrogen responsive breast adenocarcinoma), MDA-MB-231 (triple negative breast adenocarcinoma) and A549 (lung alveolar adenocarcinoma), on the basis of its ability to induce apoptosis, DNA damage and inhibition of cell cycle and proliferation. The effect of the complex on the intracellular redox state was also investigated.

The results indicated selective anticancer activity of diruthenium-1 toward MCF-7 cells at the nanomolar IC_{50} concentration (77 nmol/L) vs. the micromolar concentration IC_{50} (>1 μmol/L) for the rest of the cell lines.

The molecular mechanisms responsible for the anticancer activity of the complex in human breast cancer cells are mainly conditioned by its ability to: *i)* generate ROS burst; *ii)* induce apoptosis and necrosis of cancer cells*; iii)* interact with DNA and cause its extensive damage; *iv)* inhibit the cell cycle by arresting cancer cells at the G2/M check point. These changes are related to the concentration of diruthenium-1, the duration of the cell treatment and the post-treatment time.

LHCII TRIMER TO MONOMER TRANSITION AS A RESPONSE OF THE PHOTOSYNTHETIC APPARATUS TO MODERATE LIGHT INTENSITIES

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Photosynthesis operates efficiently thanks to several antenna pigment-protein complexes located in the thylakoid membranes responsible for sun light absorption and energy excitation transfer to the reaction center of photosystems. It is known that the yield of photosynthesis changes strongly along with absorbed light intensity. In the natural environment, the light condition varies highly in intensity and quality on a broad time scale as a result of different environmental factors change. Illumination of plants with high light intensity frequently leads to overexcitation of the reaction center of photosystems resulting in photoinhibition of the photosynthetic apparatus. The largest photosynthetic antenna complex of Photosystem II (LHCII) have a crucial role in plants protection from photoinhibition. Several reports indicate that, under optimal light condition, LHCII exists as a trimer which plays an important role in light energy harvesting. The LHCII trimer is evolutionarily preserved in thylakoid membranes of plants because of the capacity to efficiently transfer excitation energy from chlorophyll *b* to chlorophyll *a*. However, an increase in light intensity is followed by LHCII reorganization to monomeric forms capable of dissipation of excessive excitations. Such molecular LHCII reorganization is controlled by the xanthophyll cycle pigments. Violaxanthin stabilizes LHCII trimeric form and zeaxanthin preserves LHCII monomers. During the talk, the problem of light-driven regulation of the photosynthetic antenna function via the LHCII trimer to monomer transition will be addressed and discussed in terms of the photoprotective mechanism in response to moderate light intensities. Such considerations will be based on studies of a LHCII-lipid model system using "native" electrophoresis and molecular spectroscopy techniques.

TEMPOROMANDIBULAR JOINTS VIBRATIONS MEASURED BY LASER DOPPLER VIBROMETER - THE PILOT STUDY

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Temporomandibular joints are the only one that exist in human body that work symmetrically. These joints connect temporal bone with a jaw. Temporomandibular joints are the basis for the masticatory system functioning. When it acts properly it works smoothly and quietly. Problems with temporomandibular joints can cause pain in neck and ear pain or headache. Clicks and cracking can be also observed in case of temporomandibular joint dysfunctions. Pain in temporomandibular joint is an obvious sign of dysfunction. Dysfunction with pain concerns about 4-12 % of population. Dysfunction of temporomandibular joint can be caused by for example removal of molar teeth and premolar teeth. But it is not necessary, also people with a complete set of natural teeth have disorders of these joints.

Vibrations caused by temporomandibular joint were measured by Laser Doppler Vibrometry (LDV). The LDV is non-contact method which is based on Doppler effect. It is an useful method of biological vibrations measurements because disturbances related to mass of sensors and temperature of the object are eliminated. Laser Doppler Vibrometer used helium-neon laser, is

classified as safe (second class laser). The laser beam was directed toward the joints and vibrations caused by movements of the mandible were measured. Measurement of vibrations were carried out during: raising and lowering the mandible, moving the mandible to the left-right side and ejecting forward.

The difference between frequency of healthy temporomandibular joints and joints with dysfunction were observed.

THE INFLUENCE OF AMPHOTERICIN B (AMB) ON POTASSIUM CHANNELS FROM CELL MEMBRANE OF THE FUNGUS CANDIDA ALBICANS AND HUMAN DERMAL FIBROBLASTS (NHDF)

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A patch-clamp method was used to study the influence of AmB on the activity of potassium channels in *Candida albicans* and human dermal fibroblasts (NHDF). The measurements were carried out in the outside-out configuration. AmB was applied to the extracellular side of the membrane.

Potassium channels TOK1 (Two-Pore Outward Rectifier) recorded previously in *Candida albicans* [1] were selected for detailed examination. Application of 50 nM AmB caused a decrease in the number of active channels recorded at +80 mV, but did not change the unitary conductance of the channels (3.42±0.05 pS, n=4 in standard conditions and 3.47±0.09 pS, n=3 after application of AmB) and their open probability (0.171 in standard conditions and 0.177 after AmB application). Such results indicate an indirect interaction between AmB and TOK1 channels.

The effect of AmB on potassium channel activity in NHDF was studied in a gradient of K^+ promoting efflux of this cation from the cell, since the same direction of K^+ flux is observed during apoptosis in many cell types [2]. The value of the reversal potential was close to the reversal potential for K^+, indicating K^+ selectivity of the channels. AmB applied at 1 μM and 10 μM concentrations caused an increase in the channel conductance. The value of this parameter recorded at +80 mV in standard conditions reached 172.1±3.51 pS (n=5) and, after application of 1 μM and 10 μM AmB, increased to 182.82±2.66 pS (n=5) and 180.97±3.32 pS (n=5), respectively. AmB also caused an increase in the open probability of the channels from 0.179 in standard conditions to 0.187 and 0.195 after application of 1 μM and 10 μM of this antibiotic, respectively. These results indicate that AmB can accelerate the efflux of K^+ through potassium channels in NHDF, which in turn can lead to disruption of K^+ homeostasis of the cell and its apoptosis.

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EFFECT OF DIFFERENT CALCIUM CHANNEL INHIBITORS ON THE ACTIVITY OF SLOW ACTIVATING VACUOLAR CHANNELS IN THE LIVERWORT *MARCHANTIA POLYMORPHA*

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One of the features of slow activating vacuolar channels (SV) is low selectivity to monovalent and divalent cations. Since the role of SV channels in calcium signaling of plant cells is discussed, an influence of three different calcium channel inhibitors - gadolinium, ruthenium red, and verapamil, on the activity of this channel was examined.

1 mM gadolinium was the most effective in SV channel blockage. This inhibitor applied from the cytoplasmic side of the membrane caused complete inhibition of SV channel activity. The amplitude histogram prepared from long (16 s) recordings carried out at +40 mV indicated that the open probability of the channels decreased from 0.24 in the absence of gadolinium to 0 in the presence of this inhibitor. Such effective blockage of SV channels was not observed after application of 50 μM ruthenium red, but this inhibitor evoked clustered bursts of rapidly flickering of SV channels between open and close state. Clusters of bursts of the channel activity was also observed after application of 50 μM verapamil, but the periods of open and close states were longer than after ruthenium red. According to the amplitude histograms, an open probability obtained at +40 mV increased from 0.29 to 0.43 after application of ruthenium red. A much higher increase of open probability was observed after verapamil - from 0.21 to 0.53 after application of this inhibitor.

The results of this study indicate that SV channels in the liverwort *Marchantia polymorpha* can share some features with other calcium channels sensitive to gadolinium, ruthenium red, or verapamil, which were found in endomembranes from different plants [1-3].

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EFFECT OF COLLAGEN ADMINISTRATION ON ELECTRICAL PROPERTIES OF SKIN *IN VIVO*, IN PATIENT WITH VENOUS UCLER – A PILOT STUDY

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Collagen due to its superior biocompatibility is the primary resource in medical applications and is more effective in management of chronic non healing ulcers than conventional dressings. Collagen heals by forming an early granulation tissue supporting the skin development [1,2].

The progress in healing can be evaluated *in vivo* by means of different methods. Except wound size evaluation, measurement of elasticity of novel superficial tissue and its electrical properties are carried out. They are helpful in the understanding of the healing process.

The measurement of elastic properties were carried out by means of Cutanometer® [3], whereas skin electrical properties were measured in the frequency range 500 Hz -2 MHz using the Hioki dielectric spectrometer [4]. Measurement were carried out at the beginning of the experiment, next at the third week of collagen administrations. Recorded spectra show differences between patients, and were related to the stage of the healing process and duration of collagen application. This conclusion was supported by the results of cutanometry.

The experiment was approved by the Local Bioethical Commission.

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FEMTOSECOND MEASUREMENTS OF OPTICAL KERR EFFECT IN tRNA SOLUTION

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Transfer rybonucleic acid (tRNA) is a small biological molecule which plays a significant role in the protein translation process. It is well known that the role of all biomolecules is directly connected with their structure. Strong optical electric field (10^7 V/m) can change the behavior of the particles in solution and can induce the specific conformation of single molecule in its native state.

We have performed OKE measurements in tRNA solution and in distilled water. The OKE has been induced and sounded by femtosecond pulses of linearly polarized pump (λ_i=800nm) and probe (λ_p=800nm) light. For single molecule we have calculated the nonlinear optical parameters: K - Kerr constant, $X^{(3)}$ and c^{pi} – the macroscopic and molecular nonlinear third order optical polarizability. The femtosecond OKE measurements were compared with previous studies performed in ns and ps time range [1,2].

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BIOELECTROCHEMICAL ANALYSIS OF *DE NOVO* DESIGNED PROTEINS HAVING DIFFERENT MOTIFS BINDING IRON-SULFUR CLUSTER AND IRON-PORPHYRINS.

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Artificial redox-active proteins are excellent objects for studying electron transport. Here we applied electrochemical methods to study redox properties of these protein-bound cofactors. We used four artificial proteins. The first of them is RCM (redox chain maquette) and binds a porphyrin inside the helices bundle, and iron-sulfur cluster within the loops [1]. Second protein called CCIS (coiled-coil iron sulfur) binds iron-sulfur cluster within the protein core [2]. Third protein, called NCIS (N- and C-terminal iron-sulfur) is a modification of the protein HP7 [3] with addition of FeS binding motif from nitrogenase. This protein is able to bind two heme cofactor within core and FeS cluster by terminal parts of peptide. Last protein ISB (iron-sulfur binding), is also derivative of HP7, but can bind FeS within the loop between helices.

The proteins were attached by HisTag to the surface of gold electrode modified with NTA (Nα,Nα-Bis(carboxymethyl)-L-lysine hydrate) [4]. The presented data were obtained in a three-electrode cell that consists of working (WE), counter (CE) and reference (RE) electrodes. The binding of heme occurred under aerobic conditions, while assembly of the iron-sulfur cluster was done under anaerobic conditions. Data obtained by cyclic voltamperometry allowed us to determine midpoint redox potential of bound cofactors and compare them to values obtained from spectrophotometrical titration.

We are discussing the differences between obtained values and the original ones, of protein from which the motifs were excreted.

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DNA-DUPLEX FORMATION WITH PHYSICS-BASED COARSE-GRAINED MODEL

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DNA is one of the most important molecules of a living matter. Biologically important phenomena involving DNA covers time-scales from femtoseconds (electronic rearrangements) to days (chromatine reorganization) or even years (DNA aging). Correct description of these processes requires different theoretical methods, varying from quantum chemistry via atomistic and coarse-grained classical mechanics based models to mesoscopic models. Here a middle-resolution coarse-grained model of DNA, which can simulate behavior of DNA on a micro-miliseconds time-scale, is presented. The model of DNA chain, designed in a “bottom-up” fashion [1], is built of spherical and planar rigid bodies connected by elastic virtual bonds. The bonded part of the potential energy function is fit to potentials of mean force of model systems. The rigid bodies are sets of neutral, charged, and dipolar beads. Electrostatic and van der Waals interactions are parametrized by a special procedure designed in our laboratory [2]. Interactions with the solvent and an ionic cloud are approximated by a multipole−multipole

Debye−Hückel model. A very efficient R-RATTLE algorithm, for integrating the movement of rigid bodies, is implemented. It is the first coarse-grained model, in which all interactions were parametrized *ab initio* and which folds stable double helices from separated complementary strands, with the final conformation close to the geometry of experimentally determined structures. The model predicts two mechanisms of DNA hybridization – zippering and slithering [3]. The importance of backbone dihedral and rotamer potentials for dsDNA hybrydization will also be discussed.

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SPECTROSCOPIC STUDIES OF THE FLUORESCENCE EFFECTS OF SELECTED 1,3,4-THIADIAZOLE IN BIOLOGICALLY IMPORTANT MODEL SYSTEMS

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Resorcyl substituted 1,3,4-thiadiazoles are very promising compounds, particularly in treatment of neoplastic diseases. Literature presents this compound group as promising antifungal, antibacterial and, primarily, anti-cancer agents. This study focused on two analogues of 1,3,4-thiadiazoles differing considerably in the structure of the substituent (C7 – substituted with a seven-hydrocarbon chain and C1 – with only one carbon atom in the substituent used).

The analyses of the selected 1,3,4-thiadiazoles in organic solvents revealed presence of different spectral forms. Depending on the changes in the compound structure, substantial differences in the equilibrium between the spectral forms were observed. It was also noted that the observed fluorescence forms largely depended on the type of the environment, changes in its pH, and mainly on the concentration of the compound used, which suggests a considerable effect of molecular aggregation on the spectral forms of the analysed molecules. The subsequent phase of the investigations consisted in measurements of fluorescence emission spectra of the selected compounds in the liposomal environment in DPPC. It was observed that, depending on the changes in the length of the substituent used, the fluorescence emission spectra exhibited substantial differences. In the case of compound C7, there were three different spectral forms (with a fluorescence emission maximum at ca. 370, 430, and 500 nm). In turn, only two spectral forms with a fluorescence emission maximum at 370 and 430 nm were noted for compound C1 with a shorter substituent. FTIR spectroscopy measurements showed a clear effect of the substituent length on the molecular organisation of the analysed compounds. Furthermore, it was noted that the change in the spectral forms was associated with main phase transition in DPPC. Elucidation of the molecular organisation of the analysed compounds may significantly contribute to the understanding of their neuroprotective properties, which may be helpful in development of new effective pharmaceuticals in the future.

EFFECT OF ALPHA-TOCOPHEROL ESTERS ON PHYSICAL PROPERTIES OF DPPC LIPOSOMES. A DPH FLUORESCENCE ANISOTROPY STUDY

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The effects of alpha-tocopherol (a-Toc) ester derivatives: alpha-tocopherol maloneate (TM), alpha-tocopherol succinate (TB) and alpha-tocopherol oxalate (TS), on the membrane fluidity of dipalmitoylphosphatidyl choline (DPPC) liposomes were studied by measuring the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH). DPH is one of the most popular fluorescence anisotropy probes for bilayer membranes. DPH molecule due to its hydrophobic character is usually assumed that it reside between the lipid chains of the hydrophobic membrane core. Thus any modification in the movement of the chains or packing is sensitively reflected in the fluorescence anisotropy (r) of DPH. An increase in the fluorescence anisotropy values reflects a decrease in the probe mobility and an increase in the membrane structural order and a decrease in membrane fluidity.

DPPC liposomes with embedded a-Toc or its esters

were saturated with DPH and the fluorescence anisotropy as well as the steady-state fluorescence spectra of DPH were measured in the range of temperatures from 25 to 50°C. Both the fluorescence anisotropy and the fluorescence emission intensity of DPH are found to show very good response to the phase transition in DPPC vesicles and a fluidifying effect in studied concentration range of a-Toc esters.

For pure DPPC liposomes the anisotropy shows a sudden drop at 42°C, the phase transition temperature of DPPC. This transition temperature of DPPC was lowered on incorporation of a-Toc into the membrane. A similar effect was observed on incorporation of a-Toc ester derivatives into DPPC liposomes, however, a-Toc esters were more effective. The presence of a-Toc esters in proportions 2, 5, 10 and 20 mol% results in a progressive decrease in the temperature of the gel to liquid-crystalline phase transition from 42°C to about 35°C. This indicates that incorporation of a-Toc esters into the DPPC bilayer causes a disturbance in the interactions among the chains resulting in a decrease in the temperature of the gel to liquid crystal transition. Also in gel state there was significant downward trend in anisotropy with increasing derivatives concentrations.

Additionally, below the transition temperature, incorporation of a-Toc esters into the DPPC vesicles decreased the polarization of the fluorescence of DPH, whereas above the transition temperature the opposite effect was observed. This increasing the fluidity below the transition temperature was also noted in the fluorescence emission spectrum of DPH. The characteristic emission maximum of DPH in DPPC membrane in the presence of higher concentrations of a-Toc esters detected in 25°C showed a 3 nm shift toward to longer wavelength, thus indicating changes in the polarity of the environment of DPH located in acryl chains region.

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ULTRAFAST INTERNAL CONVERSION IN MIRAXANTHIN V

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Miraxanthin V is yellow dye belonging to betaxanthin family present in plants of the order of Caryophyllales. It is a dominant dye in yellow inflorescences of *Mirabilis jalapa* L., *Portulaca olreacea* L. and *Celosia argentea* L. Miraxanthin V is formed by condensation of betalamic acid with dopamine. In order to clarify the mechanism of miraxanthin V deactivation occurring after photon absorption by the dye, we applied transient UV-vis absorption technique. This is a powerful tool to characterize intermediates, photoproducts and their kinetics of formation and decay. Evolution of miraxanthin V transient absorption spectra was measured in 1 ns time window and over 330-670 nm spectral range with 200 fs temporal resolution in aqueous and alcoholic solutions. After excitation at λ_{exc} = 480 nm, tuned to the $S_0 \rightarrow S_1$ electronic transition, a positive band assigned to $S_1 \rightarrow S_n$ transition with maximum at λ = 394 nm is observed. Two negative bands correspond to S_0 bleaching band at ≈470 nm, and $S_1 \rightarrow S_0$ stimulated emission at ≈540 nm. Analysis of the data performed with a band integral reveals two time constants (4.2 ps and 24.2 ps in H_2O) that can be assigned to S_1 lifetimes of two major miraxanthin V stereoisomeric forms. Over 80 ps time window, a full S_0 ground state recovery was observed, so species as triplet excited state or persistent photoproducts are not formed upon the dye photoexcitation. The main S_1 state deactivation pathway is internal conversion, and the fluorescence quantum yield is very small (Φ_F = 0.003 in H_2O). Strong correlation between S_1 state lifetime and solvent viscosity in linear alcohols was obtained. On the basis of this correlation we postulate that the nonradiative $S_1 \rightarrow S_0$ relaxation is preceded by intramolecular rotation of the central molecular bridge bonds. The singlet excited state properties of miraxanthin V (very short S_1 lifetimes, the lowest Φ_F yield) determined make this dye exceptional from among other betaxanthins [1,2].

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NUCLEAR DNA DAMAGE AND REPAIR IN NORMAL OVARIAN CELLS CAUSED BY EPOTHILONE B

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Drugs able to induce DNA damage, such as microtubule inhibitors, play a critical role in DNA replication or in the segregation of chromosomes during cell division. One of

the compounds with these properties is PTX, which belongs to the taxane group and is utilised in the treatment of a variety tumor types. A big problem in the successful treatment with the taxanes is the appearance of the primary and acquired resistance, which has driven the search for alternative agents, such as epothilones. In this study we would like to evaluate if new chemotherapeutic microtubule inhibitor, Epothilone B (EpoB, Patupilone), can induce DNA damage in normal ovarian cells (MM14.Ov), and to estimate if such damage could be repaired. The changes were compared with the effect of paclitaxel (PTX) commonly employed in the clinic. The alkaline comet assay technique and TUNEL assay were used. The kinetics of DNA damage formation and the level of apoptotic cells were determined after treatment with IC50 concentrations of EpoB and PTX. It was observed that PTX generated significantly higher apoptotic and genotoxic changes than EpoB. The peak was observed after 48 h of treatment when the DNA damage had a maximal level. The DNA damage induced by both tested drugs was almost completely repaired. As EpoB in normal cells causes less damage to DNA it might be a promising anticancer drug with potential for the treatment of ovarian tumors.

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3-BROMOPYRUVIC ACID INHIBITS GLUCOSE TRANSPORT

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3-Bromopyruvate (3-BP) is an alkylating compound and inhibitor of glycolysis, considered as a promising anticancer agent since cancer cells, due to the Warburg effect, are expected to be more sensitive to inhibition of glycolysis. However, 3-BP has also other mechanisms of action, including induction of oxidative stress [1, 2].

The cell survival in a human hepatoma cell line, HepG2 treated with 3-BP in different media (with 5.5 mmol/dm^3 glucose and 1 mmol/dm^3 pyruvate or without glucose and pyruvate) was determined using the Neutral Red uptake assay. We studied the effect of 3-BP on glucose transport in HepG2. Cells were plated in Dulbecco's Modified Eagle Medium (DMEM) (25 mmol/dm^3 glucose) and treated with 3-BP (12.5, 25, 50 μmol/dm^3) in serum-free medium for 4 hr. After 3-BP exposure cells were washed with PBS, collected by trypsinization and suspended in glucose-free DMEM. Fluorescent 2-deoxyglucose analog, 2-[N-(7-nitrobenz-2-oxa-1,3-diaxol-4-yl)amino]-2-deoxyglucose (2-NBDG) was added to form final concentration of 5 μmol/dm^3 and fluorescence intensity was measured by flow cytometry in FITC channel. 3-BP at concentrations starting from 100 μmol/dm^3 decreased the rate of 2-NBDG uptake.

These results demonstrate that another mechanism of cellular action of 3-BP, possibly contributing to its effect on malignant cells, is inhibition of glucose uptake.

ACKNOWLEDGMENTS

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THz KERR EFFECT IN AQUEOUS SOLUTIONS

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Water is the most important liquids in biological systems. It has the smallest moment of inertia for small molecule polar liquids and as such it demonstrates the fastest solvent relaxation dynamics. On the time scale short enough the dynamic structure of water can be considered static and its molecular motions can be described by normal "solvent" modes that reflect the short-range order in liquid [1]. In this research we have measured Kerr Effect signal from deionized, distilled and buffered (PBS) water samples, using the femtosecond lasers pump-probe spectroscopic system, where the effect was induced by Terahertz radiation. The differences observed in birefringence amplitude and its dynamics can be interpreted as due to the fluctuating network of intermolecular hydrogen bonds of water molecules in the presence or absence of ions and cations in solution.

We have obtained Kerr constants of water samples of $(7\text{-}11)*10^{-14}$ m/V^2 which are comparable with the value of the static Kerr constant $K_{st}\sim3*10^{-14}$ m/V^2 deduced for water at λ_p = 800 nm from the approximation of the K(λ) values cited in literature. The obtained constants was 2-5 times higher than $K = 2.4*10^{-14}$ m/V^2 of CS_2 and 20-43 times higher than $K = 2.6*10^{-14}$ m/V^2 of benzene [2]. The smallest birefringence is found in PBS, it increases in distilled water and takes the highest value in deionized

water. This indicates that the concentration of ions in the solution play an essential role in the effect.

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APPLICATION OF FOURIER TRANSFORM INFRARED SPECTROSCOPY IN INVESTIGATIONS OF THE NEUROPROTECTIVE POTENTIAL OF SELECTED BIOACTIVE SUBSTANCES: SEARCH FOR PLASMA AND TISSUE NEURODEGENERATION MARKERS

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Acute brain ischemia/hypoxia and chronic neurodegenerative diseases are one of the most common causes of death or long-term disability in humans in developed countries. The cause of therapeutic failure is possibly the narrow a spectrum of tested medicinal substances. It seems that the future of new effective neuroprotective strategies lies in the search for drugs with different target points, i.e. with multidirectional activity. Ischemic brain damage is in fact the result of a cascade of pathophysiological events, including perilesional depolarisation inflammation, and programmed cell death.

Therapeutic hopes are provided by the investigations of the neuroprotective potential of selected compounds. The neuroprotective activity of 1,3,4-thiadiazoles and other bioactive molecules was tested in rat models of ischemia/hypoxia and excitotoxicity. Analysis of the pathophysiological processes underlying neurodegeneration in the experimental models revealed a number of biochemical markers for evaluation of anomalies detected in the brain and plasma of the experimental rats. Based on Fourier transform infrared spectroscopy, specific bands for the processes of necrosis/apoptosis associated with lipid peroxidation, inflammation and changes in the region characteristic for the nucleic acids (which could be related to damages of DNA such as single and double strand breaks) and relative changes in the secondary structure of protein (which may be associated with an increased proteolysis or demyelination processes) were analysed [1,2].

Searching for correlation between the specific spectral changes in plasma and brain and corresponding neurodegenerative processes can contribute to defining the spectral markers of neurodegeneration.

The results will be presented in a lecture at XVI Conference of the Polish Biophysical Society (PTB).

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CONJUGATION OF PHOTOACTIVE PROTEIN COMPLEXES WITH METALLIC NANOSTRUCTURES

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Fabrication of a functional artificial photosynthetic system requires optimization and refinement of the structural properties on each level of assembly of such a hybrid system [1]. In order to achieve this goal, research needs to focus on three basic processes that define photovoltaic aspects of photosynthesis: harvesting of light energy, separation of charges, and transport of charges to catalytic sites. All these processes are governed by spatial arrangement of photoactive molecules: chlorophylls and carotenoids. In this work we concentrate on assembling a plasmonic device with improved light-harvesting characteristics [1,2].

Our model system is a peripheral light-harvesting antenna, pedridinin-chlorophyll-protein (PCP) complex isolated from algae *Amphidinium carterae*. The PCP complex is equipped with streptavidin, which facilitates easy conjugation strategy with metallic nanostructures functionalized with biotin.

In this work we succeeded to assembly a hybrid nanostructure consisting of the PCP complexes conjugated with silver nanowires. For such a biocojugate we observe strong increase of the fluorescence intensity due to efficient plasmonic coupling between the constituents.

The optical properties of the PCP complexes conjugated with silver nanowires were studied using fluorescence microscopy and time-resolved spectroscopy. We find strong emission of the PCP complexes attached to the nanowire with fluorescence intensity at the ends of the nanowires being considerably stronger that for the PCP complexes along the wires. The result indicates unique properties of the silver nanowires as plasmonic structures as well as shows – perhaps for the first time – strong fluorescence enhancement for the photosynthetic complexes conjugated to metallic nanoparticles.

Following up on this result we were able to demonstrate real time conjugation of the PCP complexes monitored using sensitive fluorescence detection, as well as ability to attach individual PCP complexes to silver nanowires.

Used two types of substrates: glass coverslip and glass coverslip functionalized with streptavidin. This resulted in a different dynamics of the formation of chemical bonds between functionalized silver nanowires and PCP. In addition we find substantial differences in photostability of the PCP fluorescence on both types of substrates, which could point towards optimized architecture for potential energy conversion.

In addition to study conjugation with simple photosynthetic systems, we also attempted to conjugate Photosystem I to silver nanowires. During the presentation we describe initial results of these experiments.

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ENHANCED FLUORESCENCE OF BIOLOGICALLY IMPORTANT MOLECULES ON PLASMONIC PLATFORMS

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Nanophotonics, especially plasmonics is an interdisciplinary field that has recently revitalized luminescence. Fluorescent molecules deposited on the plasmonic platform surface are exposed to the enhanced local electric fields and are excited with higher rate constant. Due to interactions with surface plasmons the brightness of fluorophores is greatly enhanced and their lifetime is significantly shortened [1,2].

Therefore the development of new plasmonic platforms as well as new experimental setups for sensitive detection and imaging of fluorescent species located at the platforms will bring new quality to biosensing, molecular diagnostics, and detection of cardiac and cancer markers as the sensitivity of detection should be enhanced from several to about 100 times.

In this work we present both experimental setup [3] and examples of new plasmonic platforms designed to obtain spectroscopic information on Nile Red and flavones.

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SPECTROSCOPIC STUDIES AIMED AT DETECTING PHOTOACTIVE MOLECULES IN TISSUES

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One of the most important challenges related to understanding the function of molecules, both of natural origin and those synthesized in laboratory, is to develop protocols for sensitive detection of these molecules in tissues of interest. The high sensitivity is required as the concentrations of such molecules found in tissues are often at the detection limit. On the other hand, the experiments must be designed in such a way to eliminate, or at least reduce, any influence of the tissues themselves. Once possible, by correlating the results of such studies with for instance behavioral observations, it should be possible to elucidate complex impact such molecules can have on organisms.

Fluorescence spectroscopy is one of the most often used approaches aimed at detecting molecules in biological samples. Of course a prerequisite is that molecules themselves must be fluorescent.

In this work we describe an experiment aimed detecting tiadiazole molecule BHT (Butylhydroxytoluene) and xantohumol in rat brain tissues and plasma. Both molecules exhibit rather strong fluorescence upon 380 nm excitation, which also excites other species present in the tissues.

The samples were prepared as follows: the animals were anesthetized with pentobarbital, then the left heart ventricle was punctured and arterial blood was taken. The rats were sacrificed and perfused with ice-cold PBS. The brains were quickly removed, rinsed in ice-cold PBS and homogenized The brains homogenates and plasma were kept frozen at liquid nitrogen temperatures and thawed just before the measurement.

In order to obtain a complete spectroscopic information about the samples, we measured – in a standard way - absorption and fluorescence spectra of homogenates. Next we carried out spectrally- and time-resolved fluorescence measurement with excitation at 388 nm provided by a solid state laser. We compared several preparations with different doses and modes of application of the molecules to the animals.

In the presentation we shall describe the results of these studies with particular emphasis on technical aspects of the experimental setup on the one hand, and spectroscopic signatures that suggest presence of both studied molecules in the tissues. In combination with behavioural studies the results could suggest that the molecules have some potential for affecting neural system of the animals. Possible consequences of these observations will be discussed.

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INDUCTION OF APOPTOSIS THROUGH OXIDATIVE STRESS-RELATED PATHWAY IN MULTIDRUG-RESISTANT COLON CANCER CELLS BY OXICAM DERIVATIVES

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Data on potential antitumor activity of commonly used non-oncological drugs, non-steroidal anti-inflammatory drugs (NSAIDs) often appear in literature, recently. Among the many NSAIDs studied as chemopreventive agents, there is a distinguished group of oxicams, identified by their benzene(thieno)thiazine heterocyclic system containing an enolic group in position 4. NSAIDs are inhibitors of cyclooxygenase-2 (COX-2), the enzyme expressed in the most of solid tumors. COX-2 is also involved in multidrug resistance - the expression of COX-2 is well correlated with overexpression of multidrug resistance transporter MDR1/Pgp. Elevated level of COX-2 is connected with poor prognosis of many neoplastic diseases. It has been suggested that COX-2 inhibitors can sensitize cancer cells to anticancer drugs by inhibition of MDR transporters, such as P-gp, MRP1 and BCRP.

In our studies human colorectal adenocarcinoma cell line LoVo and its doxorubicin-resistant subline Lovo/Dx were chosen to study multidrug reversal activity of newly synthetized oxicams. Expression of MDR transporters in LoVo and LoVo/Dx human colon adenocarcinoma cell lines was determined in our previous studies. Results of studies presented here have shown the growth inhibition of LoVo and LoVo/Dx colon cancer cells in the presence of designed piroxicames. The expression level of the multidrug resistance proteins and their corresponding genes (mRNA determination) have been analyzed by immunodetection and PCR, respectively. The studies with use of confocal microscopy have confirmed the influence of new derivatives on cytoskeleton structure in studied cancer cell lines and MDCK-Pgp cells. On the basis of the observed effect of the compounds as potentially active multidrug resistance modulators we chose the most active oxicames and they were further studied on lipid peroxidation process. In our studies the concentration of malondialdehyde (MDA), a lipid peroxidation product, was determined in order to assess the progress of free radical processes. The analysis of cell membrane extracts from the colon adenocarcinoma cells of the lines LoVo and LoVo/Dx showed that newly synthesized derivatives of oxicam have an influence on the level of MDA. The increase of intracellular MDA concentration leads to damage of DNA in LoVo/Dx cells. It has been confirmed that caspase 3 is activated in the presence of studied oxicams.

PHOTOTHROMBOTIC ISCHEMIC STROKE MONITORING WITH THE USE OF OPTICAL COHERENCE MICROSCOPY

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Stroke remains one of the most frequent causes of death and permanent disability worldwide [1]. However, the mechanisms of its initiation and development are not recognized at the level where effective prevention and neutralization would be possible. Monitoring of the stroke in animal models is believed to provide results which potentially can be translated to the human case. We propose the comprehensive approach based on photohrombotic stroke model in mice and brain monitoring with the use of Optical Coherence Microscopy. This combination provides detailed insight in the structure and function of the brain upon stroke progress in time.

Photothrombotic stroke model involves the use of the phototoxic agent (bengal rose) whose molecules become highly reactive upon illumination [3]. It starts to interact with endothelium of blood vessel, causes its rupture following by local platelets aggregation and finally production of thrombus. The process can be monitoring in 3D with micrometer resolution with the use of Optical Coherence Microscopy setup allowing for both – functional imaging of blood flow in vessels and simultaneous observation of the actual cortex tissue up to the level of neurons. High quality of data is assured by the use of Bessel beam in microscopic setup providing extended depth of focus while maintaining high axial resolution [4].

We designed and constructed customized experimental setup and developed the stroke induction procedure suited for required measurement protocol. The results of preliminary research show high correlation between detectable changes of optical properties of brain tissue and flow dynamics in blood vessels. We expect that systematic studies on these effects will help to understand better ischemic stroke and will allow to establish reliable platform for drug-testing.

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THE INFLUENCE OF SELECTED PLANT-DERIVED POLYCYCLIC COMPOUNDS ON THE ACTIVITY OF Kv1.3 CHANNELS IN HUMAN T CELL LINE JURKAT

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Voltage-gated potassium channels of the Kv1.3 type are widely expressed in many cells, both normal and cancer. Kv1.3 channels participate in many processes, including proliferation and apoptosis of normal and cancer cells. Inhibitors of Kv1.3 channels may potentially find a clinical application to support chemotherapy of some cancer disorders characterized by an over-expression of Kv1.3 channels. Promising candidates for a clinical application in the future may be some polyphenolic compounds, since they combine ability to cancer cell elimination with a good bioavailability and a low cytotoxity [1].

Studies performed in our laboratory applying the whole-cell patch-clamp technique showed that a plant-derived prenylated flavonoid - 8-prenylnaringenin, was an effective inhibitor of Kv1.3 channels in a human cancer T cell line – Jurkat [2]. Studies on prenylated flavonoids were extended ~~on~~ for another derivative of naringenin – 6-prenylnaringenin (6-PR). Obtained results provide evidence that 6-PR is also an effective inhibitor of Kv1.3 channels in Jurkat T cells. The inhibition occurred in a concentration-dependent manner. The calculated half-blocking concentration (EC_{50}) was about 5 μM. Application of 6-PR at a maximal concentration of 30 μM inhibited the whole-cell Kv1.3 currents to about 10% of the control value. The inhibitory effect was reversible. It was accompanied by a significant acceleration of the channels' inactivation rate. 6-PR is much more effective inhibitor of Kv1.3 channels than two non-prenylated flavonoids selected for this study: acacetin and chrysin. Application of both compounds at a concentration of 30 μM caused an inhibition of Kv1.3 channels' activity to about 50% of the control value. The inhibitory effect of acacetin and chrysin was reversible. The inhibitory effect

of acacetin was accompanied by an acceleration of the channels' inactivation rate, however, this acceleration was much less significant than in the case of 6-PR. Obtained results may confirm previous hypothesis that prenyl-derivatives of flavonoids are much more effective inhibitors of Kv1.3 channels in Jurkat T cells than non-prenylated compounds.

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DEVELOPMENT OF BIOPHYSICS IN LITHUANIA

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Biophysical research in Lithuania started during cold war period. Biophysical research in Lithuania started at mid of XX century under influence of breaking discoveries in molecular biology and biomedicine such as determination of double helix structure, explanation of the biophysical mechanism of nerve pulses origin, understanding of 3D structure and conformational dynamics of proteins, finding of the genetic code, etc. This stimulated spontaneous appearance of small biophysical groups of young scientists in Kaunas medical institute and Vilnius University. Because activity of such groups was inspired by achievements of scientists US and western Europe, Soviet authority at beginning tolerated them in Moscow and Leningrad academic institutions but wasn't happy with it in Baltic countries. Nevertheless groups in Lithuania existed and after Lithuania joined EU their number significantly increased. Currently there are about 20 research groups that identify themselves as mostly related to biophysics.

Biophysical teaching at the bachelor and MS levels has been started in 1960 at Kaunas medical institute and few years later at Vilnius state University. Currently studies in Biophysics are proposed at Vilnius University at both bachelor and master levels as well as at Vilnius University and jointly at Lithuanian Health Sciences University and Vytautas Magnus University at Ph.D. level.

Lithuanian Biophysicist Club and Lithuanian Biophysical Society. At the end of XX century there were more than 100 scientists - professionals dealing with biophysical ideas and methods and seeking their application for solving various biomedical problems. This was a reason to organize annual meetings to have opportunities to discuss the news taking place in the World of biophysics and the life of National research groups in biophysics. Enthusiasm and activity of prof. M.S. Venslauskas led to foundation of the Lithuanian Biophysicist Club in 1999 year. The elected president M.S. Venslauskas chaired LBC for 14 years. With years scientific interests of LBC and biophysicists in Lithuania has crossed National boarders which gradually drove LBC to join European Biophysical Societies' Association (EBSA). The main part of LBC members and a number of young biophysicists has joined reorganized LBC to Lithuanian Biophysical Society. Prof. S. Šatkauskas has been elected to chair Lithuanian Biophysical association, which has been kindly accepted as EBSA member in 2013.

MULTIELECTRODE BIOSENSOR SYSTEM FOR SIMULTANEUOS MEASUREMENTS OF ION FLUXES ACROSS THE EPITHELIAL CELLS.

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Epithelia lines all wet surfaces of the body and form a barrier for water and ion transport in lungs, pancreatic ducts and sweat glands. There are different ion channels, transporters and pump molecules present in epithelial cell membranes. The defects in channels are responsible for ion channels-related diseases such as epilepsy, myotonia, cystic fibrosis and many others. Cystic fibrosis (CF) is the most common fatal genetic disorder among Caucasian race, affecting 1:3000 newborns. CFTR ion channel is responsible for passive osmotic water transport across epithelium. In the lungs, the change in water transport leads to dense mucus formation prone to opportunistic bacterial infections.

Multiple ions are transported across the epithelial cell monolayer i.e. sodium, potassium, chloride, hydrogen and bicarbonate. Measurement of all these ions as fast as possible is the key to understand the mechanism of cystic fibrosis. In our laboratory we built and successfully tested the small volume multielectrode ion selective electrode biosensor system which can measure transport of all these transported ions (Toczyłowska-Maminska, Lewenstam & Dołowy, 2014).

Measurements made on our multielectrode biosensor showed, that sodium is transported via paracellular route while chloride by transcellular one (defective in CF). Experiments show that ion transport across 16HBE14σ is very complex phenomenon. After medium change all ions were transported simultaneously through the cell layer.

Gradient introduction of one particular ion across the cell monolayer causes not only causes transport of particular ion but also affects transport of other ion species, in example: introduction of chloride gradient across the cell monolayer causes not only transport of chloride ions but also transport of potassium ions and pH change. Blocking of one particular ion channel species also affects transport of all ions across the cell layer. The other observation was that activation of CFTR channel by forskolin enhances the chloride ion transport and addition of $CFTR_{inh}$-172 (specific CFTR channel blocker) lowers it. Experiments with sodium and chloride gradient showed, that the cell line is chloride secretive.

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