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Posters

AN EFFECT OF CAROTENOIDS ON ION TRANSPORT ACROSS MODEL LIPID MEMBRANES

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Carotenoids are ubiquitous pigments present both in the plant and animal kingdoms, playing important physiological roles. Among diverse biological functions of carotenoids protection against oxidative damage and light harvesting in the photosynthetic apparatus are the most frequently reported. The photoprotection of carotenoids is realized via quenching of the triplet states of photosensitizers, quenching of singlet oxygen and scavenging free radicals. These mechanisms are essential for maintaining integrity of both the functional membrane proteins and the lipid phase. Protection of lipid membrane by carotenoid pigments is also realized via decreasing fluidity of the membrane.The polyene chain of carotenoid pigments incorporated into lipid membranes is localized in the hydrophobic core of the bilayer. Polar carotenoids have to adopt localization in the lipid membranes, such that the hydrophilic groups remain in contact with the polar head-groups of the lipid bilayer (in the opposite polar zones).

In the present work we analyze the effect of zeaxanthin, β-carotene and violaxanthin on transmembrane proton transfer. A pH sensitive fluorescence label, piranine trisulfonate, entrapped inside small unilamellar liposomes formed with egg yolk phosphatidylcholine, was applied to investigate effect of carotenoids on proton transport across lipid membranes. Time dependencies of fluorescence-monitored pH changes inside lipid vesicles, upon sudden acidification of the liposome suspension, were analyzed. It appeared that addition of xanthophylls to the liposomes, suppressed rapid pH changes. The effect was not observed in the case of β-carotene addition. The effect of the xanthophylls on transmembrane proton transport con be interpreted in terms of modification of the lipid phase fluidity. An alternative explanation is based upon binding of protons to the transmembrane molecular structures formed by the xanthophylls.

CALCIUM REGULATED POTASSIUM CHANNEL IS PRESENT IN ENDOTHELIAL MITOCHONDRIA

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In the present study, we describe the existence of a large-conductance Ca^{2+}-activated potassium (BK_{Ca}) channel in the mitochondria of the human endothelial cell line EA.hy926. A single-channel current was recorded from endothelial mitoplasts using the patch clamp technique. A potassium-selective current was detected with a mean conductance equal to 270 pS in a symmetrical 150/150 mM KCl isotonic solution. The channel activity, which was determined as the open probability, increased with the addition of calcium ions and the potassium channel opener NS1619. Conversely, the activity of the channel was irreversibly blocked by paxilline and iberiotoxin, BK_{Ca} channel inhibitors. The open probability was found to be voltage-dependent. The substances known to modulate BK_{Ca} channel activity influenced the bioenergetics of mitochondria isolated from human endothelial cells. In isolated mitochondria, 100 μM Ca^{2+}, 10 μM NS1619 and 0.5 μM NS11021 depolarized the mitochondrial membrane potential and stimulated non-phosphorylating respiration. These effects were blocked by iberiotoxin and paxilline in a potassium-dependent manner. Under phosphorylating conditions, NS1619-induced, iberiotoxin-sensitive uncoupling diverted energy from ATP synthesis during the phosphorylating respiration of the endothelial mitochondria. Immunological analysis with antibodies raised against proteins of the plasma membrane BK_{Ca} channel identified a pore-forming α-subunit and an auxiliary β2 subunit of the channel in the endothelial mitochondrial inner membrane. In conclusion, we show for the first time that the inner mitochondrial membrane of human endothelial cells EA.hy926 contains a large-conductance Ca^{2+}-activated potassium channel with properties similar to those of the surface membrane BK_{Ca} channel.

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ORGANIZATION OF THE THYLAKOID MEMBRANE MODEL AND LHCII PHOSPHORYLATION

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In higher plants capturing and conversation of light energy, to the form that can be used to perform the metabolic processes of living organisms during photosynthesis, takes place in the thylakoid membranes of the chloroplasts. This inner system of membranes is highly dynamic and able to adapt to variable environments condition.

The lipid multi-bilayer formed with plant lipids: monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) and modified by the largest photosynthetic antenna complex LHCII have been studied. In the research, there have been used two types of LHCII antenna complex: non-phosphorylated (isolated from dark-adapted spinach leaves) and phosphorylated (isolated from spinach leaves pre- illuminated by strong light).

X-ray diffraction, spectroscopic and microscopic measurements revealed, that membranes which contained the non-phosphorylated complexes were formed trans-layer, supramolecular structures, which are stabilized by hydrogen bonds. These structures provide a scaffold for lipid bilayers and allow the formation of the grana structures. In the lipid bilayer which contain phosphorylated LHCII have been observed aggregated lamellar structures in the plane of the membranes. The obtained results show that the process of antenna protein phosphorylation and its side reorganization in the thylakoid membranes constitute a regulating mechanism of grana formation from lipid-protein bilayers. Moreover, LHCII phosphorylation facilitates creation of aggregate structures, capable of quenching of excess excitation via non-radiative excitation energy dissipation.

The results have been used as a basis for creating a model of the thylakoid membranes of a chloroplast under normal light and under light stress co

THE INFLUENCE OF *Scutellaria baicalensis* MAIN FLAVONOIDS ON THE PROAPOPTOTIC ACTIVITY OF ANTICANCER DRUGS IN CANCER AND NORMAL HUMAN CELLS

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The beneficial effects of flavonoids in cancer therapy are often linked with their lack of toxicity against normal cells, the possibility of oral administration, low cost and general acceptance.

Accumulating evidence demonstrates that *Scutellaria baicalensis*, multi-purposed herb used in traditional Chinese medicine, possesses potent anticancer activity. In particular, its clinically important active compounds baicalin (BLIN), baicalein (BLEIN) and wogonin (W) have been reported to be primarily responsible for the cytotoxicity of this herb toward a range of cancer cell lines.

In this study the effects of above mentioned flavonoids on the proapoptotic activity of doxorubicin (DOX) and taxanes: docetaxel (DTX) and paclitaxel (PTX) in estrogen-responsive MCF-7 breast cancer cell line has been investigated. Human endothelial cell line HUVEC-ST as a model for normal cells has been also included in the experiments.

Externalization of membrane phosphatidylserine as an early marker of apoptosis was estimated by flow cytometry. Cells were preincubated for 24 hours with IC_{10} and IC_{50} concentrations of flavonoids and then incubated for 2 hours with DOX, DTX or PTX. After treatment the cells were cultured in fresh medium for 0, 12, 24 and 48 h.

Our studies showed that flavonoids did not hamper the proapoptotic activity of DOX and taxanes. Moreover, baicalin enhanced cytotoxic effect of chemotherapeutics. In contrast, combinations of anticancer drugs with BLIN displayed the lowest toxicity towards normal cells. These results showed an attractive activity of baicalin in combination with DOX and taxanes – decreased toxicity of anticancer drugs toward normal cells and enhancement of their proapoptotic activity in cancer cells.

POLYUNSATURATED FATTY ACIDS AND THEIR METABOLIC PRODUCTS IN THE DIAGNOSTICS OF HUMAN BREAST CANCER TISSUE

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Techniques commonly used in breast cancer diagnostics are: mammography, ultrasound, and finally confirming diagnosis the histopathological examination of tissue taken during a biopsy. We will present results obtained by: Raman spectroscopy, Raman imaging, and IR spectroscopy, on the identification and spectroscopic analysis of benign and malignant human breast cancer changes. Particular attention will be paid to the spectral ranges characteristic for lipids, carotenoids, proteins and water. In the analysis of the vibrational spectra characteristic for lipids range, will be presented a comparative analysis of the spectra of the human noncancerous and cancerous breast tissues with spectra of fatty acids: oleic acid, representatives of n-6 fatty acids: linoleic acid, g - linolenic acid, arachidonic acid and representatives of n-3 fatty acids, such as a-linolenic acid, eicosapentaenoic and decosahexaenoic acids and products of lipid peroxidation, [1-4].

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CHANGES IN THE VIABILITY AND ANTIOXIDATIVE SYSTEM OF ERYTHROCYTES AND HUMAN BLOOD MONONUCLEAR CELLS CAUSED BY BROMFENVINPHOS CONTAMINANT

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Bromfenvinphos is organophosphorus insecticide, which is considered to be effective in controlling of ectoparasite in farm animals, dogs and cat's fleas as well as other domestic insects. Since 2003, bromfenvinphos (as Apifos preparation) has been successfully used against the *Varroa destructor*, mite that causes bees varroosis in the species *Apis mellifera* and *Apis Cerana*. Unfortunately, this preparation was withdrawn due to lack of its specific MRL (*maximum residue limit*) values. In order to indroduce Apifos in the market again, it is necessary to conduct additional toxicity tests of bromfenvinphos and its impurities.

We investigated the effect of bromfenvinphos contaminant: 1-bromo-2-(2,4-dichlorophenyl)-2-etho-xy ethene on erythrocytes and human blood mononuclear cells (*in vitro*). The cells were exposed to different concentrations of these compound (0.1; 0.5; 5; 10; 50; 250 and 500 μM) for 1 and 4 h.

The following parameters were determined in the erythrocytes: the level of methemoglobin and reduced glutathione, reactive oxygen species formation, size and shape of the cells and the activity of acetylcholinesterase.

In human peripheral blood mononuclear cells the following parameters were analyzed: viability, size and granulation of the cells and lipid peroxidation level.

The bromfenvinphos contaminant induced a slight increase in hemolysis level (about 2%), oxidation of hemoglobin (about 20%) and oxidation of R123 (about 200%) and a slight change in shape and size of the erythrocytes. No effect was observed in acetylcholinesterase activity and the concentration of reduced glutathione. This compound showed high toxicity to human peripheral blood mononuclear cells as evidenced by the decrease in viability (about 60%) and considerable changes in size (decreased about 55%) and granularity (increased about 35%) of the cells studied.

THE NANOSTRUCTURE OF PECTINS DURING THEIR PHYSIOLOGICAL DEGRADATION

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Pectins are cell wall polysaccharides which undergo dynamic changes during growth, pre- and postharvest ripening. Pectin network is considered as a matrix surrounding cross-linked glucans. It also links neighboring cells through middle lamella, plays a role of plastificator and is a main water binding factor in the cell wall. In pectins dynamic enzymatic transformations occur such as de-esterification and depolymerization, what has a significant influence on the texture of fruits and vegetables.

The aim of the work was characterization of structural changes in pectins during natural postharvest ripening. Pectins were isolated from carrot during three months of storage as three fractions: water soluble pectins (WSP), chelator soluble pectins (CSP) and

diluted alkali soluble pectin (DASP). Atomic force microscopy and infrared spectroscopy were the main tools applied to observation of pectins structure.

Direct visualization of the pectin fractions clearly depicted structural differences. The WSP molecules characterized granular structure with very rare chain-like molecules. The CSP fraction contained branched molecules chains of several hundred nanometers long and diameter of few nanometers. Fibers extracted in DASP fraction were the longest and had a capability to create regular network-like structures. During storage a reduction of dimensions of WSP was observed as a result of enzymatic degradation. In CSP fraction both decrease of fibers length and number of junction zones between fibers was found. DASP fraction clearly lost regular cross-linking between main and side chains during postharvest ripening. Enzymatic degradation proceeded sequentially, starting from demethylation following de-polymerization and separation of side chains.

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AMPHOTERICIN B - AN OLD DRUG, NEW IDEAS

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Very high efficiency, but also the high toxicity of amphotericin B lead to the search for modifications of the drug to obtain more favorable pharmacological properties. These modifications can be made at the appropriate antibiotic formulation or modification of its chemical structure. Another possibility of the modification is the complexation of the antibiotic with transition metal ions such as ions of copper (II). Analysis of the electron absorption spectra as well as the circular dichroism indicate that the amphotericin B in aqueous solutions at pH values ranging from 10.5 to 11.0 complexes with Cu^{2+} ions with 2:1 stoichiometry. These complexes are stable at physiological pH values. In the Raman spectra of the Cu $(AmB)_2$ complex it was observed the ν_1 band shift towards lower frequencies, which was associated with the change of the polarizability of the AmB chromophore, induced by Cu^{2+} ions. The change of the chromophore polarizability may increase the hydrophobicity of the complex, which can influence its biological activity. In studies on standard strains of *Candida albicans* increase in fungistatic and fungicidal properties of the Cu $(AmB)_2$ complex was observed as compared to Fungizone - the formulation currently used in therapy. The increased antifungal activity of the complex did not result from the sum of the toxic effect of AmB and copper ions, but it is a unique feature of this complex. Among factors influencing the increased biological activity of the complex may be a different spatial structure of the complex, compared to AmB. Another factor can be transport of Cu^{2+} ions, together with complexes, directly to the cell membrane of the fungal cell.

COMPARISON OF TRANSPORT POLYMER MEMBRANE MODIFIED WITH ONE- AND TWO-SIDED METHOD OF ION IMPLANTATION

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The topic of research is to compare the transport properties of polymer membranes with a thick-ness of 12μm made of polycarbonate (PC) and poly (ethylene terephthalate) (PET), single and double-sided modified by ion implantation. Implantation ion in polymer samples using ion beam implanter UNIMAS 79. In these studies, we use N^{+1} ion beams with energies 180 keV and a dose of $1 \times 2 * 10^{14}$ (modified single-sided) and $2 \times 1 * 10^{14}$ ions/cm^2 (double-sided modifica-tion). The idea of this task is to obtain the dou-ble-sided modified membrane whose surface is modified by ions of the same dose per unit area of the membrane, as in the case of one-sided membrane.

The aim of research is to explain the conduc-tivity changes of diffusion of polymer membra-nes resulting from implantation. In this study, we have shown previously that a change in membrane surface topography (increase the active surface area) as well as the change of surface wettability improves conductivity coefficient of diffusion. Current research is aimed to explain how the coefficient changes depending on the method of implantation. In order to explain this the polymeric films were subjected to infrared spectroscopy studies (FTIR), atomic force microscopy (AFM) and the contact angle (CA). However, transport prope-rties, i.e. conductivity coefficient of diffusion have been studied using laser interferometry.

THE IMPACT OF NEAR-INFRARED RADIATION (NIR) ON THERMO-TOLERANCE IN HUMAN ERYTHROCYTES

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Human erythrocytes exposed to temperature 44°C become resistant to second thermal shock at a temperature 48.5°C. The phenomenon of thermotolerance in human erythrocytes reaches a maximum after 3 hours incubation at 37°C between the shock and disappears the after 7, 8 hours.

The aim of the study was cause thermotolerance in human erythrocytes by NIR (wavelength of 700-200 nm). Human erythrocytes (2% hematocrit) were subjected to radiation of 6.9 mW/cm^2 in 5-20 minutes

Obtained results indicate that 10 and 15 minute exposure to NIR causes resistance to temperature 48.5°C (2nd shock). The kinetics of appearance and disappearance of the thermotolerance caused by NIR is different than caused by thermal shock. Increase in ATPase activity is accompanied with thermotolerance caused by thermal shock and by near-infrared radiation.

EFFECT OF PIROLIN AND DOXORUBICIN ON MITOCHONDRIAL MEMBRANE POTENTIAL IN MCF-7 AND MDA-MB-231 BREAST CANCER CELLS

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Breast cancer chemotherapy employing doxorubicin (DOX) is associated with numerous side effects, especially severe cardiotoxicity. It is believed that oxidative stress generated by DOX is the main reason of its high cardiotoxicity due to naturally impaired antioxidant system of heart myocytes. In this context low molecular, nonimmunogenic and cell permeable compounds with antioxidant and chelating properties, such as nitroxides, seem to be good candidates for serving as cytoprotectors. A desirable property of such compound is to not hamper anticancer activity of chemotherapeutic.

In this study the effect of pyrroline nitroxyl derivative Pirolin (PL) on the collapse of mitochondrial transmembrane potential (DY_m) of DOX-treated breast cancer cells was investigated. Impaired mitochondrial potential is one of the fastest response of cells to proapoptotic stimuli. Changes in DY_m were assessed using the fluorescent probe JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolocarbocyanine iodide). Fluorescence of JC-1 monomers and dimmers was measured over 0-180 min period after the treatment in order to estimate the kinetics of DY_m changes caused by the investigated compounds. Cells preincubated with 5 μM of carbonyl cyanide m-chlorophenylhydrazone (CCCP), an uncoupler of oxidative phosphorylation, served as a positive control.

Pirolin used alone and in combination with DOX caused both depolarization and hyperpolarization of mitochondrial membrane, nevertheless cells treated with PL recovered faster their DY_m than cells incubated with DOX. Preincubation with PL did not improve DY_m alterations caused by DOX. Changes in DY_m persisted longer in estrogen-negative MDA-MB 231 cells which suggested their higher sensitivity to the investigated compounds.

APPLICATION OF QUANTUM DOTS TO DETECT ANTIOXIDANT PROPERTIES OF PHENOLS

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Absorption, emission and fluorescence lifetimes of CdTe quantum dots in aqueous and deuterated solutions as well as in the presence of some polyphenols with antioxidant properties have been measured. It has been shown that fluorescence quenching of cadmium telluride quantum dots occurs in the presence of substances with antioxidant properties. We observed the linear relation between fluorescence quenching of CdTe quantum dots and content of polyphenols. The obtained results were used to set up a simple and fast method for determination of total antioxidant activity and total content of naturally occurring polyphenols. The possible mechanisms responsible for the observed interaction between CdTe quantum dots and catechin have been discussed.

Applied fluorescence quenching method of CdTe quantum dots to estimate the total antioxidant activity, due to its better specificity, may be alternative to more elaborate Folin-Ciolteau method.

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MOLECULAR TRANSPORT OF AMINO ACIDS IN GELS PROBED BY INTERFEROMETRIC TECHNIQUE

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We study molecular transport of amino acids in a diffusion system consisting of two cells separated by a horizontally located polymer membrane. We have filled the upper cuvette of the diffusion cell with agarose hydrogel solvent while in the lower one there has been an aqueous gel solution of transported substance. Then, the substance diffuses from the lower cuvette to upper one. Since the concentration gradient is in vertical direction only, the diffusion is expected to be one-dimensional. We follow the diffusion process of amino acids. For each measurement we prepared different concentration solution of agarose in water (gek samples) and the same gel dripped by the amino acid. The diffusion can be characterized by a time evolution of the so-called near -membrane layer (NML) or concentration boundary layer (CBL) where the concentration of diffusing substance drops *k* times [1]. When the thickness of NML- %u0111, grows in time as $t^{ß}$ with ß *= 0.5* we deal with normal or gaussian diffusion. If ß *> 0.5* there is superdiffusion and when ß *< 0.5* we have a subdiffusive behaviour [1,2,3]. To observe the time evolution of CBL we employed the laser interferometric technique [1,2,3]. The analysis of interferograms allows reconstructing the time dependent concentration profiles of the substance transported in gels.

Our results show that the thickness concentration boundary layers are smaller than in normal diffusion, and transport is consequently said to be subdiffusive.

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THE EFFECT OF DIAMOND NANOPARTICLES ON THE LEVEL OF GLUTATHIONE, AND ENZYMES INVOLVED IN ITS METABOLISM IN LUNG CANCER CELLS

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With the development of nanotechnology, the new methods of synthesis and surface modification of diamond nanoparticles are seen. Diamond nanopowders are diamond particles with sizes below 100 nm, although in practice, this limit is about 10 nm. Nanodiamonds with the smallest sizes could penetrate cell membranes, which would affect the functioning of the cells, causing changes in redox homeostasis. Cells have antioxidant system which protects them against changes in redox homeostasis and involves the sweeping of free radicals, which includes glutathione and enzymes involved in the metabolism.

In this study we used lung cancer cell line (A549), which was treated by nanodiamonds at concentration 0-100 μg/ml for 24, 48 and 72 hours. We observed changes in the level of glutathione, as well as the activity of glutathione peroxidase, glutathione reductase and glutathione transferase. The changes in the enzyme activities were founded which was associated with glutathione in a tested cell line, and these changes were dependent on the concentration of this diamond and time incubation.

UTILIZATION OF MAGNETOTHERAPEUTIC DEVICE IN THE STIMULATION OF THE SEED GERMINATION AND DEVELOPMENT OF ONION (*Allium cepa* L.)

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The alternating magnetic field is widely used in rehabilitation and therapy from many years. Magnetotherapy and magnetostimulation are used as supporting therapy as well as individual therapies.

Utilization of magnetic field in stimulation of seeds and plants development is a topic of long standing experiments carried out in many research centres. Application of magnetic field leads to better germination capacity and plant development. It affects also some taste values of selected, economically significant plants.

In the carried out researches the VIOFOR JPS device used in medicine and exploited in Poland was utilised for magnetostimulation of onion seeds.

Obtained results show the improvement in germination capacity and taste values of some varieties of onion such as *Allium cepa* L. cultivated in Poland.

INFLUENCE OF PHOTOSYSTEM II ANTENNA COMPOSITION ON THE TRAPPING RATE OF ELECTRONIC EXCITATION

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Photosystem II (PSII) is a large membrane protein complex composed of a core, consisting of two reaction centers and core antenna, and peripheral antenna. LHCII complexes (consisting of apoproteins Lhcb1, 2, and 3) are the major part of the peripheral antenna and are connected to the core via minor peripheral antenna, called CP29, CP26, and CP24 (the apoproteins of these antenna are called Lhcb4, 5, and 6, respectively). The antenna absorb the light, convert its energy into electronic excitation of chlorophyll , and transfer it to reaction centers, where it is utilized to initiate the electron transfer. Thus, reaction centers act as quenchers of electronic excitation. Consequently, fluorescence decay measurements in PSII allows determination of excitation lifetime in antenna. In the presented work, influence of Photosystem II peripheral antenna composition on the trapping rate of electronic excitation by reaction centers was studied. Modifications of the antenna composition were introduced by mutations. The polypeptide composition in each mutant was determined biochemically. PSII-enriched membrane fragments were then studied by fluorescence time-resolved technique and correlations between polypeptide composition and fluorescence decay lifetimes were observed in order to determine the role of particular polypeptides in excitation energy transfer from the peripheral antenna to reaction centers.

STRUCTURAL CAUSES OF SEMI-LAMELLAR AGGREGATION OF PROTEOLIPOSOMES

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Chloroplast membranes which conduct the „light" photosynthetic phase are the most common type of membrane bilayer in the world. The specific mechanism of protein-lipid interactions within these membranes is essential for photosynthetic, energetic efficiency of the process. Photosynthetic efficiency is directly bound to the degree of membranes' stacking. Membrane aggregation and fluidity are varying due to environmental conditions for example. That's why it is very important to create simpler, useful model of such a membrane for more elementary study.

The attempt was made to create such a model. We used proteoliposomes build with plant galactolipids, with incorporated LHCII trimeric antennae. This is our basic model, with some modifications like the level of saturation acyl lipid chains or the level of aggregation each of the components (lipid and protein alike), it could be very useful for explanation of mechanisms which occurs in natural, thylakoid membranes.

Our preliminary model of aggregating proteoliposomes was based on spectroscopic analyses (Fourier Transformed Infrared Spectroscopy, fluorescence in 77K), as well as on microscopic studies (confocal laser scanning microscopy, atomic force microscopy). For the very first time such a model was used. We shown that proteoliposomes' structure is very similar to the native ones, so we think that this model can be used with success as thylakoids' research simplifier.

EXCITATION ENERGY TRANSFER AND PRIMARY STEPS OF ELECTRON TRANSFER IN PHOTOSYSTEM I FROM GREEN ALGA *Chlamydomonas reinhardtii*

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Photosystem I (PSI) is a large pigment-protein complex embedded in the thylakoid membrane, which uses light energy to drive the transmembrane electron transport. It consists of the core and the peripheral light harvesting complexes (LHCI). PSI core has its own antenna system containing ~90 chlorophyll *a* molecules bound to the protein matrix. The central part of the PSI core, called the reaction center (RC), is provided with two quasi-symmetrical branches of electron carriers (A and B). The purpose of the antenna chlorophylls is to absorb the light and transfer the excitation energy to the RC, where in the charge separation process the electron transport is initiated.

The aim of our research is to obtain a consistent description of the electronic excitation migration and charge separation in Photosystem I, using two complementary ultrafast spectroscopic techniques: femtosecond transient absorption and time-resolved fluorescence measurements by streak camera. Our measurements are carried out for preparations based on green alga *Chlamydomonas reinhardtii*, both for isolated PSI core and PSI-LHCI complexes, as well as whole living cells.

So far we have managed to clarify some controversial issues relating to the functioning of Photosystem I, such as: (1) reversibility of the primary charge separation leading to reproduce the excited state, (2) the involvement of each of the electron carriers branches in the charge separation process, (3) the time of excitation energy transfer from LHCI to the PSI core, (4) the presence of so-called *red chlorophylls* in the PSI antenna systems, (5) the emission spectrum of the excited RC.

EXCITATION ENERGY TRANSFER BETWEEN FMN MOLECULES MONITORED BY FLUORESCENCE INTENSITY DECAY

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Flavins play an important role in biological processes. The existence of FMN dimers in biological and photoreceptor systems has been shown by many authors. Presence of dimers may be important in photoreception phenomena. Therefore it is very interesting to examine the nonradiative energy transfer processes in the ensemble of FMN monomers and dimers.

The task of this paper was to abtain additional information on the mechanism of monomer – dimer energy transfer for FMN in water using time-resolved techniques. The FMN concentrations (from $1.05 \cdot 10^{-5}$ M to $2.84 \cdot 10^{-1}$ M) were prepared in water (pH 7.0). Mean fluorescence lifetimes were determined from fluorescence intensity decays using the Fluotime 200 spectrofluorometer (Picoquant) ($lambda_{ex}$=473nm).

It was found that at low concentrations, in the absence of energy migration and trapping, the FMN fluorescence intensity decay is single exponential. From C= $1.98 \cdot 10^{-2}$M the decay is accelerated with initially slight deviations from single exponential character because of multistep energy transfer between FMN monomers and trapping. It was found that fluorescence intensity decays are strongly accelerated in the presence of dimers due to excitation energy trapping. The mean fluorescence lifetime of FMN at low concentration t= 4.67 ns, whereas at the highest concentration it attains 0.43 ns. Mean localization time of excitation energy and the number of its jumps between FMN molecules were calculated versus concentration by Monte–Carlo method.

The localization time $\tau_{loc}=\tau_0 /(n+1)$, where n is the mean number of excitation energy jumps. The mean fluorescence lifetime was significantly longer than the mean localization time at monomers because excitation energy walks randomly within the set of monomers and is transferred finally to dimers. The effect of material diffusion enhances the efficiency of energy migration.

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THE PHOTOACOUSTIC SIGNAL OF SCOTS PINE NEEDLES FROM DIFFERENT ENVIRONMENTS

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At present, photoacoustic spectroscopy is one of the measuring techniques providing detailed information on the photosynthesis system and structure of plants. Unfortunately, the applicability range of most of parameters is limited to the homogeneous samples. However, as evidenced in our previous studies (Szurkowski, 2001), such a condition is not fulfilled in the case of Scots pine (*Pinus silvestris* L.) needles.

The aim of the work was to demonstrate the differences in the photoacoustic signal of Scots pine needles from different environments. The needles used in the studies were collected in the spring of 2011. The sampling stations were situated in the Tri-city (Gdańsk) area and in a place about 100 km from the agglomeration (Sominy).

Since the measurements were performed at the modulation frequency of 20 Hz, the photoacoustic signal originated from mesophyll of parenchyma cells containing chloroplast, for Scots pine needles. Both the signatures of the photoacoustic signal amplitude, in the presence of the strong light background (i.e., equivalent of adsorption spectrum in photoacoustic spectroscopy), and of the signal phase, measured under the condition of the sample illumination with the measuring light beam alone, exhibited an apparent variability with the needle age and place of the sample collection.The variability of the photoacoustic spectra had already been studied earlier, for Scots pine needles, although the phase spectra were obtained and interpreted here for the first time. These phase spectra allow one to determine the change in oxygen evolution yield with the light wavelength of the irradiation. With the needle aging, the ratio of phase for the Soret to red bands decreases. For the samples collected in Sominy the ratio was significantly higher than determined for the samples originating from Gdansk center.

THE VOLTAGE AND FLUX CHARACTERISTICS OF TWO-MEMBRANE SYSTEMS UNDER DIFFUSIVE AND CONVECTIVE CONDITIONS

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The study was conducted for the two-membrane system with bacterial cellulose membrane (CB), and acetate cellulose membrane (N) and ternary solutions of water, ethanol and KCl. The membranes were placed in horizontal planes and separated three compartments with solutions of equal volumes . The Ag|AgCl electrodes located in outer chambers, 5 mm from the membranes surfaces, were connected to a voltage measurement circuit coupled with a computer. The outer chambers at the initial moment were filled with KCl solutions having a concentration of 10^{-5} mol l^{-1} whereas the chamber between the membranes was filled with ternarny solution with varying concentrations of ethanol and KCl at initial moment (turning off mechanical stirring of solutions). The time characteristics of the voltage between the electrodes were measured after turning off mechanical stirring of solutions. The membrane systems with upper membrane – CB and the lower membrane - N (and vice versa) were analyzed. After turning off mechanical stirring of solutions, the concentration boundary layers (CBLs) were formed at surfaces of the lower and upper membrane. In the case when density of the solution in the chamber between the membranes was lower than density of solutions in external chambers, the convection conditions in CBLs could appear at upper membrane surfaces and did not appear at surfaces of the lower membrane. The convective stirring of the solutions was the cause of voltage pulsation in time due to the periodical changes of KCl concentrations at the surface of one of the electrodes. Analyzing of received voltage characteristics it can be stated that the moment of appearance of voltage pulsations (connected with hydrodynamic instabilities in the membrane system) depend non-linearly on the initial concentrations of ethanol and KCl in the chamber between the membranes. Moreover, the nature and temporal evolution of voltage in the membrane system depend on the initial concentrations of ethanol and KCl in the chamber between membranes. The characteristics of the initial time after which the pulsations arise as a function of concentration of ethanol have also complex and non-linear character.

CONVECTION DISORDERS OF KCl CONCENTRATIONS IN THE MEMBRANE SYSTEM: TIME CHARACTERISTICS OF VOLTAGE FOR DIFFERENT ELECTRODE DISTANCES FROM THE MEMBRANE

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The studies of the membrane system with bacterial cellulose membrane, placed in horizontal plane, with aqueous solutions of KCl were carried out. Both Ag|AgCl electrodes were placed in the chamber with an initial KCl concentration equal to 10^{-5}mol l^{-1}. One of the electrodes (the reference electrode) was located 14 cm from the surface of the membrane and the second electrode (active) was placed at different distances from the membrane in the range from 1 to 12 cm. Initial KCl concentration in the second chamber was 10^{-2} mol l^{-1}. After filling the chambers with solutions of KCl and equalization of the pressure in the chambers the voltage between the electrodes was measured. The solution with higher KCl concentration (solution with higher density) was placed above the membrane. In this case, diffusive formation of concentration boundary layers (CBLs) at the surfaces of the membrane could lead to the hydrodynamic instability and could cause convective stirring of solutions in chambers. These instabilities occur when the solution density gradient in CBLs, directed oppositely to the gravitational field vector, reaches sufficiently large value for which the calculated Rayleigh number for CBL reaches critical value (1700) . The disorders of KCl concentration at the electrode surface, observed as voltage pulsations in time, were caused by hydrodynamic instability in chambers of the membrane system. The determination of the dimension of the convection cell (by changes of position of active electrode) and analysis of time needed to the appearance of voltage pulsations was the aim of the study. According to the preliminary measurements, sift of the active electrode further away from the membrane causes increase of time after which the pulsations of voltage caused by hydrodynamic instabilities appear. The time required for the appearance of concentration disorder at the surface of the active electrode, connected with the convection stirring of solution, is proportional to the distance of electrode from surface of the membrane.

TIME-RESOLVED SPECTROSCOPY OF *Arabidopsis thaliana* LEAVES

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Light absorbed by plants is split into three major parts: photosynthesis, fluorescence, and heat. In this work we describe the results of time-resolved fluorescence spectroscopy obtained for Arabidopsis thaliana wild-type and recessive mutant *npq4-1*, deprived in the PsbS protein. Prior to the experiment parts of the plants (approximately halves of rosettes) were exposed to excess light at the wavelength of 620 nm. In the case of the mutant, the fluorescence decay exhibits drastic shortening, and measured transients are independent upon the excitation power. In addition, decays measured for non-illuminated parts of the plants are generally longer than for the illuminated parts. Wild-type plants feature much weaker response to the excess light. This result indicates a key role of PsbS protein in regulation of excess energy dissipation.

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PROTEIN-QUANTUM DOTS CONJUGATES

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Colloidal quantum dots (QD) are crystals of semiconductor materials (e.g. CdSe or CdTe), of diameter from few to several nanometers. QDs are of special interest of (bio)nanotechnology because of its spectral properties - broad absorption spectrum, size-tunable and narrow emission spectrum and significant resistance for photobleaching [1]. Surface of quantum dots may be modified with other molecules. When biological molecules are used, the product is called nanohybrid. Application of proteins for modification of QD surface increase usefulness of hybrid, depending of the protein properties. Here we present covalent conjugates of QD with enzymatic protein, ferredoxin-$NADP^+$ oxidoreductase (FNR) [2], examining the changes in protein and QD properties by several techniques. FNR is photosynthetic protein, involved in electron transfer from photosystem I to NADPH or plastoquinone pool. FNR is also oxidoreductase of potential biotechnological application. We are showing that FNR-QD hybrid sustained its activity, however parameters of enzyme kinetics were impaired.

We also present noncovalent, stable conjugates of QDs and membrane-scaffold proteins (MSP) [3] as novel option for hydrophobic QDs solubilization. Application of MSP as a QD cover was possible due to amphipathic character of these proteins, usually used for formation of lipid nanodiscs. We developed a procedure, resulting in nanohybrids of monomeric QDs, stable for several days in water solutions as well as sustaining its fluorescent properties. Such methods may results in easier preparation of water-soluble QD-protein conjugates.

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ANTICANCER ACTIVITY OF NOVEL FERROCENYL-FLAVONE COMPLEXES

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Bioorganometallic chemistry focuses on the biological function of organometallic compounds. A special efforts have been dedicated toward ferrocene-containing anticancer agents. In this field compounds based on the conjugation of the ferrocenyl moiety with a biologically relevant molecule are of special interests. Recently conjugates of ferrocenes with flavonoids have focused the attention of scientists as a new promising class of biologically active compounds for medical application.

In this study we have evaluated the cytotoxic and cytostatic properties of novel ferrocenyl-flavone compexes (Fc): (*E*)-6-ferrocenylvinyl-chromen-4-one (4), (*E*)-6-ferroce-nylvinyl-2-methyl-chromen-4-one (5), (*E*)-6-ferrocenyl-vinyl-2-phenyl-chromen-4-one (6) and (*E*)-6-ferrocenylvinyl-chromen-4-one-3-propionic acid (7) against a range of human cancer cell lines derived from estrogen-responsive (MCF-7) and estrogen-negative breast adenocarcinoma (MDA-

MB-231), hepatocellular carcinoma (HepG2) and T lymphoblast-like polymorph cells (CCRF-CEM). The cells were exposed for 24-72 h to a range of Fc concentrations (0-120 μM) and the fraction of viable cells were estimated by MTT test.

CCRF-CEM cells were the most sensitive to investigated ferrocenyl-flavones showing the lowest IC_{50} concentration (37.5 ± 0.9 μM) for compound 4. The remaining cell lines expressed little response (IC_{50} > 120 μM). Prolonged exposure to low doses of Fc promoted HepG2 cell proliferation, which was an undesirable effect in the context of the assumed anticancer potential of these compounds. A different degree of ferrocenyl-flavone cytotoxicity and differences in their antiproliferative properties suggest both SAR (*structure activity relationship*) and the specific response of human cancer cells to these bioorganometallic compounds.

COMPARISON OF ANTIBODY BINDING WITH NATIVE *Proteus mirabilis* (S1959) O3 LIPOPOLYSACCHARIDE AND ARTIFICIAL EPITOPE LYS-GALA-PAA

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The focus of the presented studies is lipopolysaccharide isolated from *Proteus mirabilis* (1959) O3 strain, one of human opportunistic pathogens. The peculiar feature of O3 LPS is the presence of 6 N^{alpha}-(D-galacturonoyl)-L-Lysine residues recognized by human and rabbit antibodies (Kononov L.O., et al. Clycoconj J, 1991, Knirel Y.A, et al. Inn.. Immun. 2011). Immune complexes of rabbit antibodies with native O3 LPS and synthetic, artificial epitope Lys-GalA coupled to polyacrylamide (Lys-Gal-PAA) were tested by a label-free optical detection techniques. Two methods - Total Internal Reflection Ellipsometry (TIRE) and atomic force microscopy (AFM) were used. The TIRE experimental was set-up with SE 800 SENTECH spectroscopic ellipsometer operating in the spectral range of 280-850 nm. The lowest amount of LPS *Proteus mirabilis* (S1959) O3 and artificial epitope Lys-GalA-PAA that bind anti-O3 antibodies were 0.5 and 0.00195 mg/ml, respectively. The measurements of adsorbed immunocomplexes were carried out and ellipsometric parameters $\Psi(\lambda)$ and $\Lambda(\lambda)$ for different incident wavelength in a spectral range between 400 and 850 nm were obtained. The wave shift changes between phases, depending on the two antigens (O3LPS and Lys-GalA-PAA) were observed. Atomic force microscopy (AFM) experiments were carried out using Magnetic AC mode. The images were acquired at 20^{o}C in a PBS aqueous solution on the samples previously used for ellipsometric experiments. The RMS surface roughness was determined from AFM topography for the O3 LPS or Lys-GalA/-PAA and anti O3 rabbit serum immobilized on a gold surface. The measured values were 3.02 nm and 3.84 nm respectively and reflected the differences in a molecular structure of the components forming immunocomplexes. In conclusion, TIRE and AFM methods confirmed their efficiency in immuno-complexes studies.

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THE REGULATION OF LIPOSOME AGGREGATION PROCESSES IN MOLECULAR CROWDING CONDITIONS FOR LIPOSOMAL TRANSDERMAL DRUG FORMULATIONS

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Liposomes as enhancers of transdermal drug delivery have been firstly described in 1980. Mezei (Mezei M., Gulasekharam V., Life Sciences, 1980, 26 (18), 1473-77) presented experimental data showing that the concentration of steroids in the skin was five times higher when liposomes were used in the formulation. Since then it has been demonstrated in numerous studies that liposomes can serve as a permeability enhancers for anesthetics, antibiotics and many other biologically active compounds.

Despite years of studies the exact molecular mechanisms of liposome permeability enhancement have not been determined. In order to correctly identify the liposome mode of action, the rarely used parameter needs to be considered, namely the molecular crowding. The high density of macromolecules, aggregates or polymers results in altered water activity therefore the state of biological structures can be modified and this in turn will change the activity of the compound of interest, mainly by altering its pharmacokinetic profile.

The dermis is a highly crowded space therefore the crowding effect needs to be accounted for regardless on the exact physicochemical nature of the compound-skin interaction. It has been shown, for example, that liposomes aggregate and fuse when dehydrated using highly hydrophilic polymers.

Having all that in mind, the method to measure the liposome (LUV) aggregation process induced by the macromolecular crowding is proposed. The method is based on the fluorescence resonance energy transfer (FRET) so the membrane fusion can be detected and quantitated. Using this method the liposome composition, which ensures their stability in crowded spaces

therefore making the liposomal formulation stable, has been determined. Controlling the aggregation process opens the door for designing the encapsulated compound release triggering mechanism, which would depend exclusively on the molecular density of the environment.

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LUMINESCENCE OF UPCONVERTING Gd_2O_3: (Zn^{2+}, Er^{3+}, Yb^{3+}) NANOPARTICLES

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Gadolinium oxide (Gd_2O_3) due to its chemical stability, thermal stability, high melting point (~2320^oC) and low phonon energy (phonon cutoff 600 cm^{-1}) works very well as a host matrix for upconversion [1]. Low phonon energy decreases the probability of non-radiative relaxation thus increasing the quantum yield of upconversion process. The wide bandgap - 5,4 eV allows on easily doping with rare earths (RE) luminescence ions [2, 3].

Such RE doped material is, due to the high density (ρ=7.6 g/cm^3), suitable for use in X-ray detection and imaging. The properties of Gd_2O_3 make it applicable in contrast-enhanced magnetic resonance imaging (MRI) technique. RE-doped Gd_2O_3 opens up new perspectives for selective treatment of local tissues and for early diagnosis neoplastic diseases.

We synthesized Gd_2O_3 nanoparticles doped by Er^{3+}(1%) Yb^{3+}(18%) ions by the solution combustion method with adding Zn to starting materials. Entering of zinc ions into the Gd_2O_3 matrix introduces oxygen vacancies, leading to an increase of photoluminescence intensity due to reduction of the site symmetry of rare earth ions [4].

Transmission electron microscopy, scanning electron microscope and X-ray diffraction served for characterization of the studied nanoparticles. Using photoluminescence techniques we investigated the relationship between the intensity of emission and the excitation power, providing information on the amount of photons involved in the upconversion process. The quantum yield of photoluminescence was determined for Gd_2O_3: Er^{3+},Yb^{3+} nanoparticles as a function of Zn concentration in the starting materials and of excitation power. The highest quantum yield of the studied nanoparticles is 0.09% upon 980 nm excitation (continuous wave) for nanoparticles about 70 nm diameter.

Because of the good quantum yield this material is adequate for biomedical imaging. The nanoparticles were passivated with PVP and introduced into HeLa tumor cells. We examined their location inside HeLa cells for various incubation times and nanoparticles concentra-tions.

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THE EFFECT OF EXTRACT FROM PRIMROSE (*Oenothera paradoxa*) ON ERYPTOSIS INDUCED BY TERT-BUTYL PEROXIDE *in vitro*

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Oenothera paradoxa is a rich source of polyunsaturated fatty acids, particular linoleic acid. Human organism is not able to synthetize those acid, thus they must be supplied with diet. Because polyunsaturated fatty acids plays different functions in human organism

including regulation of various biochemical processes they are commonly used in the prevention of numerous diseases, which are associated with oxidative stress.

The purpose of the study was to assess if extract from primrose has protective role on eryptosis induced by tert-buthyl peroxide in vitro. The erythrocytes (5% hematocrit) where preincubated with extract from primrose at concentrations ranging from 5 to 20 mg/ml for 30 min. Then, the samples were washed and incubated with tert-butyl peroxide in the concentrations of 200, 300 and 400 μM.

Treatment of the erythrocytes with different concentrations of primrose or tert-butyl peroxide at 200 μM and 300 μM did not enhance eryptosis in comparison to control sample, where tert-butyl peroxide at 400 μM caused 10% eryptosis.

Preincubation of the erythrocytes with different concentrations of oil from primrose for 30 min and 1 hour incubation with tert-butyl peroxide at 200 and 300 μM did not cause eryptosis. However, the treatment of the cells with tert-butyl peroxide at 400 μM and different concentration of oil from *Oenothera paradoxa* induced 30% eryptosis. These results show that the oil from *Oenothera paradoxa* seeds synergistically with the highest concentration of tert-butyl peroxide

THE EFFECT OF EXTRACT FROM PRIMROSE (*Oenothera paradoxa*) ON HUMAN ERYTHROCYTES EXPOSED TO OXIDATIVE STRESS INDUCED BY TERT-BUTYL PEROXIDE in vitro

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Oenothera paradoxa is a rich source of polyunsaturated fatty acids. Human organism is not able to synthetize those acid, this they must be supplied with diet. Diet supplementation with oil from primrose has beneficial effects because it plays a essential role in the immune response reactions, with are associated with alterations of pro- and antioxidative balance. Oxidative stress occurs when reactive oxygen species formation exceeds antioxidative capabilities of the cell, with contributes do damage to cellular components.

The purpose of the study was to assess whether extract from primrose may protect cells from oxidative stress induced by tert-buthyl peroxide in vitro. The erythrocytes (5% hematocrit) where preincubated with extract from primrose at concentrations ranging from 5 to 20 mg/ml for 30 min. Then ,the samples were washed and incubated with tert-butyl peroxide in the concentrations of 200, 300 and 400 μM. In the study hemolysis, lipid peroxidation, methemoglobin formation the level of hydroksyl radical and catalase activity were assessed.

The conducted analysis showed that the oil from primrose did not cause any changes in hemolysis, lipid peroxidation, hemoglobin oxidation, catalase activity or hydroxyl radical level. Preincubation of the erythrocytes with extract from primrose and following incubation with tert-butyl peroxide at 300 and 400 μM, caused an increase of all parameters studied.

The obtained results show synergistic effect of the extract from primrose and tert-butyl peroxide in human erythrocytes.

EFFECT OF Ca^{2+} IONS ON THE ACTIVITY OF VACUOLAR ION CHANNELS IN *Physcomitrella patens* MOSS

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A majority of environmental stimuli cause an increase in the cytoplasmic calcium $[Ca^{2+}]_{cyt}$ concentration in plant cells. Changes in the concentration of this ion initiate a series of processes, including regulation of the activity of ion channels in cell organelle membranes. One of the largest organelles with calcium-dependent ion channels is the vacuole.

Previous research on vacuolar channels in higher plants indicates their high dependence on $[Ca^{2+}]_{cyt}$. SV and VK are among channels that open at high $[Ca^{2+}]_{cyt}$. Both types of channels are activated upon binding of Ca^{2+} to the EF motifs located on the cytoplasmic side of the vacuolar membrane (tonoplast). The dependence of SV and VK channels on varies $[Ca^{2+}]_{cyt}$. SV channels open at concentrations higher than 10 μM, whereas VK channels are active at concentrations of up to 5 μM. The activity of SV channels is additionally influenced by vacuolar calcium $[Ca^{2+}]_{vac.}$, whose increased concentration causes a decrease in channel activity.

Until now, there have been no investigations of SV and VK channels in the vacuoles of lower plants. The moss *Physcomitrella patens*, whose genome comprises protein coding sequences similar to the SV and VK channels in higher plants, is a model organism.

The investigations were carried out using the patch-clamp method at different concentrations of $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{vac}$. The measurement conditions applied facilitated simultaneous observation of the activity of the SV and VK channels. SV channels were shown to be more dependent on $[Ca^{2+}]_{cyt}$ than the VK channels, which opened in the absence of Ca^{2+} in the cytoplasm. This trait was not observed in the VK channels investigated previously in higher plants. Experiments performed in the absence of Ca^{2+} in the vacuole revealed increased numbers of active SV channels, which more likely to open in these conditions.

CHANGES IN THE BIOELECTRICAL POTENTIAL GENERATED IN *Arabidopsis thaliana* LEAVES AND STEM BY AN INJURY STIMULUS

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Arabidopsis thaliana is a model organism and a highly valued research material for investigations of excitability and transmission of the bioelectrical potential. Bioelectrical changes propagating over long distances include action potentials (AP) and variation potentials (VP). AP and VP differ from each other considerably, but both have a fundamental signalling role in plants [Fromm, Lautner 2007].

The present investigations were conducted on three strains of *Arabidopsis thaliana* ecotype Columbia: a wild-type strain (WT) and two strains with a knocked out gene encoding the potassium channel, i.e. AKT2-2 and Gork [Michard et al. 2005]. We used an extracellular method. The investigations were carried out in two experimental systems, in which electrodes were inserted into plant stem or leaves. An injury stimulus (burn) was applied, which evoked action and variation potentials recorded in all the analysed strains. The mean velocity of propagation of the bioelectrical changes elicited by leaf blade burning was 2 $mm \cdot s^{-1}$ (AKT2-2), 1 $mm \cdot s^{-1}$ (GORK), and 0,4 $mm \cdot s^{-1}$ (WT). The mean value of the amplitude of bioelectrical changed generated in the leaves was 85 mV (AKT2-2), 47 mV (GORK), and 40 mV (WT). The bioelectrical changes induced by burn injury in the stem of the AKT2-2 strain were primarily variation potentials. The mean amplitude of these changes was 65 mV and its propagation velocity was 2 $mm \cdot s^{-1}$.

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THE INFLUENCE OF FULLERENOL $C_{60}(OH)_{36}$ ON HUMAN PERIPHERAL MONONUCLEAR BLOOD CELLS

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The aim of this work was to assess the influence of fullerenol ($C_{60}(OH)_{36}$) on human peripheral mononulear blood cells (MNBC) under oxidative stress induced by hydrogen peroxide.

MNBC ($1x10^6$cells/ml) were incubated with fullerenol (75 mg/L or 150 mg/L) and/or H_2O_2 (0.5 mM). Cell viability by Trypan Blue assay, LDH release, caspase 3 activity, mitochondrial membrane potential with potentiometric dye $DiOC_{6(3)}$ as well as cytofluorimetric assay of size (FSC) and granularity (SSC) of MNBC were determined. On the basis of changes in the viability it was assessed that fullerenol was toxic to the cells but the decrease in viability was lesser than after H_2O_2 treatment. Fullerenol combined with H_2O_2 did not produce synergistic effect on MNBC. It was observed that $C_{60}(OH)_{36}$ neither influenced the LDH release nor caspase 3 activity. However, when MNBC were treated with fullerenol combined with H_2O_2 the caspase 3 activity decreased. Fullerenol at 75 mg/L did not influence the mitochondrial membrane potential of the cells, whereas fullerenol at 150 mg/L decreased the potential. Fullerenol at both concentrations protected from H_2O_2-induced mitochondrial potential decrease after 3-hour incubation. Fullerenol did not influence the size of MNBC whereas the cells granularity increased proportionally to the fullerenol concentration. Fullerenol combined with H_2O_2 induced the greater increase of the granularity of the cells than H_2O_2 itself.

In conclusion, fullerenol at 150 mg/L decreased the viability and mitochondrial potential of the cells and increased granularity of MNBC. Changes induced by fullerenol, especially at 150 mg/L, were comparable to changes induced by H_2O_2. However, fullerenol at both concentrations protected from H_2O_2-induced mitochondrial potential decrease.

APPLICATION OF INHIBITORS OF SELECTED METABOLIC PROCESSES FOR IDENTIFICATION BIOLOGICAL SOURCE OF BIOSPECKLE PHENOMENON IN APPLE TISSUE

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The biospeckle phenomenon – dynamic interference pattern formed by scattering of coherent light on living objects – is used in experimental, non-destructive methods of evaluation of fruit and vegetables quality. The physics of biospeckle is well-developed, but biological background of biospeckle of plant tissues is not clearly defined. Biospeckle activity (measure of biospeckle dynamics) is the result of movement inside the tissue, therefore physical (diffusion, Brownian motion) and biological processes (cytoplasmic streaming, organelle movement, cell division and growth) as a sources of biospeckle activity are considered.

The goal of this study was to investigate the effect of: cytochalasin B (CB), lantrunculin B (LB), colchicine (CO), cycloheximide (CY) and a mixture of ion channel inhibitors (ICI) as a substances non-

destructively and selectively influencing the processes associated with the movement in the cell, on biospeckle activity. CB inhibits polymerization and LB causes depolymerization of actin filaments, and both cease transport associated with the actin cytoskeleton as well as cytoplasmic streaming. CO prevents microtubule polymerization, stopping their reorganization. CY - an inhibitor of translation - blocks protein synthesis and can potentially reduce the number of emerging and moving scattering centers in the cytoplasm in the form of the protein. Since changes in intracellular ions concentration alter cytoplasmic streaming, an ICI, blocks the transport of H^+, K^+, $Ca^{2+}$and A^-, and can affect movements of cytoplasm and a number of secondary processes.

Results indicate that about 74% of biospeckle activity is caused only by biological processes and reconstruction of actin filaments and functioning of ion channels are a main sources of biospeckle activity in case of apple tissue.

HEMOLYTIC AND OXIDATIVE PROPERTIES OF METABOLITES AND IMPURITIES OF GLYPHOSATE

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The toxicity of herbicides is an issue of worldwide concern. Glyphosate [*N*-(phosphonomethyl) glycine] is used all over the world to protect agricultural and horticultural crops and it's not safe as it had been considered before. Poisonings still pose a challenge and problems for toxicological investigations.

The present study was undertaken to evaluate the toxic potential of a widely used glyphosate, its metabolites: aminomethylphosphonic acid, methylphosphonic acid and impurities: *N*-(phosphonomethyl)iminodiacetic acid, *N*-methylglyphosate, hydroxymethylphosphonic acid and bis-(phosphonomethyl)amine. The analysis of noxious effects of metabolites and impurities seem to be very important to evaluate the toxicological risk that is exerted by these substances (EU regulations 1107/200/EC).

The erythrocytes were exposed to different concentrations of these compounds (0.01; 0.05; 0.1; 0.25; 0.5; 5mM) for 1 and 4 h.

We evaluated the effect of these compounds on hemolysis, hemoglobin oxidation and reactive oxygen species formation in human erythrocytes. Moreover, the changes in the size (FSC-A) and the shape (SSC-A) of red blood cells were assessed using flow cytometry.

It was proven that glyphosate at the highest concentration 5 mM during 4 h incubation changed the parameters examined in human erythrocytes, except FSC-A and SSC-A parameter. Glyphosate metabolites and impurities increased hemolysis (about 1 %) and methemoglobin level (about 1.5 %) but did not change the size and shape of the erythrocytes. The changes were observed only for the concentrations ranging from 0.5-5 mM. Most of the investigated compounds induced ROS formation from 0.25 mM (increase ROS level about 20%), except the *N*-methylglyphosate that caused changes from 0.5 mM. The investigated metabolites and impurities caused stronger damage to human erythrocytes than glyphosate itself after 4 h incubation.

BASIC HIPPOCAMPAL CELL RESPONSES TO VIOLOGEN-PHOSPHORUS DENDRIMERS

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Two zero generation (G0), water-soluble viologen-phosphorus dendrimers (VPD) were tested on murine hippocampal cells. VPD belong to the new class of dendrimers characterised by the presence of viologen moieties and phosphorus atoms in their structure. Several biological properties of this group of dendrimers have been already discovered, among others, the antibacterial activity, toxicity to B14, N2a cell lines and erythrocytes, as well as the effect on cholinoesterases involved in neurodegenerative diseases. Nevertheless, due to the lack of data on the influence of these new type of dendritic compounds on cell processes, we analysed chosen cell responses after 24 h treatment with five concentrations of VPD (1 - 20 μM). Performed tests comprised cytotoxicity assay, generation of reactive oxygen species (ROS), oxidative activity of mitochondria, mitochondrial membrane potential ($\Delta\Psi m$) alterations, and changes in catalase activity. The results revealed only small changes in cellular processes, indicating that VPD are only slightly toxic to mouse hippocampal cells. Interestingly, in contrast to other well-known classes of dendrimers, VPD seem to possess weak antioxidative activity, which may be very useful feature in the context of their potential biomedical applications. In general, these compounds can be considered good candidates for further studies.

VIOLOGEN-PHOSPHORUS DENDRIMERS DO NOT INDUCE CELL DEATH IN MOUSE HIPPOCAMPAL CELLS

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Viologen-phosphorus dendrimers (VPD), possessing both phosphorus atoms and viologen groups in their architecture, are new class of dendritic compounds. The biomedical potential of VPD is dependent on their cytotoxicity but so far, only MTT test was performed to examine the impact of these dendrimers on cell viability. For this reason, we performed a number of

tests to check if VPD induce apoptosis or necrosis, which included double staining methods, DNA fragmentation assay, cell cycle analysis, and assessment of cell morphology. We chose embryonic mouse hippocampal cell line (mHippoE-18) to analyse the cell condition after 24 h treatment with two zero generation (G0) VPD (VPD1 and VPD2). These two dendrimers differ in the core structure, the number of viologen units and surface groups. The results show that, at the tested range of concentrations, VPD caused only slight induction of apoptosis and alterations in the cell cycle phases distribution in mouse hippocampal cells, while no changes in the cell morphology were observed. These findings indicate that VPD can be considered as relatively safe compounds, useful for further biomedical investigations.

EVALUATION OF THE OXIDATIVE PROPERTIES OF HYBRID NANOSPHERES IN HUMAN BREAST CANCER CELLS

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Development of novel and effective non-toxic compounds with anticancer activity has been receiving a considerable attention because of the increasing number of deaths caused by cancer. Recently, the casein kinase CK2 has been shown to play crucial role in proliferation and apoptosis of cancer cells. Overexpression of this enzyme has been demonstrated as associated with the progression of cancer. Nanoparticles like polyoxometalates (POM) act as effective inorganic inhibitors of protein kinase CK2 and thus have ability to neutralize its activity. Little is known about other mechanisms of their activity in cancer cells.

This study aimed at evaluating the ability of PA66/POM hybrid nanospheres (POM clusters stabilized within non-toxic polyamide 66) to generate oxidative stress in cancer cells. For this purpose GSH, -SH groups and total antioxidant potential of estrogen-responsive MCF-7 breast cancer cells treated with PA66/POM have been estimated.

The cells were incubated with different amount of PA66/POM for 0, 24, 48 and 72 h and then subjected to analysis. Microplate spectrofluorimetric method with O-phthaldialdehyde (OPA) has been used for estimation of cellular GSH. OPA reacts with GSH which generates fluorescence allowing its specific quantification. Content of –SH groups was determined by the EPR spectroscopy using spin labeling with RSSR [bis(2,2,5,5-tetramethyl-3-imidazoline-1-oxyl-4-il)disulfide], a stable biradical nitroxide containing disulfide bond. The total antioxidant potential of cells was determined using 2,4,6-tripirydyl-s-triazine (TPTZ) method based on reduction of Fe^{3+} to Fe^{2+} in low pH conditions which yields formation of a colored complex with 2,4,6-tripirydyl-s-triazine (TPTZ). The investigation showed that PA66 and PA66/POM hybrid materials caused a time-dependent decrease in the level of all of the investigated parameters which could suggest the generation of oxidative stress in cancer cells by these nanomaterials.

ELECTRON PARAMAGNETIC RESONANCE (EPR) SPECTROSCOPY IN THE INVESTIGATION OF OXIDATIVE STRESS IN PLANTS

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Electron paramagnetic spectroscopy (EPR) is a technique used in the study of paramagnetic materials such as organic and inorganic radicals, transition metal ions and rare earth metal ions, appearing in solids and solutions. Due to the high sensitivity, EPR is widely used in biology. Not being a destructive method, it allows measurement of biological material without damage of the integrity and structure of the tissue. The aim of our experiment was the characteristics of EPR spectra of wheat grains, which show different sensitivity to oxidative stress and leaves of the seedlings obtained from these grains, cultured in stress conditions. Oxidative stress was induced in plants by differentiating the water uptake by root system by application in the hydroponic conditions of polyethylene glycol (PEG 6000) or NaCl.

EPR studies and elemental analysis of grains and seedlings showed the presence of Mn(II), Fe(III) and Cu(II) in higher contents in sensitive genotypes. Besides, in investigated tissues some stable organic radicals, localized in protein and carbohydrate structures were found. The content of radicals differentiated sensitive and tolerant wheat genotypes. The number of radicals in grains and seedlings of control plants (not stressed) of sensitive wheat genotypes was higher and increase in the stress conditions. The dependence between content of radicals and osmotic stress intensity was also established.

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COUPLING OF GOODWIN'S LOOPS OF REPRESSION AND INDUCTION

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Circadian rhythms are generated in a cell by oscillatory systems in the net of transcription factors. Negative feedback loops constitute necessary components of such systems. We consider two coupled Goodwin's loops. The loop of repression has negative feedback and the loop of induction has a positive feedback. Both loops are coupled by a common promoter. Transcription of the two respective genes takes place simultaneously at high concentration of the inductor and low concentration of the repressor. We analyzed numerical solutions of the mathematical model in two kinds of systems. 1. Both loops have the same number of elements and have identical rate constants. 2. The loop of induction is shorter or longer than the loop of repression. Oscillations have the shortest period when the induction loop is by one or two elements longer than the loop of repression. The period increases and approaches to a constant value at longer induction loops. The oscillations are slower at shorter loops of induction. They are fully damped when the induction loop is by three or four elements shorter than the repression loop.

METAL-ENHANCED FLUORESCENCE OF AMPHOTERICIN B

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In this work we used confocal fluorescence microscopy to investigate the influence of plasmonic excitations in metallic nanoparticles on the fluorescence intensity of Amphotericin B (AmB), a molecule with important pharmaceutical function, in particular for serious systemic mycoses. For AmB molecules placed in the vicinity of silver nanowires synthesized in aqueous solution we observed strong increase of fluorescence emission when using 405 nm excitation wavelength. This excitation wavelength is resonant with plasmon excitation in the silver nanowires. We find that the emission intensity for the Am molecules located at the ends of the nanowires is stronger as compared to the AmB molecules located along the wires. Fluorescence decays measured for the AmB molecules coupled with silver nanowires remain unaffected by the coupling, suggesting increase of excitation rate as a source for the observed increase of fluorescence intensity. The results can be a starting point for further optimization of the design of a hybrid nanostructure composed of AmB and silver nanowires for achieving more efficient fluorescence detection and describing in detail molecular arrangement of AmB in biologically relevant architectures.

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EFFECT OF TIME ON LIPOSOME MEMBRANE FLUIDITY DOPED BY LIPOPOLYSACCHARIDES OF *Hafnia alvei* STRAIN PCM 1200: ESR STUDY

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Liposomes prepared from egg yolk lecithin (EYL) by sonication method were doped with lipopolysaccharide (LPS) of *Hafnia alvei* strain PCM 1200 in following concentrations: 16.8, 5, 0.5% in molar ratio to lecithin. To monitor the fluidity at different depths and different regions of the membranes two spin probes were used. Spin probe 2, 2, 6, 6-tetramethylpiperidine-1-oxyl (TEMPO) can freely diffuse in the membrane and provide information about both the water and lipid phases. Thus, the ESR spectrum of TEMPO in a membrane is a superposition of two components coming from TEMPO in water and in lipid phase. The relative extent of partitioning of TEMPO between the hydrophobic and hydrophilic phases can be measured from the ra-tio of signal intensities. In this study the partition coefficient F was measured. It varies as a function of fluidity of surface membranes over time. Spin probe 2-(14-Carboxytetradecyl)-2ethyl-4,4-dimethyl-3-oxazolidinyloxy (16DOXYL) was used to measure fluidity change in the deep hydrophobic region of the lipid bilayer. The data was obtained by calculating the rotational correlation time τ which various over time. Studies conducted for 80 hours gave following results:

1. surface membrane stabilizing effect was observed in all LPS concentrations;
2. the strongest surface membrane stabilizing effect was induced by 5% LPS;
3. 5% LPS induced also a very strong stabilization effect deep in the lipid bilayer.

Liposomes doped with 5% LPS formed very stable structure with low sensibility over time in studied 80 hours period. Due to their properties liposomes modified in such way may be used as drug carriers.

MICRO- AND MACRORHEOLOGY OF NEWTONIAN FLUIDS AND COMPLEX MACROMOLECULAR SYSTEMS – - COMPARISON OF DYNAMIC LIGHT SCATTERING, OPTICAL TWEEZERS AND ROTARY RHEOMETRY

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Rheological properties of substances used in pharmaceutical industry are essential for production process and functionality of the final product. In this paper, a comparison of techniques determining the rheological properties at macro and micro scale is presented. Dynamic light scattering (DLS), optical tweezers and rotary rheometry were used in order to determine viscosity coefficient of medium. DLS method uses tracking of nanoscopic objects of known size to determine viscosity of studied medium while optical tweezers uses thermal fluctuations of probe of known properties. These two techniques allow to determine rheological properties of medium in microscale whereas rotary rheometry enables to obtain information about global viscosity. Measurements were carried out for two substances which differ in viscoelastic properties: propylene glycol and aristoflex AVC. Propylene glycol was measured at concentration in a range 10–50% and aristoflex AVC in concentration range 0.05–0.35%. In DLS and optical tweezers measurements polystyrene nanobeads as probes were used. Diameters of nanospheres were 50, 100, 200, 500, 800, 1000, 1500 and 2000 nm. The viscosity coefficient of propylene glycol obtained with DLS and rotary rheometry were consistent with tabulated values. The viscosity values of artistoflex AVC obtained with rotary rheometry were different from values obtained with DLS and optical tweezers technique. Moreover viscosity coefficient determined with DLS using probes of different diameter were significantly different. The results provided information on both mechano-elastic properties of complex materials as well as their level of structuring at the microscale.

THE EFFECT OF THE DUAL FLUORESCENCE IN SELECTED 1,3,4 THIADAZOLES

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The spectroscopy researches of the new and biologically active compound (4-fluorophenylamino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole (FABT) have been recently done. The compound is derived from the group of 1,3,4-thiadazoles. The compounds are characterized by antibacterial and antifungal features as well as they show a quite high level of the nerves protection and anticancer protection.

The researches which were done by using the fluorescence spectroscopy, allowed to observe the effect of the double fluorescence which was induced by the concentration of the hydrogen ions, the changes of the temperature as well as the aggregations effects in water environment. Regarding the analogical measurements which were done in methanol (as well as other solvents) only the single fluorescence was noticed.

After the process of the methanol acidification till pH 1, two separated, partly covered with each other bands were observed. Regarding the crystallography data as well as the fluorescence researches two types of the conformations FABT molecules were noticed: the conformation ”S” – type (including – OH group from resorcil ring which is placed on the side of the sulphur atom from 1,3,4-thiadazol ring. The “S” – type shows the single fluorescence band. The second type is the “N” – type with the same group as mentioned above, however, on the side of the nitrogen atom showing the double fluorescence.

The calculations which were done by using DFT methods show the differences in the energy between the two above conformations which is 3.2 kcal / mol. The rotary barrier in case of tested molecule, according to calculations is 12.6 kcal /mol. The analysis using oscillatory and spectroscopy method – FTIR as well as Roman method indicates that pseudo-hetero–aromatic system may have been created in the case of the “N” – conformation in FABT molecule.

This system may induce the setting inner molecular transfer of the CT load. Additionally this may led to the changes in chemical as well as biological features in the element. FABT molecules (as well as others from 1,3,4-thiadiazols group can be used as fluorescence probes which sensitive to pH changes and the polarization of the environment.

CHLORINATED PERSISTENT POLLUTANTS INDUCE APOPTOTIC ALTERATIONS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES (*In vitro* STUDY)

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Persistent Organic Pollutants (POPs) are chemical substances that persist in the environment, bioaccumulate through the food web, and pose a risk of causing adverse effects to human health. In this study, we have assessed the effect of selected POPs, i.e. 1,2,4-trichlorobenzene (1,2,4-TCB), hexachloro-benzene (HCB), lindane and dieldrin on apoptosis induction in human peripheral blood lymphocytes.

The cells were incubated with xenobiotics for 2 h for ROS analysis and for 4 h for analysis of other parameters. Using fluorescent probe H_2DCFDA we observed an increase in ROS formation in lymphocytes incubated with all of the compounds examined in the concentrations range from 0.05 to 5 μg/mL.

Analysis of changes in transmembrane mitochondrial potential ($\Delta\Psi m$) was conducted using JC-9 fluorescent probe. It was noted that chlorobenzenes, and particularly lindane and dieldrin in the concentrations ranging from 0.2 to 10 μg/mL increased the number of cells, which were characterized by $\Delta\Psi m$ reduction. ROS formation and changes in mitochondrial membrane potential could have affected caspase-3 activation, which was observed in the lymphocytes incubated with all of the compounds studied, particularly with lindane and dieldrin in the concentrations of 5 and 10 μg/mL.

Changes in cell's membrane permeability (test with YO-PRO-1) and translocation of phospha-tidylserine (test with Annexin-V conjugated with fluorescein) were assessed to confirm apoptotic alterations in human lymphocytes. It was found that all compounds studied from 0.2 to 10 μg/mL increased the above parameters in the incubated cells.

The observed apoptotic changes in human lymphocytes provoked by relatively low concentrations of 1,2,4-TCB, HCB, and particularly lindane and dieldrin suggest that these compounds can disturb function of immunological system among people environmentally, and in particular occupationally exposed to these substances.

TOWARD THE STANDARDIZATION OF OBTAINING CRITICAL MICELLE CONCENTRATION, ENTHALPY OF MICELLIZATION AND MICELLE IONIZATION DEGREE FROM ISOTHERMAL TITRATION CALORIMETRY AND CONDUCTOMETRY MEASUREMENTS

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Micellization is one of the most widely studied phenomenons occurring in solutions of amphiphilic molecules. Thermodynamics of micellization is characterized first of all by the critical micellar concentration (CMC). It is a measure of stability of the micellar form of solutes, like surfactants or amphiphilic polymers, with respect to their monomeric form in solution. The basic thermodynamic functions of the micellization process: Gibbs free energy, enthalpy and entropy of micellization, are obtained from measurements directly or indirectly, being calculated from the values of CMC and other parameters like e.g. degree of micelle ionization for ionic amphiphiles. Among a wide variety of experimental methods used for study micellization processes the isothermal titration calorimetry (ITC) and conductometry (for ionics) are of special interest. ITC allow obtaining in one measurement CMC and enthalpy of micellization, and conductometry CMC and degree of micelle ionization. Micellization is not an abrupt transition in the solution properties, but rather some continuous process of formation of molecular aggregates from monomers. Therefore there is not unique definition of CMC, and the obtained values depend also on the experimental method used. Additional problem in determining CMC from measurements arise due to very diverse behavior of different compounds during micellization. In the present work an attempt is presented to standardize the method of determining CMC, enthalpy of micellization and degree of micelle ionization based on data obtained from ITC and conductometry measurements, by fitting some mathematical functions. The proposed approach is compared with methods used in literature.

DETERMINATION OF A GLASS-TRANSITION TEMPERATURE FOR SOME MAMMALIAN ALBUMINS

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The temperature at which the properties of material change from liquid-like to solid-like is called glass-transition temperature T_g. In the present paper T_g for human serum albumin (HSA), equine serum albumin (ESA), ovine serum albumin (OSA), porcine serum albumin (PSA) and rabbit serum albumin (RSA) has been obtained from viscosity measurements of aqueous solutions of the albumins and from the Avramov's model. The viscosity measurements have been performed with an Ubbelohde-type capillary microviscometer at temperatures ranging from 278 K to 318 K and over a wide range of concentrations. For each protein the viscosity-tempera-ture dependence, for a fixed concentration, is analyzed on the basis of equation resulting from the Avramov's model. The model gives three-parameter dependence of liquid viscosity on tem-perature, and one of those parameters is T_g. It appears that the glass-transition temperature of a solution, for each studied albumin, increases with increasing concentration. To establish the glass-transition temperature of a particular albumin, in turn, a modified Gordon-Taylor formula is applied. The formula shows that the glass-transition temperature of a solution depends on both T_g for a dissolved albumin $T_{g,a}$ and for water $T_{g,w}$, and on a parameter describing the strength of the albumin-water interaction k. The glass-transition temperature for pure bulk water is well-known and its the most frequently cited value is $T_{g,w}$ = 136 K. The quantities $T_{g,a}$ and k has been taken as adjustable parameters in a modified Gordon-Taylor formula. Thus obtained numerical values of the parameters are as follows: $T_{g,a}$ = (245.5 ± 3.8) K, k =

0.2616 ± 0.0294 for HSA at pH 4.7; $T_{g,a}$ = (245 ± 6.2) K, k = 1.473 ± 0.154 for HSA at pH 7.0; $T_{g,a}$ = (217.1 ± 4.3) K, k = 1.435 ± 0.159 for ESA; $T_{g,a}$ = (217 ± 6.1) K, k = 0.7702 ± 0.1198 for OSA; $T_{g,a}$ = (215.5 ± 5.8) K, k = 0.5707 ± 0.089 for PSA; $T_{g,a}$ = (215.4 ± 7.0) K, k = 0.6624 ± 0.1102 for RSA.

SOME HYDRODYNAMIC PROPERTIES OF HUMAN SERUM ALBUMIN IN SOLUTIONS AT ISOELECTRIC POINT

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Paper presents a viscometric study of human serum albumin (HSA) aqueous solutions at isoelectric point (pH 4.7). Viscosity measurements were made with an Ubbelohde-type capillary microviscometer for concentrations from 9.5 kg/m^3 up to 328 kg/m^3 and at temperatures ranging from 278 K to 318 K. The viscosity-temperature dependence, for each concentration, was analyzed on the basis of a modified three parameters Arrhenius equation. Each of those parameters increases with increasing of a solution concentration. Analysis of these relations showed that, for HSA in solution at isoelectric point, the activation energy of viscous flow E = 8.77 10^5 kJ/mol, the entropy of the process of viscous flow S = 5.58 10^6 J/mol K and the effective specific volume x=1.78×10^{-3} m^3/kg. Hydrated HSA molecule was approximated by an ellipsoid of revolution with one long semi axis a = 8.2 nm and two shorter semi axes b = 2.1 nm. Dependence of the proteins solutions viscosity on concentration can be quantitatively described by the Mooney equation. One of the parameters in these equation is the self-crowding factor. Its numerical value for HSA at isoelectric point is 1.74 and it lies in the range (1.35 - 1.91) which was originally obtained by Mooney. At low concentrations, the intrinsic viscosity and the Huggins coefficient were obtained. The intrinsic viscosity, which measures a contribution of albumin to the viscosity of the solution, decreases from 6.46×10^{-3} m^3/kg (5^{0}C) up to 6.29×10^{-3} m^3/kg (45^{0}C). The Huggins coefficient, in turn, increases from 0.775 (5^{o}C) up to 0.782 (45^{o}C). The existence of three ranges of concentrations: dilute, semi-dilute and concentrated, was proved. In the semi-dilute regime, the Mark-Houvink-Kuhn-Sakurada (MHKS) exponent was calculated. It slightly increases with increasing temperature from 0.333 (5^{0}C) up to 0.343 (45^{0}C).

VISCOMETRIC STUDY OF BOVINE, OVINE, AND RABBIT SERUM ALBUMIN IN DILUTE, SEMI-DILUTE, AND CONCENTRATED AQUEOUS SOLUTIONS

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The viscosity of bovine serum albumin (BSA), ovine serum albumin (OSA) and rabbit serum albumin (RSA) aqueous solutions has been measured at temperatures ranging from 278 K up to 318 K in the mono-disperse range i.e. in a range of concentrations up to 363 kg/m^3 for BSA, up to 317 kg/m^3 for OSA, and up to 300 kg/m^3 for RSA. The measurements were conducted with an Ubbelohde-type capillary microviscometer. A convenient method of data presentation, in the case of viscosity-concentration relationship, consists of using reduced variable [hc], where [h] is the intrinsic viscosity in m^3/kg and c is the solute concentration in kg/m^3. For each studied albumin and at each measured temperature the log-log plot of the specific viscosity h_{sp} versus [hc] gives a master curve, which shows the existence of three ranges of concentrations: dilute, semi-dilute and concentrated region. In the dilute and concentrated region the above mentioned plot is linear, and in the semi-dilute one is non-linear. In the semi-dilute region the relation between the relative viscosity and concentration can be described by Lefebvre's relation. One of the parameter in this relation is the Mark-Houvink-Kuhn-Sakurada (MHKS) exponent. In general, it is considered as an indicator of protein conformation in solution. The obtained values of the MHKS exponent change slowly from 0.35 ± 0.006 (5^{0}C) up to 0.353 ± 0.006 (45^{0}C) for BSA, from 0.355 ± 0.005 (5^{0}C) up to 0.349 ± 0.006 (45^{0}C) for OSA, and from 0.346 ± 0.006 (5^{0}C) up to 0.35 ± 0.006 (45^{0}C) for RSA. It indicates that for each studied albumin the MHKS exponent is, in the frame of error estimation, constant. The slope of the master curve in the concentrated region is an indicator of protein stiffness. It decreases from 6.64 (5^{0}C) up to 5.61 (45^{0}C) for BSA, from 4.91 (5^{0}C) up to 4.39 (45^{0}C) for OSA, and from 4.78 (5^{0}C) up to 4.26 (45^{0}C) for RSA. The above results shows that stiffness of the studied albumins decreases with increasing temperature.

MOLECULAR DYNAMIC OF DONUT-LIKE FORM OF HUMAN CYSTATIN C IN SOLUTION

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Cystatin C (HCC) is small (MW=13343 Da, 120 amino acid residues), nonglycosylated protein with amyloidogenic properties [1-3]. The structure is stabilized by four cysteine residues forming two disulfide bridges. Due to the presence of HCC in most of human body fluids and tissues, cystatin C is excellent marker for various diseases e.g. rheumatic disorders and osteoporosis or as flag of kidney transplant rejection and function. This protein is also considered to be a guard of central nervous system. For the proper performance of its functions, cystatin C should occur in the form of monomers. Due to low structural stability of wild type monomers, cystatin C exhibits a tendency to aggregation. This tendency to form oligomers as well as the Leu68Gln pathological mutation causes the formation of HCC fibrils in cerebral blood vessels (hemorrhagic amyloidosis).

The aim of this work was a structural characterization of HCC dodecamers and a attempt to create model of this system through molecular dynamic. The molecular dynamic simulations were performed using AMBER program package and several structural models of HCC dodecamers were created.

Obtained structural models of HCC dodecamers were also compared with microscopic data. Obtained micro-images revealed that HCC dodecamers have donut-like morphology.

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SMALL ANGLE X-RAY SCATTERING (SAXS) STUDIES OF HUMAN CYSTATIN C IN SOLUTION

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Human cystatin C (HCC) is an inhibitor of papain-like and legumain-like cysteine proteases. This protein is present in many body fluids like urine, cerebrospinal fluid and in tissues such as cerebral cortex. HCC was observed as coprecipitate of pathological amyloid fibrils in the brains of patients with Alzheimer's disease. For correct functioning HCC should occur in the monomeric form. In the crystal, native HCC forms dimers via the domain swapping mechanism [1,2].

The study presented was aimed at developing low-resolution structure of monomeric, dimeric and trimeric forms (stabilized by disulfide bonds) of human cystatin C in solution and comparison with the HCC crystal structures.

The small angle X-ray scattering (SAXS) data were obtained using BioSAXS system and synchrotron radiation (beam line BLi911-4 [3], MAXII storage ring of the MAX-Lab Lund, Sweden; l=0.091 nm). Low-resolution structures of the HCC oligomers (covalently stabilized monomers, dimers and trimers) in solution were restored using program DAMMIN [4]. This study clearly indicated that the preferred conformation of monomeric form of HCC in solution, is almost identical with the crystal structure. HCC dimer structure is compatible with structure with elongated conformation as in tetragonal crystal form, and low-resolution structure of HCC trimer shows the structure with 3-fold axis of symmetry.

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CHANGES IN SECONDARY STRUCTURE OF WHEAT GLUTEN AFTER USING Ag NANOPARTICLES

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Crops storage processed in inadequate conditions causes heavy losses in crops quality. Post-harvest losses are mainly due to bacteria and fungi infections. Silver in form of nanoparticles is well-known from its antimicrobial properties. For this reason, silver nanoparticles (AgNPs) were used as a protective layer on the grain surface against bacterial and fungal infections (antimicrobial agent). Silver nanoparticles stabilized by trisodium citrate were used. Trisodium citrate is commonly applied as a food additive in the food industry but is not regarded as antimicrobial agent. Fourier transform infrared (FT-IR) spectroscopy was used to examine conformational changes in secondary structure of wheat gluten washed out from grain treated by aqueous solution of silver nanoparticles (AgNPs) stabilized by trisodium citrate. Analysis of the amide I band revealed significant changes in secondary structure after using both kinds of AgNPs. It was observed a slight increase in β-sheet content (from 36.2% to 39.2%) at the expense of α-helix and beta-turns content. The percentage distribution of beta-turns decreases from 13.1% for control sample to 11.7% for AgNPs-treated sample. To find factors causing these changes, the wheat grain were treated by aqueous solution of trisodium citrate and water. Obtained results indicated that the changes in gluten structure were connected mainly with the trisodium citrate action due to presence of small amount of free molecules of the stabilizer in AgNPs solution. Additionally, the conformational changes in gluten pointed out that gluten flexibility increased (decrease in α-helix/beta-sheet ratio from 1.40 for control sample to 1.26 for AgNPs-treated samples) as well as solubility of gluten decreased.

SPECTROSCOPIC CHARACTERISTIC OF ESTER-TYPE DERIVATIVES OF α-TOCOPHEROL IN HOMOGENOUS ENVIRONMENTS

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Alpha-tocopherol (α-Toc) is one of the most potent natural antioxidant known in nature, which can protect the membranes from damages induced by lipid peroxidation. However, alpha-tocopherol is very unstable under the influences of light and oxygen. Therefore, less susceptible to oxidation than α-Toc many ester-type derivatives of this vitamin were developed. The synthetic esterified forms of α-Toc are frequently added to many foodstuffs, pharmaceuticals and cosmetics.

In this study, spectroscopic properties (absorption and fluorescence) of α-Toc and two ester-type derivatives of α-tocopherol: a novel ester, di-α-tocopheryl maleate (TM), and commercially available α-Toc derivative: α-Tocopheryl succinate (TS), in some organic solvents were measured.

In organic solvents with different physical properties, the absorption maxima for TS and TM were located at similar positions (285-286nm), with extinction coefficients ranging from $2350M^{-1}cm^{-1}$ for TS in methanol to $5150M^{-1}cm^{-1}$ for TM in hexane. The investigated ester-type derivatives exhibit a blue shift of 7-10nm compared to α-Toc. The fluorescence maximum of TS and TM in investigated solvents is found at the wavelengths range 304 to 308nm, which is blue shifted at about 18nm compared to α-Toc. The positions of maxima of investigated derivatives are held within a wide fluorophores concentration range. Increasing the concentration of these esters results in the linear fluorescence increase only in initial range. At higher esters concentrations (about 80μM) the increase becomes non-linear with further fluorescence quenching. Such behavior may indicate the formation of esters dimers or aggregates.

The measured absorption and emission spectra of α-Toc esters show that esterification of α-Toc modifies its spectroscopic and physico-chemical properties compared to parent tocopherol compound. Observed electronic energy increase of the esters is very probably due to electron rearrangement in the chromanol ring due to attached moiety.

MULTI-METHOD APPROACH TO STRUCTURE AND FUNCTION OF THE RNA 5' cap-BINDING PROTEINS RESPONSIBLE FOR REGULATION OF EUKARYOTIC GENE EXPRESSION: eIF4E AND PARN

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A biophysical bases of molecular mechanisms underlying the recognition of the mRNA 5' terminal structure called "cap" by proteins is crucial both for understanding of the complex process of regulation of eukaryotic gene expression at the levels of translation and mRNA surveillance, as well as for putative drug design.

Recognition of the 5' cap by the eukaryotic initiation factor 4E (eIF4E) is the rate limiting step of protein biosynthesis, while poly(A)-specific ribonuclease (PARN) is a 5' cap-dependent enzyme that plays a key role in 3' deadenylation, is involved in nonsense-mediated mRNA decay, and also in regulation of cytoplasmic polyadenylation.

The goal of the studies was to find structural requirements for the affinity of the cap-binding proteins to the cap [1], thermodynamic driving forces [2,3] and kinetic characteristics of the intermolecular recognition, as well as to gain an insight into the structure and structural dynamics [4], that are biologically relevant.

We have established a precise method of the protein-ligand binding constants determination [5] and found that eIF4E exploits conformational changes to provide tight binding of the cap and the synergy of interactions with eIF4G/4E-BP1 [1-4], while PARN is the only one among 3' exoribonucleases which interacts with the 5' mRNA terminal structure [6] to provide the processivity of deadenylation. PARN is thus the minimal protein context to bind two mRNA termini concurrently [7]. eIF4E and PARN share similar structural cap-binding motif (Trp-m^7G) but they have different thermodynamic and kinetic binding properties that correlate with the biological functions of these proteins. We have also visualized single PARN molecules by Atomic Force Microscopy in liquid [8] that provided mesoscopic structural description complementary to the protein fragments known from cryslallography.

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EVALUATION OF THE PHYSICAL PROPERTIES OF THE ANIMAL BONES AFFECTED BY LEAD

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Lead exposure is an important public health problem. There are many sources of lead, including: ceramic glazes, electronic waste, cosmetics, toys, water pipes, solder in canned food and lead from soils. However, lead contaminated dust and lead-based paints are the main sources of lead poisoning. Clinical studies have shown that lead is devastating to the human body. This chemical element enters the human body from the environment by inhalation and through the digestive system and one is accumulated in the kidneys, liver, brain, lungs and muscles. More than 90% of the Pb in adult human body and 70% in child body is stored in the bones. Lead is released very slowly, from calcified tissue. According to Rabinowitz et al. the elimination half-life of Pb in cortical bone is approximately 10 to 30 years. The aim of researches were evaluation of the changes in the bone tissue in rats intoxicated with lead acetate. To determine the possibility of bones quality reduction by Pb two studies were conducted: biomechanical strength assay and FTIR spectroscopy measurement. Femur strength was measured in three-point bending test, whereas infrared spectroscopy was used to measure molecular structural changes, specifically, to study ratio of area of two types of vibrational transitions, determining to mineral to matrix ratio. The results of the biomechanical study show that femurs of rats treated by Pb-acetate appear to be weaker than bones of the control group and may produced condition for development of higher risk of fractures. FTIR spectra of the processed rat femoral head samples show significantly differences in the mineral to matrix ratio between the control and lead-treated bones.

The lower bone mineral content and the weaker mechanical properties of bones from Pb-treated rats are associated with the pathologic state dependent of the exposure of lead.

CHANGES IN PHYSICAL PROPERTIES OF THE BISPHOSPHONATE-ENRICHED BONE CEMENT

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Bisphosphonates (BPs) are a class of drugs that has very efficient antiresorptive properties. The use of bisphosphonate include the prevention and treatment of ostheoporosis and similar diseases. Benefits that accrue from use of bisphosphonates are better mineral density, bone microarchitecture, strength and quality of bones.

PMMA (methyl polymethacrylate) bone cement is widely used material to anchor artificial joints. The bone cement fills the free space between the prothesis and the bone, that means that this material should be highly biocompatible and biotolerant.

The goal of the presented study was to assess whether the enriching bone cement with bisphosphonate has changed its physical properties. Investigation of pure bone cement and bisphosphonate-enriched bone cement sample's biomechanical parameters included compressive test and three point flexural test. During the three point flexural test we recorded the load and stress to the point of maximal load which caused fracture and deflection and strain of the sample to this point. From obtained characteristics the transverse Young's modulus, energy which was absorbed by the sample before the fracture occurred and stiffness of the samples were determined. Longitudinal Young's modulus and stiffness was determined from compressive test. Besides the biomechanical parameters the density and the Fourier-transform infrared spectra of the samples were recorded.

The studies have shown that enrichment of bisphosphonates cause yielding of the bone cement material which results in increase of elastic region. It also did not change the density of the samples. Any significant differences between FTIR spectra of pure bone cement and bisphosphonates-enriched bone cement were recorded, which means that BP-enrichment did not cause any visible changes in the chemical composition of bone cement.

STUDY ON FINITE ELEMENT ANALYSIS OF PLANT TISSUE MICROMECHANICS

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The goal of this research was to create a computational model that incorporates micro-scale geometrical features of plant tissue, and which will provide qualitative and quantitative predictions of mechanical properties. The proposed technique of simulation of micromechanical cellular systems was demonstrated on case study of onion (*Allium cepa* L.) upper epidermis. Onion epidermis was chosen due to its simple single-layer structure, the lack of intercellular spaces and ease of sample preparation. The geometry of the FEM model was created on basis of images obtained using a confocal scanning laser microscope CSLM (OLYMPUS FluoView300, Olympus Corporation, Tokyo, Japan). The geometrical features of onion tissue were reconstructed in FEM environment by means of vectorization procedure. Then, uniaxial tensile test were carried out to determine the mechanical parameters of tissue samples. Mechanical testing was carried out up to 50% of strain with a deformation speed of 1.5 mm/min. During mechanical test the tensile force and elongation of the sample were recorded. Both values were converted into stress and strain respectively.

The uniaxial tensile tests were simulated in FEM environment using created virtual models of onion epidermis. The qualitative validation was based on the comparison of the force-strain curves from laboratory tensile test and the simulations. On the basis of calculated mechanical parameters we were able to provide a qualitative and quantitative validation of FEM models. The values of cell wall mechanical properties from the experiments were compared with those from FEM models that gave the best fit of the force deformation curves.

The developed model showed good qualitative and quantitative agreement with experimental results. The curves obtained through simulation preserved all the key characteristics of the real object. The FEM model was able to predict mechanical properties of cell wall with average estimation error of 15%.

INTERACTION BETWEEN POLYPHENOL COMPOUNDS OF BILBERRY FRUIT EXTRACTS AND MODEL LIPID MEMBRANES

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Phenolic compounds contained in plant extracts are nowadays extensively studied, because they exhibit many beneficial effects on living organisms, mostly due to their antioxidant properties. The subject of the study was extracts from the fruit of low, high and black (wild) bilberry of genus *Vaccinium.* The UPLC–ESI/MS and HPLC-DAD analysis showed that these fruits are rich in many nutrients, including polyphenols. The most predominant phenolic group was anthocyanin derivatives that constituted ca. 25 % in high

blueberries, 28 % in low blueberries, and 34 % in wild blueberries of fruit extract.

The aim of the study was to determine changes incurred by polyphenol compounds from blueberry fruit in model lipid membranes. In particular, the effect of the extracts on the packing order in the hydrophilic lipid phase and fluidity of the hydrophobic phase was studied. Model membranes were formed of DPPC, egg phosphatidylcholine, and lipids extracted from erythrocyte membranes. The interaction of the extracts with the lipids was examined with differential scanning calorimetry (DSC), infrared spectroscopy (ATR IR), and fluorometry, using the Laurdan, Prodan, and DPH probes. All the experimental results indicate that the biggest changes occurred in the hydrophilic part of the lipid bilayer. The polyphenol compounds had practically no influence on fluidity in the hydrophobic region of the membranes. No changes in temperature of the main phase transition of DPPC were observed and only a small change in pretransition temperature for high concentration of the compounds. The results obtained with the ATR IR method did not reveal any changes in the alkyl chain region of the bilayer; however a small shift of bands was observed for the phosphate and choline groups, the broadest shift being for wild bilberry.

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INFLUENCE OF PLASMONIC EXCITATION ON THE ENERGY TRANSFER IN PERIDININ-CHLOROPHYLL-PROTEIN COUPLED TO SILVER NANOWIRES

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The motivation of this work was to investigate the influence of plasmon excitations in silver nanowires upon the dynamics of the energy transfer in photosynthetic complex peridinin-chlorophyll protein that was reconstituted with both, Chl *a* and Chl *b* (Chl *a*/*b*-N-PCP). In order to control the strength of this interaction, we separated the silver nanowires from the PCP complexes with silica spacers with thickness of 5 nm and 40 nm.

The PCP complex from *Amphidinium carterae* is unique in a sense, that it features bidirectional energy transfer, that is from Chl *a* to Chl *b* and from Chl *b* to Chl *a*.

The samples were prepared by spin-coating a solution of Chl *a*/*b*-N-PCP on substrates with both 5-nm-thick and 40-nm-thick silica spacers covering the silver nanowires. The fluorescence properties of such hybrid nanostructures were examined using wide-field fluorescence microscopy for both Chl *a* and Chl *b* emissions out of the same locations across the sample. z In this was we obtain spatially resolved maps of fluorescence intensity ratio of both Chl emissions.

In the case of the sample with the thinner silica spacer we observe strong interaction between the pigments comprising the PCP complex and plasmon excitations in the silver nanowires. The intensity ratio between Chl *a* and Chl *b* emissions is equal to 2,7, while it amounts to 4 away from the nanowires. In contrast, in the case of the thicker silica spacer, the intensity ratio is identical across the whole sample, regardless of whether it is monitored on or off a nanowire. This result indicates that the plasmon excitation in silver nanowires significantly influences the energy transfer between chlorophylls in the PCP photosynthetic complex.

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INCORPORATION OF LHCII INTO CHLOROPLAST LIPID MONOLAYERS

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LHCII is a light-harvesting pigment-protein complex of photosystem II , responsible for the absorption of light energy and regulation of its transfer in the photosynthetic apparatus. The efficiency and mecha-nism of these processes depend on the specific of the molecular complex in the thylakoid membrane . The monolayer formed from chloroplast lipids (MGDG and DGDG) with built-in LHCII complex reflects well the organization of LHCII in the thylakoid membrane.

Langmuir-Blodgett technique enables the formation of collated lipid monolayers with controlled thickness and accurate composition.

LHCII complexes were incorporated into the lipid monolayer using two methods:

1) a chloroplast lipid mixture was applied to the subphase surface, and was then compressed to the required pressure surface. LHCII complex suspension was injected under this stable monolayer lipid . The increase of the molecular surface with kept constant pressure was inter-preted as incorporation of protein-pigment structures into the lipid membrane.

2) monolayers were prepared out of a protein-lipid mixture and then applied to the subphase surface. The

mixture was compressed to the surface pressure as mentioned earlier.

Afterward, the-prepared samples were trans-ferred to a solid substrate while monitoring the size of the transfer. The resulting structures were visualized with Atomic Force Microscopy (AFM) and fluorescence-lifetime imaging spectroscopy - (FLIM)**.** A lipid membrane containing LHCII complexes built-in with subphase showed the presence of monomeric, trimeric and aggregated forms of LHCII. A monolayer received from applying a prepared protein-lipid mixture on the subphase surface showed the presence of aggregated configurations in form of multilayer units.

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THE INFULUENCE OF HUMIC AND FULVIC ACIDS ON THE CONCENTRATION OF FREE RADICALS IN AQUEOUS ENVIROMENT: ESR TECHNIQUE

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The aqueous solutions containing humic substances (fulvic and humic acids extracted from peat) and free radicals (using probes 2,2,6,6 - tetramethylpiperidine - 1 - OXYLANE - TEMPO, by the ESR method was studied. The concentration of the probe in relation to the water molecules was 0.1 ppm , the concentration of humic substances: humic or fulvic acids was in a weight ratio of water to 0.1 %. In order to precise stir, each of the samples before measurement were shaken 60 seconds and then placed in the measuring chamber ESR spectrometer. ESR spectrometer operating parameters were: sweep time t = 128 s, sweep range Δ H = 7mT, amplitude modulation dH = 0.08mT. The total duration of the measurement was 60 hours. Based on the spectra of the probe TEMPO, the changing of the concentration of the free radicals in the solution was determined (according to the time). Our research shows that the probe is strongly " poisoned " by humic substances , both the larger (humic acids) and less (fulvic acids) molecular weight.

This provides that both fractions of humic substances are characteristic of free radical sweepers. In the initial phase of the experiment, the more activity were humic acids, which showed intensively impact on the signal recording by the spectrometer.

However, after a longer period - 40 hours of the experiment, stronger effect of the fulvic acids was observed. The observed antioxidant properties of humic substances, especially in the context of the production of paramedical preparations derived from peat and used in herbal medicine. Humic substances could be a large natural reservoir of free radicals and could be important in controlling the decomposition processes of organic matter in the soil.

DISORDERS OF ERYTHROCYTE'S ANTIOXIDANT SYSTEM IN PEOPLE WITH CORONARY ARTERY DISEASE TREATED WITH STATINS

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Oxidative stress is one of the most important factors in the development of cardiovascular disease. Increased level of free radicals is also observed in coronary artery disease (CAD). The reactive oxygen species cause pro- and antioxidant imbalance. That imbalance can cause disorders of natural antioxidant system.

The aim of our study was to evaluate the effects of oxidative stress associated with CAD on the selected parameters in erythrocytes and the effect of statin therapy to improve of disorders parameters. The study included 34 patients with CAD after myocardial infraction within 6 months at the age of 62.8 ± 6.1 years. Qualified patients were divided into two groups. The first group has taken atorvastatin in dose 40 mg/day and the second group has taken rosuvastatin in dose 40 mg/day. Control was healthy individuals in appropriate age. In the erythrocyte were determined antioxidant enzymes activity (catalase, glutathione peroxidase and superoxide dismutase), lipid peroxidation and concentration of thiol groups.

The results show disorders of antioxidant system in patients with CAD whose are manifested in the reduction of antioxidant enzyme activity (11% catalase and 22% superoxide dismutase) compared to the control group and 16% higher level of lipid peroxidation. Monthly treatments statins resulted in statistically significant increase in catalase activity: 13% atorvastatin and 14% rosuvastatin and superoxide dismutase: 19% atorvastatin and 16% rosuvastatin. Both treatments have also influence on reduction of lipid peroxidation level: 18% atorvastatin and 20% rosuvastatin.

In conclusion, statin treatment has a positive effect on the balance of pro-and antioxidant properties of erythrocytes in patients with CAD.

PLASMA LIPID PEROXIDATION IN PEOPLE WITH CORONARY HEART DISEASE (CAD) AND STATIN TREATMENT

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Coronary artery disease (CAD) is associated not only with increased cholesterol levels but also oxidative stress. In the treatment of lipid disorders as well as in primary and secondary prevention of CAD the most important role plays statins.

In our study, we tried to determined changes in plasma antioxidant capacity in patients with CAD before and after treatment with statins. The study involved 30 patients with coronary artery disease after myocardial infarction within 6 months, at the age of 63.5 ± 5.9 years Qualified patients did not have high blood pressure, no burning and alcohol abusers. Patients were divided into two groups in which one group have taken, for a period of one month , atorvastatin in dose 40 mg/day and second rosuvastatin in dose 40 mg/day. Red blood cells from healthy individuals were a control group. In the plasma were determined: lipid peroxidation and total antioxidant capacity depends on the fast and the slow antioxidants.

The results show that patients with CAD have any statistically significant changes in plasma total antioxidant capacity but show 25% increase in plasma lipid peroxidation level. Monthly treatment atorvastatin resulted in reduction of lipid peroxidation level by 20%, while treatment rosuvastatin by 23% compared to the results before treatment. Both the treatments did not cause statistically significant changes in total antioxidant capacity of plasma.

These data suggest that CAD is accompanied by high lipid peroxidation but does not change the total antioxidant capacity of plasma.

EFFECT OF TREATMENT OF HIPOLIPEMIC DRUGS ON THE STRUCTURE OF ERYTHROCYTES IN PATIENTS WITH CORONARY ARTERY DISEASE (CAD)

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Statins, as inhibitors of 3-hydroxy-3-methyl-coenzyme A (HMG-CoA) reductase are used as hipolipemic drugs. But not always they are able to decrease of the desired lipid parameters, especially in monotherapy. Combining statins with hipolipemic drugs with different mechanism of action, which are representative by ezetimibe, allows by lower dose of statins achieving a similar reduction in cholesterol.

Our study was aimed to investigate the effect of combination therapy to improve the structural parameters of erythrocytes, that disorder observed in CAD. The material was erythrocytes isolated from the blood of patients with CAD after myocardial infarction within 6 months. The study group included 20 patients, at age 64.2 ± 5.9 years. Patients qualified for the study were divided into two groups: the first has taken 40 mg/day atorvastatin and the second 10 mg/day atorvastatin + 10 mg/day ezetimibe. Control was healthy individuals in appropriate age. The structure parameters determined erythrocytes: lipid peroxidation, concentration of thiol groups in membrane proteins, total cholesterol, and erythrocyte membrane fluidity.

Our results show disorders in the structure of erythrocytes in people with CAD. In these patients, we observe an increased level of lipid peroxidation (18%), total cholesterol (19%) and a decrease in erythrocyte membrane fluidity (in the subsurface layers of 14%, in the deeper layers of 7%). Monthly treatment with atorvastatin resulted in reduction of lipid peroxidation (12%), while the monthly atorwasatyną + eztymibem therapy resulted in reduction of lipid peroxidation (19%) and increase membrane fluidity in subsurface layers (9%).

The results suggest that combination therapy of statin with hipolipemic drugs with different mechanism of action, allows achieving similar results as the use of statin in monotherapy in larger doses.

INTERACTIONS OF XANTHOPHYLL PIGMENTS WITH PROTEINS

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Carotenoid pigments are important constituents of human eyes and they are responsible for vision. Deficiency of macular xanthophyll's, lutein and zeaxanthin, has been correlated with macular retinopathy referred to as AMD (Age Related Macular Degeneration). It seems very likely that xanthophyll difference in eyes is associated while impeared transport across the blood-macula barrier. in the current work we study interactions of carotenoid pigments (lutein, zeaxanthin and β-carotene) with proteins which can play role of carotenoid transporters in the blood: BSA and GST. The results of electronic absorptions and FTIR studies show strong interactions of carotenoids with protein studied.

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SCOTS PINE NEEDLE SURFACE WETTABILITY PARAMETERS AS INDICATORS OF AIR POLLUTION IMPACTS

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Wettability represents a fundamental property of any solid material, it reveals information on the chemical structure and surface architecture of the surface-modified biological substrata strongly correlated to the environmental pollution stress. An investigation of water contact angles (CA), contact angle hysteresis (CAH) was carried out for 1-year to 4-year old needles (Pinus sylvestris) collected in urban (Gdansk) and rural (Karsin) locations using an original measuring technique based on the geometry of the drop on a vertical filament. Concentrations of air pollutants (SO_2, NO_x, O_3, F and SPM-suspended particular matter), currently considered to be most important in causing direct damage to vegetation, were simultaneously monitored. A set of the surface wettability parameters: the apparent surface free energy, adhesive film pressure, work of adhesion, and spreading were determined from CAH data using the approach developed by Chibowski (2003) to quantify the surface energetics of the needle substrata. Since CAH depends on the outermost wax layer surface roughness and spatial physicochemical heterogeneity of a solid surface, CA data were corrected according to the Wenzel and Cassie equations, respectively using surface architecture profiles. It was found that the roughness parameter r is significantly negatively correlated (R=-0.74) with the needle age (collected at Karsin). The needle surface becomes smoother with an increase in sample ages in the village area whereas such a relation does not appear (R=-0.24), for samples collected in industrialized regions (Gdansk). The wettability parameters were closely correlated to pollutant concentrations as evidenced from Spearman's rank correlation procedure ($R = 0.63\text{-}0.91$; $p < 0.05$). The aim of the study was to validate the established CA methodology to create a new non-invasive, low cost technique suitable for monitoring of structural changes at interfaces of biological systems.

EFFECT OF RESVERATROL ON NEUROBLASTOMA (Neuro-2a) AND HIPPOCAMPAL CELLS (mHippoE-18) UNDER OXIDATIVE STRESS CONDITIONS

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Resveratrol (RSV) is a polyphenol, produced naturally by some plants in response to several harmful factors such as attack by pathogens, UV radiation, or increased oxidative stress. Resveratrol has been shown to exert anticancer, neuroprotective and cardiovascular effects.

The main aim of this research was to investigate the effect of resveratrol on neuroblastoma (Neuro-2a) and hippocampal cells (mHippoE-18) under conditions of oxidative stress.

Cells were treated with hydrogen peroxide and incubated for 24 hours with or without the presence of resveratrol. Different concentrations of resveratrol (2.5 μM to 40 μM) were added to cell culture 3, 6 or 12 hours prior to H_2O_2 treatment. The cytotoxicity of the studied compounds was checked with MTT assay. To estimate percent of apoptotic cells, the Annexin V Detection Kit was used.

The concentration of the hydrogen peroxide that caused about 50% reduction in cell viability was 20 μM for Neuro-2a and 30 μM for mHippoE-18 cells. Comparing Neuro-2a viability, it decreases in cells treated with both – resveratrol and hydrogen peroxide (especially with higher concentrations of RSV) than in cells treated by hydrogen peroxide only. Higher doses of resveratrol combined with hydrogen peroxide reduces cells viability substantially.

Our data show that resveratrol does not demonstrate any statistically significant effects on cell viability of hippocampal cells. We also observed that resveratrol in low concentrations (7.5 μM preincubated for 6 hours) reveal antioxidant effect.

In conclusion, resveratrol in combination with hydrogen peroxide increases apoptosis and cellular cytotoxicity of tumor cells (Neuro-2a). It does not influence on normal, hippocampal cells (mHippoE-18).

THE BEHAVIOR OF THE POLAR HEAD GROUP OF NEW 2-(ALKYLDIMETHYL-AMMONIO)ETHYLGLUCONAMIDE BROMIDES IN WATER SOLUTION

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Sugar-based surfactants are known for their improved surface and performance properties, and reduced environmental impact due to easy biodegradation. They may be also considered as main molecular factors in the gene delivery. The newly synthesized 2-(alkyldimethylammonio) ethylgluconamide bromides(C_nGAB) with different chain lenghs (n = 10, 12, 14, 16) were proved to be able to compact plasmid DNA with great efficiency. This happens due to a number of intermolecular interactions in which the polar head group plays an significant role. Thus, the exploration of the behavior of the polar head group of C_nGABs in water solution is very important. It is also interesting for defining structure-activity relationships.

The enthalpies of dilution of C_nGABs were measured by means of Isothermal Titration Calorimetry at 298 K and 313 K. The calorimetric curves were normalized to infinite dilutions, which approximately correspond to relative partial molar enthalpies of dilution. Analyzing and comparing the trends with those of other quaternary ammonium bromides with long chains, we draw the conclusion that the polar head group of C_nGABs in comparison with trimethylammonium group decreases the hydrophobicity of the surfactant to the same extent as shortening its alkyl chain by one methylene group. This may be due to the presence of amide group. The heat capacity of micellization for C_nGABs and alkyltrimethylammonium bromides was also estimated and the number of "dry" hydrogens in micelles was calculated. This suggests that in micelles of C_nGABs are hydrated for one methylene group deeper than alkyltrimethylammonium bromides. This may be also attributed to amide group, especially to its ability to form hydrogen bonds with water.

These findings are supported by the results of molecular modeling studies performed for the two types of surfactants in solution.

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TRANSPORT OF 3-BROMOPYRUVATE ACROSS THE HUMAN ERYTHROCYTE MEMBRANE

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3-Bromopyruvic acid (3-BP) is a promising anticancer compound as it is a strong inhibitor of glycolytic enzymes, especially hexokinase II and glyceraldehydes 3-phosphate dehydrogenase. Since malignant cell are much more dependent on glycolysis than normal cells due to the Warburg effect, they are more sensitive to this compound. However, potential complication of anticancer therapy with 3-BP are the side effects of this compound due, i.a., to interaction of 3-BP with normal cells, especially erythrocytes.

The aim of our study was the kinetic characterization of 3-BP transport into human erythrocytes. Erythrocytes (hematocrit of 5%) in phosphate-buffered saline (PBS) were added with various concentrations of 3-BP containing 10 μM 14C (carboxyl)- 3-BPA (15 mCi/mmol, Perkin-Elmer). After 1-min incubation at room temperature, the suspensions were centrifuged through a layer of dibutyl phthalate and washed 2 times with ice-cold PBS. Radioactivity of the red cell sediment was measured after sample treatment with 0.5 M NaOH/10% H2O2 in a dioxane-based scintillation cocktail. The Km and Vm values for 3-BP transport were 0.89 mM and 0.94 mmol/min*l cells, respectively. The transport was inhibited competitively by pyruvate and significantly inhibited by SITS and 1-cyano-4-hydroxycinnamic acid.

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PHYSICOCHEMICAL STUDIES OF INTERACTIONS BETWEEN MAIN COMPOUND OF *Oenothera gigas* TANNINS AND LIPOSOMES

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Tannins are ones of plant metabolites with wide biological activity including antioxidant, antitumor, anti-inflammatory and antibacterial properties. Despite many different experiments focused on their biological activities the physicochemical mechanisms responsible for interactions between tannins and biopolymers are still unclear. There are only a few papers concerned tannins-lipids interactions. At present studies the interaction between 1-O-galloyl-4,6-hexahydroxydiphenoyl-β-D-glucose (OGβDG; main compound of *Oenothera* plant tannins) and 3 types of liposomes (with different lipidograms) was analyzed. The main goals of experiments were to verify how different lipid compositions influence liposomes-OGβDG interactions and what is biophysical and physicochemical nature of this reactions. Liposomes (100 nm diameter) were prepared using extrusion methods and composed from lecithin, DMPC and DPPC phospholipids in three different weight ratios. The hydrolysable tannin OGβDG possessing 11 –OH groups, 3 aromatic rings and glucose ring was dissolved in water. Spectrofluorimetric measurements (using TMA-DPH and DPH dyes) were used to analyze the interaction of OGβDG with liposomes outer and inner monolayer. The measurement parameter was fluorescence anisotropy. Zeta-size and Zeta potential measurements were applied to estimate the changes in liposomes diameter and charge induced by OGβDG. Fourier Transform Infra-Red (FTIR) analysis was used to study what chemical groups are engaged in liposomes-OGβDG interactions. Anisotropy analysis showed that OGβDG enhanced rigidity of inner monolayer and decreased rigidity of outer monolayer in liposomes. This observation demonstrated that OGβDG enters the liposomes and penetrate into structures of fat globules. Zeta-size and Zeta potential analysis revealed increase of liposomes diameter and decrease of zeta potential.

Changes in FTIR spectra show that CH_2 alkanes groups (from phospholipids chains) and hydroxyl –OH groups (from OGβDG molecules) are responsible for liposome-OGβDG interactions. Obtained results demonstrate that OGβDG strongly interacts with lipids but should note that effects depend on the composition of used liposomes.

REGULATION OF TRANSCRIPTION: NEGATIVE AND POSITIVE FEEDBACK LOOPS COUPLED BY A COMMON PROMOTER

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We consider a hypothetical system of two genes, whose transcription is controlled by a common promoter. According to our assumption, transcription of the both genes takes place when the promoter is free of the repressor and bonds the inductor (~Rep^Ind). Protein precursors of the repressor and inductor are encoded in the two genes. Transient transformations of these two proteins lead to products which can function as transcription factors. We are modeling this system by a set of ordinary differential equations. The two equations, referring to mRNA synthesis, are nonlinear. Values of derivatives of the nonlinear terms in equilibrium, which are dependent on system's parameters, determine qualitative features of the evolution. Characteristic equation and conditions of saddle-node bifurcation have been obtained in general form. Hopf bifurcation and the possibility of oscillatory solutions have been derived in some special cases. The oscillatory solutions exist if the difference between Hill coefficients of repression and induction is sufficiently high. Highly cooperative repression promotes oscillations. In contrast, highly cooperative induction suppresses them. The oscillation are impossible, if the time of turnover in the loop of induction is too much shorter than that in the loop of repression.

MULTIFUNCTIONAL $NaYF_4$: Er^{3+}, Yb^{3+}, Gd^{3+} NANOPARTICLES UP-CONVERING INFRARED LIGHT TO VISIBLE AND ULTRAVIOLET RADIATION FOR USE IN CANCER IMAGING AND PHOTODYNAMIC ANTICANCER THERAPY

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Multifunctional NaYF4 nanoparticles doped with Er^{3+}, Yb^{3+}, Gd^{3+} ions with optical and magnetic properties were synthesized. These nanoparticles absorb infrared (IR) light and as a result of up-conversion, emit visible (VIS) and ultraviolet (UV) light. This allows for their application in biology and medicine as fluorescent markers (VIS) and as a potential agent for anticancer photodynamic therapy, through the production

of reactive oxygen species (ROS), under the influence of ultraviolet (UV) light.

Nanoparticles containing Gd^{3+} ions exhibit paramagnetic properties that allow cancer cells imaging using magnetic resonance imaging (MRI). Another field of the nanoparticles application is a selective killing of cancer cells in living organisms by hyperthermia. Superparamagnetic materials, placed in an external high-frequency magnetic field, heat up to the temperature at which protein denaturation occurs, thus eventual cancer cells death.

NaYF4: Er^{3+}, Yb^{3+} nanoparticles, after functionalization by polyvinylpyrrolidone (PVP), were introduced into the HeLa cancer cells. Location of the nanoparticles was determined in the cells as a function of incubation time, concentration of nanoparticles and presence of transfection agent Lipofectamine 2000.

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PHOTO-OXIDATION OF *cis*-PARINARIC ACID

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Non-oxidized conjugated fatty acids are found in minor concentrations in vegetable oils and improve their value due to their health-promoting impact on the human body. This paper analyzes whether oxidation with triplet oxygen was the only process occurring in the samples of *cis*-parinaric acid (CP) exposed to UV radiation at 250-300 nm. The study was carried out using samples of CP dissolved in n-hexane and containing oxygen and those de-oxygenized with argon. The efficacy of de-oxygenation was approximately 79%. The samples were radiated and the absorption and fluorescence spectra were simultaneously measured. The spectra of both de-oxygenized and non- de-oxygenized samples changed as a result of radiation: the specific CP acid bands disappeared. Although the decays were similar, they differed in the rate of changes. The rate constants for the processes were calculated based on both absorption spectra and on fluorescence excitation spectra. The calculations included the amount of absorbed photons. The calculations demonstrated that the decays were two-exponential (consistent with first-order reactions) and the rate constants for the argonized samples were smaller by approximately 50%. As the fading of spectra was two-exponential and de-oxygenation insufficiently inhibited this process, it was concluded that *cis*-parinaric acid also underwent photo-degradation, most probably under the influence of UV radiation.

THE EFFECT OF DIAMOND NANOPARTICLES AND THEIR FORM AFTER CHEMICAL MODIFICATION ON THE ANTIOXIDANT SYSTEM IN LUNG CANCER CELLS.

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Nanotechnology is one of the fastest growing scientific areas in the contemporary world. Particles received at the nanoscale have completely different optical, structural and electrical properties compared to materials in the macroscale, that is why they are used in biology, biotechnology, cosmetology and pharmacology. Nanodiamonds with sizes below 10 nm can easily uptake through cell membranes and may cause changes in the cell. One of the mechanisms of their action may be associated with the induction of oxidative stress which we observed by the increased production of free radicals. That's why cells have the antioxidant system with the protective function. The antioxidant enzymes in cells are catalase, superoxide dismutase and glutathione peroxidase.

In this study we used lung cancer cells line A549, which were incubated with diamond nanoparticles (D - nanodiamond and D+OH – nanodiamonds after the chemical modification with connected hydroxyl groups on the surface of nanodiamond) for 24, 48 and 72 hours, at concentrations 0 - 100 □g/ml. We observed changes in the antioxidant activity of the enzymes because of the dependence on the type and concentration of nanoparticles and time incubation.

LHCIIb IN LIPID BILAYER ENERGY MINIMIZATION AND MOLECULAR DOCKING OF ASCORBIC ACID

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The light harvesting complex II (LHCII) is the most abundant membrane protein present in the biosphere. The X-Ray crystallographic structure has been resolved for a number of species. However, so far no attempt has been made to describe LHCII in the natural environment within a lipid membrane. With the use of the program YASARA a MGDG/DGDG lipid bilayer was created using a modified version of the default macro. A LHCIIb trimer was placed in the lipid bilayer and the system energy was minimized. The resulting protein and crystallographic structure was compared. Molecular docking of ascorbic acid to the energy minimized LHCIIb structure from the lipid bilayer was performed using Molegro Virtual Docker v5.5. The docking results suggest three probable spaces of interaction between ascorbic acid and LHCIIb located near the monomer-monomer contact sit

TIME RESOLVED FLUORESCENCE SPECTROSCOPY OF ANTIBIOTIC AMPHOTERICIN B

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Amphotericin B (AmB) is a popular drug from the group of polyenes, applied in the treatment of mycosis. It is envisaged that as a metabolite of *Streptomyces nodosus* recognizes and destroys lipid membranes of the fungi. Unfortunately, its activity is also characterized by high toxicity, which identify. Because biological activity of AmB depends on the molecular organisation of AmB system, understanding of molecular mechanisms that govern the organisation of AmB is important, not only for the understanding of different biological effects, but also for minimizing the toxic side effects of the drug.

Knowledge of the dynamics of excited states, which is associated with molecular organisation of fluorophores, is a key factor in understanding aggregation processes.

Realization of this goal is based on the **Time Resolved Fluorescence Spectroscopy** which measures very precisely fluorescence life-time and as a result easily detects different molecular organisation forms of the drug. Fluorescence lifetime is a handy parameter in distinguishing various types of structures (monomer, dimer, aggregate). Fluorescence lifetime - associated spectra of amphotericin B emission was presented and discussed.

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STUDY OF RELS AND FRET IN Cyt c AND MITOCHONDRIA MODIFIED SPHERICAL AuNP

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The oxygen deprivation to the brain, heart, and peripheral tissues is one of the most important causes of serious illnesses (stroke, angina pectoris, heart attack, obesity, diabetes, cancer, autism, Alzheimer's disease), disability, and mortality. Injuries caused by oxygen deficit and reperfusion are deleterious. Hence, it is important to gain better understanding of the cellular events during hypoxia and uncover the mechanisms of cellular defenses against two opposite conditions: oxygen deficit and oxidative stress. During hypoxia, the mitochondrial matrix is swelling and cytochrome c (Cyt c) is released to cytosol, marking the beginning of cell apoptosis. In this work, we have investigated electrostatic interactions of mitochondria with gold nanoparticles (AuNP) using resonance elastic light scattering spectroscopy (RELS). The responses of the novel mitochondrial system to various drugs influencing the potassium ion-channel opening have been investigated. The interactions of hemoprotein Cyt c with nanoparticles have also been investigated using RELS and fluorescence spectroscopy (FL) techniques.

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CIRCUMNUTATION TRACKER – NEW SOFTWARE FOR ANALYSIS OF CIRCUMNUTATIONS

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Circumnutations are endogenous, helical movements of growing plant organs with an ultradian rhythm. Time-lapse video is the basic method for investigations of circumnutations. *Circumnutation Tracker* (*CT*) is novel software for analysis of time-lapse recordings in the Win environment. Standard parameters, i.e. the period, length, rate, shape, angle, and direction of circumnutations are determined automatically, which reduces duration of the analysis. The function and capabilities of *CT* were evaluated by analysis of circumnutations of *Arabidopsis thaliana* inflorescence stems. The inflorescence stem growing under constant light was found to exhibit a strong ca. 90-min. rhythm and several-hour long fluctuations of circumnutation length. Varied shapes of individual circumnutations with sequences arranged in a characteristic rosette-like pattern were recorded. Additionally, the circumnutation direction changed from clockwise into anti-clockwise and vice versa. The results obtained show usefulness of the new *CT* software for measurements of *Arabidopsis thaliana* circumnutations. We expect that *CT* will be a convenient tool for investigations of circumnutations in various plant species as well as growth, gravitropism, biological clock, and membrane transport, i.e. processes involved in the mechanism of circumnutation.

EGGSHELLS OF GREY HERON (*Ardea cinerea*) AS A TOOL FOR BIOINDICATION OF RIVER VALLEY

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One of the mechanisms for the elimination of substances toxic to the bird's embryo is the process of their deposition in the eggshell. The main factor justifying the usage of the *Ardea cinerea* eggshells for the bio-indication purpose is their diet which consists of different water organisms such as fish, amphibians, mammals and invertebrates. Additionally, high population of birds in a unit area, mobility, longevity, group preying and nesting makes this species a useful marker for environmental monitoring.

The survey was focused specifically on 5 Heron colonies located adjacent to the largest rivers in the Lublin area.

The main contaminants present in such areas are the remains of agricultural activity and the pollutants common to industry deposited into the rivers.

Concentrations of heavy metals in the Grey Heron eggshells were estimated by means of ICP-OES (i.e. inductively coupled plasma optical emission spectrometry) technique.

An important part of the conducted experiments was the determination of toxic element concentrations such as Chromium (Cr), Lead (Pb) and Cadmium (Cd). Their concentrations showed the following sequence: Cr > Pb > Cd. Our survey confirmed this pattern in 4 out of 5 Grey Heron colonies examined.

One of the examined elements having a negative influence on the bird's reproductive process is Strontium (Sr). *Aredea cinerea* eggshells contained 150.72 μg/g dry weight (dw) of Sr on average. The maximal level of Sr was found in the eggshell originating from the heronary localized in Chodlik near Opole Lubelskie (above 255 μg/g). The concentration(s) of the elements determined in eggshells were compared with the respective concentrations of elements from the sediments in the rivers closest to the birds' preying areas. Interestingly, it was found that Strontium (Sr) and Aluminium (Al) concentrations in eggshells are many times higher than the respective concentrations found in river sediments. The highest differences were observed among the Grey Heron colony in Wólka Michowska, where a 4-fold concentration increase of Sr and 48-fold higher concentration of Al were found in the Grey Heron eggshells.

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PLASMONIC-BASED INSTRUMENT RESPONSE FUNCTION FOR TIME-RESOLVED FLUORESCENCE

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We investigated plasmonic platforms to target ultrashort fluorescence and accurate instrumental response function in a time-domain spectroscopy and microscopy. The interaction of metallic nanoparticles with nearby fluorophores resulted in the increase of the dye fluorescence quantum yield, photostability and decrease of the lifetime parameter. The properties of platforms were applied to achieve a picosecond fluorescence lifetime (21 ps) of erythrosine B, used later

as a better choice for deconvolution of fluorescence decays measured with "color" sensitive photodetectors. The response functions were monitored on two photo-detectors; microchannel plate photomultiplier and single photon avalanche photodiode as a Rayleigh scattering and ultra-short fluorescence. We demonstrated that use of the plasmonic base fluorescence standard as an instrumental response function results in the absence of systematic error in lifetime measurements and analysis.

STUDY ON SPATIAL DISTRIBUTION OF POLYSACCHARIDES IN PLANT CELL WALL BY RAMAN MICROSCOPE

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The plant cell wall is kind of the cellular skeleton that controls cell shape and determines the relationship between turgor pressure and cell volume. The cell wall is composite of many different natural polymers, mainly cellulose, xyloglucan, pectins and also lignin for secondary cell wall which forms after cell growth. Proteins, lipids, enzymes, aromatic compounds and water are another components of this part of plant cell.

It is thought that percentage of components of plant cell wall has an important influence on mechanical properties of fruits and vegetables. Therefore research on content and spatial distribution of each component of these part of cells are extremely important in studies of quality of fruits and vegetables. So far many analytical and microscopic methods of investigation of plant cell wall was developed. Nevertheless, none of this methods gives data relating to accurate distribution and amount of individual substances in microscale. Raman microscopy can resolve this problem without necessity of staining section of plant tissues.

Briefly, Raman microscope is connection of microscope and Raman spectroscopy. It allows to collect spectra at each points of sample. In this way map of spatial distribution of sample's components can be obtained.

In this work we would like to discuss the methodology of measurement using Raman microscopy and present Raman images obtained for cell walls of several plant tissues. Examples of spatial distributions of main cell wall compounds will be depicted (CH-streching region ~ 2800 cm^{-1}) Due to chosing specific band location, we were able to localize and indentify pectins (856 cm^{-1} α-glycosidic bonds in pectin), hemicellulose (1735 cm^{-1}) or lignin (1600 cm^{-1} phenyl groups in lignin).

ACIVITY OF NEWLY SYNTHESIZED PHENOTHIAZINE DERIVATIVES AS ANTIPROLIFERATIVE AND MDR REVERSING AGENTS IN COLON CANCER CELLS

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Multidrug resistance (MDR) of cancer cells which is one of the form of resistance to chemotherapy, has been extensively studied for more than 30 years. For MDR phenotype are responsible plasma membrane proteins that belong to a large superfamily of the proteins called the ATP-binding cassette (ABC) transporters, particularly:P-glycoprotein (P-gp),multidrug-resistance associated protein 1 (MRP1) and breast cancer resistance protein (BCRP).Compounds that can reverse multidrug resistance may influence on MDR transporters by action at different molecular levels – the protein,mRNA or DNA level.In our studies we have tested the ability of the newly synthesized phenotiazine derivatives to inhibit the growth of human adenocarcinoma cancer cells as well as a possibility of these compounds to reduce drug resistance of LoVo/Dx cells.It occurred that phenothiazines act as antiproliferative agents and cell growth inhibition was observed both in drug sensitive (LoVo) and doxorubicin-resistant (LoVo/Dx) cell line.Our results indicated that these derivatives are able to reverse the resistance of cancer cells against doxorubicin and may be regarded as promising agents improving doxorubicin efficacy in drug-resistant cancer cells.Doxorubicin can be used as fluorescence marker of drug accumulation inside the cells and its modification for eg. By MDR transporters' inhibition.The fluorescence signal derived from the drug accumulated inside LoVo/Dx cells increased in the presence of the phenotiazines.The effect of studied compounds on the expression of P-gp, MRP1 and BCRP has been also checked and determined by RT-PCR and immunohistochemical methods. These experiments revealed that one of the studied compounds increased the expression of BCRP in LoVo/Dx cells.Applying the QSAR methods allowed us to describe electronic, structural and topological parameters and hydrophobicity of tested compounds and to correlate these properties with ability of phenothiazines to influence on MDR phenotype.

POTENTIAL USE OF HALLOYSITE IN PHYTOREMEDIATION OF SOILS CONTAMINATED WITH HEAVY METALS

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The present study aimed to investigate the effect of the addition of halloysite to soils contaminated with heavy metals on the growth rate of common flax (*Linum usitatissimum* L.) and cock's foot (*Dactylis glomerata* L.*)* in pot culture and on the bioaccumulation of elements in these plants.

The peat-muck soil with the structure O-Mt-n-Mt-Ot-D formed on light loamy sand from the former Białogon Pond bowl contaminated with heavy metals (Pb 910,4 mg/kg d.w., Cu 121,3 mg/kg d.w., Zn 1140,5 mg/kg d.w.) was used for experiments. The influence of halloysite concentration on growth of test plants and the content of heavy metals (Pb, Cu, Zn) in biomass of plants, growing on contaminated soils enriched in different doses of halloysite (from 10% to 50%) were recorded. The content of heavy metals in soils and biomass of test plants was determined using X-ray fluorescence (XRF) method.

The highest growth rate and biomass growth were observed in plants cultivated on soil supplemented with 25 % halloysite while the slowest values were recorded for 50% halloisyte. The greatest heavy metal bioaccumulation factors were seen for lead at 50% halloysite (WB_{Pb}= 0.35), for copper at 25% halloysite (WB_{Cu}=2.2) and for zinc also at 25% halloysite (WB_{Zn}= 0.65). The addition of halloysite to the contaminated soil induced changes in many of its physico-chemical properties, e.g. decreased the contents of Pb to 80%, Cu to 30% and Zn to 20%. A positive correlation was found between biomass and Cu content in cock's foot and between heavy metal contents in plant biomass and soil pH or calcium carbonate content.

THE INFLUENCE OF SELECTED PRENYLATED CHALCONES AND FLAVONOIDS ON THE ACTIVITY OF Kv1.3 CHANNELS IN HUMAN JURKAT T CELLS

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It is known that small-molecule organic inhibitors of voltage-gated potassium channels Kv1.3 channels may potentially find a clinical application to support a chemotherapy of some cancer disorders characterized by an overexpression of Kv1.3 channels, such as breast, colon and lymph node cancer, melanoma or chronic lymphocytic leukemia. Promissing candidates may be some plant polyphenolic compounds that combine a high efficiency with a good bioavailability and a low cytotoxicity. Studies performed previously in our laboratory showed that a plant-derived prenylated flavonoid - 8-prenylnaringenin, in contrast to its precursor, naringenin, was an effective inhibitor of Kv1.3 channels both in normal human T lymphocytes and in human cancer T lymphocyte cell line - Jurkat [1]. Studies on the influence of prenylated chalcones and flavonoids on the activity of Kv1.3 channels in cancer cells were then extended on other compounds from both groups: xanthohumol, isoxanthohumol and isobavachalcone. The influence of these compounds on the activity of Kv1.3 channels in human Jurkat T lymphocytes was studied applying the whole-cell patch-clamp technique. Obtained results provide evidence that all selected compounds are inhibitors of Kv1.3 channels in Jurkat T lymphocytes. The inhibitory effect occurred, in case of all compounds, in a concentration-dependent manner. The value of a half-blocking concentration (EC_{50}) was about 3 μM for xanthohumol, 5 μM for isobavachalcone and 7.8 μM for isoxanthohumol, respectively. The inhibitory effect was reversible for all the compounds tested. These results may confirm our earlier hypothesis that the presence of a prenyl group in the molecule is a factor that facilitates the inhibition of Kv1.3 channels by flavonoids and chalcones [1].

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MULTI-ION SENSOR SYSTEM FOR REAL-TIME ION TRANSPORT MONITORING

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Cystic fibrosis (CF) is the most common human genetic disorder caused by disturbed ionic transport in cells [1,2]. The direct cause of anomalous ion transport in CF is the mutation of CFTR gene encoding chloride-conducting channel. This leads to increased thickness of mucus layer in bronchial epithelium what is advantageous for development of chronic lung infections resulting in patient death. There is a lot of contradictory hypotheses of ion transport mechanism in CF what is the result of complex interactions between numerous transporting proteins found in bronchial epithelium [3]. Usually ion transport studies

are carried out in Ussing chamber system. However, this method is limited because of lack of selectivity toward specific ion. The current signal obtained from Ussing chamber measurements results from total ion fluxes in the cell layer and it is not possible to distinguish particular ion contribution in the observed current change. Hence, conclusions drawn from Ussing chamber experiments are incomplete. Reliable ion transport mechanism may be obtained only in the system where particular ion fluxes are monitored simultaneously. Potentiometric methods based on ion-selective electrodes (ISE) meet this expectations and have become indispensable tools for the determination of ionic constituents of human body. Beside selectivity toward specific ion, simple construction and possibility of miniaturization were the main reasons for ISE to be paid much attention and gain popularity in biological and medical applications.

In this work novel ISE-based system for the simultaneous determination of K^+, Cl^-, Na^+ ions and pH in cell monolayer was described. The designed system allows for real-time monitoring of ion transport within cell layer. The constructed miniaturized electrodes were integrated with reference electrode in one system allowing direct observation of changes in the concentration of ions in surface layer of the cells. The ISE-based system for conducting continuous flow measurements was successfully applied for in vitro studying of ion fluxes in human bronchial cells monolayer.

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PROTECTIVE ROLE OF THE ELECTRIC FIELD AGAINST ACCESS TO BIOLOGICAL MEMBRANE POTENTIALLY TOXIC CATIONS

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The aim of this study is to search of an effective method of protection of biological membrane against potentially toxic cation, including organometal cations. As demonstrated by our earlier studies some amphiphilic cations from the group of quaternary ammonium salt used in properly small concentrations, in which practically do not disturb cell membrane, significantly inhibit erythrocyte hemolysis induced by organic cations of lead. This effect can be explained by changing the polarity of the membrane (increase in positive surface charge after incorporation of amphiphilic cations) and, consequently, difficult access to the membrane of organometallic ions. In this case, an electrostatic field can serve a protective function against toxic effects of organometallic cations. In this study we investigated the effect of the electric polarization of the monomolecular lecithin membrane on access to it potentially toxic cations present under the monolayer (in the water subphase). As a protective factor, positively polarizing the monolayer, dihexadecylammonium bromide ($C_{34}H_{72}N^+Br^-$) was used. Surface pressure changes were measured after addition of the test compound to the subphase (due to the relatively small changes in surface pressure caused by organometallic compounds of lead, here we used a cationic surfactant, hexadecylpyridynium chloride, $C_{21}H_{38}ClN$). It was found that the change in surface pressure monomolecular lecithin membranes, under the influence of the test compound present in the subphase, decreases with membrane electrical polarization. This demonstrates the effective inhibition (under the influence of the electric field generated by the protective cations, $C_{34}H_{72}N^+$) of access to the membrane of cations present in the subphase.

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EFFECT OF GLYCATION ON THE THERMODYNAMICS OF DENATURATION OF COLLAGEN TYPE I

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Glycation of collagen - the primary structural component of connective tissue , and accumulation of advanced glycation products (AGEs) is one of the reasons for dysfunctions affecting diabetics and elderly people. The experiment tested the hypothesis that glycation can affect the thermodynamic stability of collagen fibers.

Chemically pure fibers of the main organic component of bone tissue and tendons , collagen type I , were used. Collagen was incubated in aqueous solutions ribose or glucose in different concentrations for a period of 4-14 days. The formation of AGEs was evaluated spectrofluorimetrically at 420-440nm (370 nm excitation) and at 390-400 nm (335 nm excitation). The latter signal is specific for pentosidine –a marker for AGEs in the aging processes. A high level of pentosidine fluorescence, dependent on the incubation period and sugar concentrations, was stated. The level of fluorescence related to other AGEs was lower, however correlated with pentosidine.

Thermodynamic parameters of denaturation and melting of collagen fibers were measured using differential scanning calorimetry (DSC). The parameters depend on the configuration of molecules, their hydration and cross-linking within and between fibers. Both hydrated and dry fibers were investigated.

Glycation in ribose, even for small concentrations of sugar and a short period of incubation, resulted in a higher temperature of denaturation, both in fully hydrated, and dry samples.

Incubation in glucose, resulted in an increase of denaturation temperature only after two weeks of glycation, and only in the dry samples , where the molecules of collagen, without being surrounded by water, are more strongly influenced by cross-links between fibers. However, after the first week of glycation changes in enthalpy of denaturation and cooperativity of the thermal processes were observed.

The results obtained confirm that the measurements of thermodynamic parameters of collagen can be used to assess the changes in connective tissue in diabetes and advanced age.

THERMODYNAMIC CHARACTERISTICS OF BONE COLLAGEN DENATURATION

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The aim of the study was to examine thermodynamic characteristics of collagen in bone tissue and demineralized bone matrix and an assessment of the impact of glycation induced bonds on thermodynamic stability of bone collagen. Parameters of denaturation and decomposition of collagen were estimated on the basis of differential scanning calorimetry (DSC) performed for temperatures from 40 °C up to 220^{o}C. Unmodified cortical bone samples , samples of fully demineralized bone matrix and bone samples modified by glycation in vitro were tested. Changes of heat capacity, enthalpy, entropy and Gibb's energy during denaturation of bone collagen were calculated from the thermograms.

It was shown that, compared to other proteins, collagen in bone tissue is thermally very stable, both in natural, and in the demineralized bone matrix. Is was stated that denaturation of collagen occurs gradually in a few separate steps which are followed by meting of collagen fibers into smaller structural units. That gradual denaturation results from existence of different populations of collagen with different level of cross-links inside and between fibers. It was alsofound that the complex process of bone collagen denaturation is influenced by additional bonds induced by glycation, which can contribute to the nonphysiological changes in bone tissue and an increased fracture risk among diabetics.

PLASMONIC FLUORESCENCE ENHANCEMENT IN PERIDININ-CHLOROPHYLL-PROTEIN-SILVER NANOWIRE HYBRID NANOSTRUCTURE

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Collective elecface plasmon resonance, can affect absorption and emission of light by placed nearby fluorophores.

In our experiment we investigate the influence of plasmon excitation in silver nanowires on fluorescence of the photosynthetic complex peridinin-chlorophyll-protein (PCP) using a wide-field fluorescence microscope equipped with EMCCD detector.

First we took white-light transmission images to localize positions of silver nanowires on the surface. Then we recorded map of the PCP fluorescence off this area. We observed that in the vicinity of the silver nanowires emission of the PCP complexes is strongly enhanced. The enhancement is higher at the ends of the silver nanowires. The enhancement can be observed for both 405 nm and 480 nm excitation wavelength, and is present for samples with different concentrations and arrangement of silver nanowires versus the PCP complexes. In particular, for structures were the nanowires are mixed with the PCP complexes prior the deposition on the surface the enhancement factor values are generally higher than for a sample where the PCP complexes are deposited on previously prepared silver nanowire layer. Analysis of kinetics of fluorescence yields a surprising result that the presence of the silver nanowires has no influence upon the photostability of the PCP fluorescence.

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QUININE INFLUENCE ON THE DYNAMIC PROPERTIES OF LIPOSOME MEMBRANES MODIFIED BY N-METHYLATED PEPTIDOMIMETICS – EPR STUDY

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The susceptibility to proteolytic degradation hindering the use of peptides as drugs. Thus, extensive studies on modifications of naturally occuring peptides are carried out to overcome this difficulty. The most common modifications are incorporation of non-coded amino acids into peptide backbone and replacement of a hydrogen atom with a methyl group on the nitrogen atom [1]. The main aim of research concentrates on the improvement of the therapeutic index of biologically active substances too. One of the most promising intelligent drug carriers tend to be liposomes [2].

In the current studies we used quinine in the presensce of three peptidemimetics [two peptides with Phe residue: Ac-Phe-NHMe (1) and Ac-Phe-NMe_2 (2) and one with ΔPhe amino acid Ac-DPhe-NMe_2 (3)] to explore structural and dynamic changes for model EYL (**E**gg **Y**olk **L**ecithin) bilayer. The effect of such dopants on the plasticity and fluidity of bilayer was studied by electron spin resonance (ESR) technique enhanced by typical spin probe – TEMPO.

Based on the analysis of the EPR signal of EYL liposomes we observed a significant change of bilayer fluidity:

- Quinine in connection with studied peptides modify spectroscopic parameter F of TEMPO spin probe.
- Petide 1 in the presence of quinine liquefies liposome membrane in proportion to the concentration of quinine in the range of 0 to 7%. However, above the 7% we observed a rapid growth of membrane fluidity, which suggest a phase transition in the membrane bilayer.
- Peptide 2 with quinine cause liquidate the model bilayer in the range of 0 to 8%. Over 8% we found the similar trend as in the case of peptide 1.
- In the case of peptide 3 we didn't observe quinine effects on fluidity of liposome membrane.

These findings are important and they show a synergistic effect of both quinine and the peptides 1 and 2 as potential means of controlling liquidity of lipid bilayer. The results may have application significance in design of drugs.

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MOLECULAR ORGANIZATION OF POLYENE ANTIFUNGAL ANTIBIOTIC DRUG Amphotericin B IN STEROL CONTAINING MODEL LIPID MEMBRANE

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Amphotericin B (AmB) is a polyene antifungal antibiotic, widely used for the treatment of systemic mycoses. The risk of fungal infections is particularly high in patients with immune system weakened by long term treatment with immunosuppressive drugs or *Acquired Immuno-Deficiency Syndrome* (AIDS). According to the studies performed since 1970s, the pharmacological effect of the drug but also the toxic side effects are determined by the molecular organisation of the antibiotic in lipid membrane. As follows from the hitherto proposed and investigated models, AmB selectively forms specific structures that aggregate with ergoterol, which brings about the pharmacological effect of the drug. However, similar mechanisms of interaction with cholesterol seem to responsible for the side effects of the drug. Comprehensive understanding of molecular mechanisms responsible for organisation of the drug in model systems of biological importance is of great significance for possible design and development of a drug showing much reduced toxicity.

Amphotericin B was characterised in model lipid membranes either with or without sterols (ergosterol or cholesterol) by UV-vis linear dichroism spectra and FTIR. Results obtained by UV – Vis linear dichroism spectra revealed that AmB molecules were incorporated into the ergosterol-containing lipid membrane at a lower angle than into a cholesterol-containing membrane or a membrane without sterols. This finding has considerably increased the probability of formation of a trans-membrane channel in the form of AmB tetramer. FTIR linear dichroism analysis for the drug built into the membrane permitted to check the influence of the antibiotic and sterols on the lipid membrane at the specific sites, in the hydrophobic layer and in the region of polar heads. The outcome of the study has brought a contribution to understanding the origins of the toxic side effects of the drug.

APPLICATION OF MEMBRANE SYSTEM FOR INVESTIGATING SORPTION PROPERTIES OF HALLOYSITE

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To study the sorption properties of minerals the single-membrane system as a component of the interferometric set-up was used.The interferomeric method allows a comprehensive examination of the substance diffusion process. This method also permits the visualization of concentration diffusion layers creating process.

The absorption capacity of halloysite was investigated with reference to glucose, which is often found in industrial waste water and the glucose excess can disturb the environmental eco-balance. The sorption capacity of halloysite was thus determined indirectly, based on the comparison of concentration profiles as well as time characteristics of glucose quantities released from the control solution and from the solution incubated with a halloysite adsorbent. Glucose diffusion analysis from these solutions was carried out in a two-chamber system with the horizontally situated membrane. Concentration profiles for various times were obtained for the control glucose solution of initial concentration 0.05 M, as well as for solution exposed to halloysite. The time characteristics of glucose quantities released from the control solution and from the solution incubated with a halloysite adsorbent were also obtained. On the basis of concentration profiles the evolution of concentration field was defined, and adsorption efficiency (34%) as well as the amount of glucose adsorbed at equilibrium state (6.12 mg/g) were determined.

The obtained results confirm the halloysite good sorption properties with respect to the investigated substance and the usability of the method for this kind of investigations. The present study indicates the possibility of optimizing the measurement system so that it is possible to visualize and study the kinetics of the adsorbed substance release directly from the mineral.

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MICELLIZATION STUDY OF ENVIRONMENTAL FRIENDLY DICEPHALIC AMINE DIBROMIDE IN COMPARISON WITH GLUCONAMIDE-TYPE CATIONIC SURFACTANTS

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In recent years, the basic and applied research interest in cationic surfactants has increased, partly because of urge for cationic amphiphiles to be useful in medical applications. The most popular trend here is the pursuit to obtain environmental friendly compounds with desired physicochemical properties. We have recently studied the aggregation processes of various cationic surfactants. The worth of our special interest are sugar-based compounds (w-(alkyldimethyl-ammonium) alkylaldonamide bromides) showing unique properties such as mild production conditions, lower toxicity and higher biodegradability. As a continuation of our research we were examined the physicochemical behaviour in the water solutions of N,N- bis[3,3-(trimethylammonio) propyl]alkylamide dibromides $C_n(TAPABr)_2$ with different chain lengths (n= 12, 14, 16). The critical micelle concentration (cmc) were otained using the Isothermal Titration Calorimetry (ITC) as the main investigation technique. The thermodynamic parameters – the enthalpies, the entropies of micellization as well as the contributions of headgroups to the Gibbs free energies $\Delta G°(hy)$ were calculated. The aggregation processes of $C_n(TAPABr)_2$ were also studied by means of conductance method to calculate the degree of micelle ionization b. The obtained results were compared with those previously reported and literature data for compounds with monomeric head structure as well as sugar-based surfactants with analogical chain lengths. Sugar-based surfactants and dicephalic amine bromide studied have been considered to be promising candidates as gene- and drug-delivery vehicles for biomedical applications, as was find in other study. In the view of these practical applications the comparison of chemical-physical data among different groups of cationic surfactants can lead to better insight into the structure-property relationships.

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mRNA CAP ANALOGS – WHAT IS IN THEM FOR A BIOPHYSICIST?

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In terms of structure, mRNA cap analogs are similar to a cellular cap, consisting of 7-methylguanosine attached to the first transcribed nucleoside via a triphosphate bridge (m^7GpppN), that flanks the 5' end of eukaryotic mRNAs. However, their modifications impose various physicochemical properties. Thus, cap analogs may serve as tools specialized for the structural and functional examination of cap-binding properties. One example of such proteins is decapping scavenger (DcpS) enzyme catalyzing the hydrolysis of a cap in the 3'→5' mRNA decay, avoiding inhibition of other cap-binding proteins. Notably, DcpS is specific to very short cap-containing oligonucleotides, with the highest activity towards dinucleotides.

We applied a set of 50 intrinsically fluorescent mono- and dinucleotide cap analogs to characterize *C.elegans* DcpS. Using cap analogs modified within nucleosides, which are hydrolyzed by DcpS, the Michaelis constants and maximal velocities were determined. Selecting unhydrolysable cap analogs modified in the phosphate chain the association constants and Gibbs free energies of binding were calculated. The representative cap analogs were further used for computational docking studies, revealing enzyme residues involved in the cap-binding. This approach enabled us to find that *C. elegans* DcpS catalysis relies on the recognition of a positively charged 7-methylguanosine and on interactions with the ribose 2'O and 3'O hydroxyls of 7-methylguanosine. Diphosphate chain of a cap is sufficient for an efficient binding to DcpS, whereas triphosphate or longer one is required for hydrolysis. The second nucleoside is not absolutely necessary for hydrolysis, but plays a role in the stabilization of a cap in the cap-binding pocket. Our studies extend the knowledge about DcpS enzyme and serve as an example of potential applications of cap analogs in the biophysical studies of proteins playing a role in the cap-dependent mRNA metabolism.

APPLICATION OF MEMBRANE SYSTEM FOR INVESTIGATING SORPTION PROPERTIES OF HALLOYSITE

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Living organisms have developed many manners of ion transport, in which peptides are involved (channels, pumps, transporters). Change of osmotic pressure, caused by active ion transport across the cell membrane, causes the passive water transport through water channels existing in lipid bilayer called aquaporin's. Transport of one chemical molecule through the membrane is accompanied by transport of about 450 molecules of water. Transport across the lung epithelium is especially interesting since the defect in anion channel CFTR is responsible for the most common fatal human genetic disorder – cystic fibrosis. Through epithelial cells in lungs, ions like Na^+, K^+, Cl^-, $HCO3^-$ are transported, and exist the system of pH stabilization. Measurements of this parameters as fast as it is possible can seem to be the key to understand the mechanism of cystic fibrosis. That is why we have developed our integrated electrode system.

MATERIALS AND METHODS

Electrodes

Silver wires were mounted in poly(methyl metacrylate) capillary with ion selective membrane and filled by suitable inner solution.

Integrated electrode system for biological measurements

Build of two different poly(methyl metacrylate)-based modules containing five places for ion selective electrodes each and solution inlet/outlet. Modules are placed vis-à-vis at a distance of approximately 100 μm. The insert with monolayer of epithelial cells grown on porous support is placed between them.

Cell line

Immortal cell line of Human Bronchial Epithelium - 16HBE14-σ.

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