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## **PREFACE**

Dear Colleagues,

On behalf of the members of the Organizing and Scientific Committees of the XVII Congress of the Polish Biophysical Society I am very pleased and honored to welcome you to Olsztyn in beautiful campus of the University of Warmia and Mazury.

I am confident, that all plenary lectures, communications and discussions will bring new insights to our research, scientific collaboration and friendly relationship between members of our Society.

Please accept my invitation to read the Book of Abstracts. There are more than 40 abstracts representing broad spectrum of research interests of Polish biophysicists. I am convinced that reading of these abstracts will allow you to get good image of Polish biophysics development. Polish Biophysical Society is trying to support this development.

Zbigniew Wiczorek

President of the Polish Biophysical Society

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# Plenary lectures

## ELECTROSTATIC INTERACTION EFFECTS IN THE KINETICS OF CONFORMATIONAL TRANSITIONS OF PROTEINS

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Experimental approaches to study electrostatic effects in biomolecular processes include investigation of their dependence on the ionic strength of the medium in which they occur. One interesting class of biomolecular processes subject to such studies are conformational transitions of proteins. Such transitions range from spontaneous conformational fluctuations to more substantial transitions triggered by different factors like binding of ligands. Recently, we investigated ionic strength dependence of the kinetics of tri-N-acetylglucosamine binding to lysozyme (Antosiewicz & Długosz, 2018). We found that forward and backward conformational transitions in lysozyme, following formation of the initial encounter complex, both become faster as the ionic strength of the solvent is increased. We suggest that this might be a general feature. It is supported by a simple calculation within the Poisson-Boltzmann model of the solute-solvent system, which shows that the electrostatic free energy barrier for conformational transitions is lowered by increased concentration of low-molecular-weight salt. Here I would like to consider these issues in a more systematic manner.

### ACKNOWLEDGMENTS

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## THE HOST-GUEST COMPLEXATION BETWEEN $\gamma$ -CYCLODEXTRINS AND ETHYL 5-(4-DIMETHYLAMINOPHENYL)-3AMINO-2,4-DICYANOBENZOATE

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In recent years, considerable attention has been focused on understanding and controlling supramolecular interactions between organic molecules and well-recognized macrocyclic hosts: cyclodextrins, cucurbit[n]urils, crown ethers, calixarenes and cyclophanes [1]. Furthermore, host-guest inclusion has attracted attention for its wide applications in nano-machines and smart materials [2-3]. Recently, our group has contributed to this field of interest by introducing the role of specific solute-solvent interactions (H-bonding) and excited-state intramolecular charge (proton and electron) transfer process in the formation of inclusion complexes between fluorophore and cyclodextrins [4-6].

In the present paper, the effects of  $\gamma$ -cyclodextrins ( $\gamma$ -CDs) on the both emission modes (LE – locally excited and ICT – intramolecular charge transfer) of the fluorescence spectrum of ethyl 5-(4-dimethylaminophenyl)-3-amino-2,4-dicyanobenzoate (EDMAADCy) in DMSO and aqueous DMSO solution have been investigated using steady-state and time-resolved fluorescence techniques. Because the main purpose of this work was to investigate the influence of molecular conformation of investigated D-A dye on the formation of inclusion complexes with cyclodextrins, the basic, concentration-dependent luminescent characteristics (absorption, fluorescence excitation, and emission spectra, as well as fluorescence decay times) were measured in DMSO and DMSO-water binary mixtures in the presence of  $\gamma$ -CD. The relation between molecular conformations of EDMAADCy and the concentration-dependent spectral behaviour was interpreted in terms of concentration-induced planarization model. Performed spectroscopic studies clearly demonstrate that “perpendicular” form of EDMAADCy is considerable more included in the cyclodextrin cavity than the “flattened” form.

### ACKNOWLEDGMENTS

This work was financed within the statutory fund BMN 538-5200-B045-18.

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## CITRUS FLAVONOIDS-NARINGENIN AS AN OPENER OF MITOCHONDRIAL POTASSIUM CHANNELS

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Certain flavonoids, including naringenin, have cytoprotective properties. Although the antioxidant effect has long been thought to be a crucial factor accounting for the cellular effects of flavonoids, mitochondrial channels have emerged recently as targets for cytoprotective strategies [1,2].

In the present study, we characterized interactions between naringenin and the mitochondrial BK<sub>Ca</sub> channels recently described in dermal fibroblasts and endothelial cells. Our path-clamp study shows that naringenin in micromolar concentrations leads to an increase in mitoBK<sub>Ca</sub> channel activity. The opening probability of the channel decreased from 0.97 in the control conditions (200 μM Ca<sup>2+</sup>) to 0.06 at a low Ca<sup>2+</sup> level (1 μM) and increased to 0.85 after the application of 10 μM naringenin. Additionally, the activity of the mitoK<sub>ATP</sub> channel increased following the application of 10 μM naringenin. To investigate the effects of naringenin on mitochondrial function, the oxygen consumption of dermal fibroblast cells was measured in potassium-containing media. The addition of naringenin significantly and dose-dependently increased the respiratory rate from 5.8 ± 0.2 to 14.0 ± 0.6 nmol O<sub>2</sub> x min<sup>-1</sup> x mg protein<sup>-1</sup>.

In this study, we demonstrated that a citrus flavonoid, naringenin, can activate K<sub>ATP</sub>- and BK<sub>Ca</sub>-type channels present in the inner mitochondrial membrane of dermal fibroblasts and endothelial cells [3].

### ACKNOWLEDGMENTS

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## UNDERSTANDING THE PEGYLATION EFFECT ON BIOLOGICAL PROPERTIES OF PROTEINS AND DENDRITIC NANOPARTICLES

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Protein and nanoparticles PEGylation is a widely used technique to improve delivery and bioavailability of pharmaceuticals. The PEGylation of biological and chemical compounds have many advantages and can improve the safety of many drugs. The PEG chain possess properties such as good water solubility, lack of toxicity and low immunogenicity. The effects of PEGylation on structural, dynamical and functional stability of protein and nanoparticles have been investigated for years [1-3]. However, till now there is not sufficient knowledge about the effects of PEGylation on binding properties of nanoparticles to protein and vice versa. In our study we discover the role of PEG which is attached to protein in catching the dendrimers. The PEG attached to albumin is able to bind cationic dendrimers and transport them without creating the characteristic protein-dendrimer corona where, protein properties are disrupted. Moreover, we are going to present the first results about role of PEGylation of dendritic silver nanoparticles for binding behavior to protein with different isoelectric point. We suggest that PEGylation of cationic nanoparticles not only change the charge of nanoparticles surface but completely change the kinetics and thermodynamic of binding process between nanoparticles and proteins.

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## UNRAVELING MECHANISMS BEHIND VARIABLE PRESENTATION OF SIGNALING LIPIDS WITHIN MEMBRANES

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Lipids are key structural components of biological membranes. In addition, they are indispensable for a wide range of cellular functions, including signal transduction and modulation of membrane protein functions. Until recently, the lipid-protein recognition processes were considered as simple ligand-receptor events, based largely on specific interactions of proteins with lipid head groups. Each of signaling lipids (e.g. phosphatidic acid - PA and phosphatidylinositides - PIPs) may be involved in a broad array of cellular pathways, which suggests that regulation of their biological activity has to be precise. While the local action of numerous specific lipid-metabolizing enzymes control levels and turnover of signaling lipids, additional molecular mechanisms are necessary to tightly regulate protein-lipid recognition [1,2]. Membrane lipid composition, bilayer organization/morphology and the presence of specific ions in the aqueous phase can lead to conformational changes of lipid head group conformation, exposition to the water-bilayer interface and/or domain formation. In a consequence, these factors govern the mechanisms of lipid recognition by peripheral membrane proteins – a concept known as lipid presentation [3].

In our study we employ membrane model systems, including lipid monolayers and vesicles of different size to analyze how peripheral membrane proteins selectively recognize individual signaling lipid species in the context of membrane of different composition and in variable conditions. Using state-of-the-art biophysical approaches together with molecular dynamics simulations, we elucidate molecular mechanisms that modulate the behavior of signaling lipid and their recognition. Such approach led us to discover that calcium strongly influences PIP head group conformation, which is reflected by altered recognition of the lipid by peripheral proteins [4]. Also, cholesterol appeared to be a potent modulator of PIP-protein interactions, although the mechanism does not rely on head group conformational changes (Czogalla *et al.* unpublished). The effect of cholesterol on signaling lipid presentation appeared to be one of the major modulatory mechanisms also in case of other lipids (e.g. PA), although the consequences to protein membrane recruitment and/or activation strongly depend on structural features of membrane-binding domains. Moreover, we observed that lipid recognition depend also on its acyl chain configuration [5], which suggest that within a cell several subspecies of a particular signaling lipid may play different physiological roles.

Our results allow to decode the mechanisms, by which signaling lipids are selectively recognized by effector proteins. This is crucial to understand cellular signaling pathways and consider additional, so far poorly defined aspects of their regulation and mutual relationships.

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## THE MOLECULAR BEACONS FOR BIOANALYTICAL APPLICATIONS

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Last year Christ and co-workers proved the formation of i-motif structures in vivo and their work indicate that i-motifs may have relevance in key biological processes. On the other hand, there is growing interest in utilizing i-motif forming sequences in nanotechnology and bioanalytical platforms [1]. Molecular beacons based on cytosine-rich sequences can serve as tools for monitoring intracellular pH due to their ability to form tetraplex structure called i-motif in response to pH decreasing [2]. The transition of cytosine-rich sequence from an open state into a non-canonical DNA conformation is a consequence of forming cytosine-hemiprotonated cytosine (C-C+) base pairs [3,4]. We developed fluorescent molecular beacons, which exploited (a) pyrene excimer emission, (b) 5-(1-pyrenylethynyl)-2'-deoxyuridine emission or (c) the 1,3-diazo-2-oxo-phenothiazine (analog tC) emission for the transduction of the proton-binding event by the recognition pH-sensitive fragment of molecular beacon sequence. In latter approach, the hairpin structure contains tC analogue incorporated in cytosine-rich loop or the analogue tC is located in the stem of probe, in which, both the core and part of the loop contain cytosine repeats. The spectral behaviour of all systems were examined by

recording the UV-vis, fluorescence, and CD spectra in solutions pH range from 5.5 till 8.0. Efficient fluorescence quenching of tC fluorophore occurred upon lowering the pH from 8.0 to 5.5. The possibility of using of the sensors for monitoring pH changes are demonstrated.

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### A NEW METHODS FOR INNER FILTER EFFECT I AND II CORRECTIONS

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One of the main techniques used in biophysics is fluorescence. It has many applications due to its measurement's simplicity. However, one of the main problems in literature is using uncorrected spectra for inner filter effect I and II.

To avoid these effects, absorbancies in cuvette should be less than 0.05, which limit to great extend application of fluorescence technique. Other way is to make amendments which correct spectra for inner filters.

In this presentation we propose new method for dealing with inner filters based on novel way of finding cuvette geometry, necessary for precise corrections. Examples of such results is presented alongside with simplified method for calculating quantum yield.

### FORMATION OF THE 3+1 G-QUADRUPLEXES MONITORED BY CIRCULAR DICHROISM AND UV-VIS SPECTROPHOTOMETRY

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Continue Here

G-quadruplexes are the structures formed by DNA and

RNA strands rich in guanine residues. The research on those structures attracted great interest since their formation was confirmed in human genome [1]. G-quadruplex is formed by four strands and stabilized by Hoogsteen hydrogen bonds, stacking between nucleic bases and electrostatic interaction with metal cation (typically potassium or sodium cation). Depending on the number of molecules and direction of the strands many topologies can be distinguished. The most interesting from biological point of view are unimolecular G-quadruplexes which corresponds to the structures found in the human genome. However other structures are also investigated for various applications like DNA nanotechnology and biosensing.

One of the application of G-quadruplexes is formation of DNAzyme by complexation with hemin molecule. Such DNAzyme catalyzes the reaction between hydrogen peroxide and organic substrate. This peroxidase-mimicking DNAzyme found application in many bioassays [2]. In the presented study we decided to develop new method of signal amplification using 3+1 G-quadruplexes. Such G-quadruplex should be formed by two DNA oligonucleotides: one with three guanine tracts (probe) and the other one with one guanine tract (target). The idea of this project is to form many G-quadruplexes on one long target. Such approach will allow on double amplification of the signal: by the formation of many 3+1 G-quadruplexes on one strand and DNAzyme activity.

The first part of this project included spectral characteristic of designed probes. We designed two probes and two targets. First system allowed on the study of 3+1 G-quadruplex. The second system included formation of 3+1 G-quadruplex stabilized by duplex formation. The third system included elongated target which in theory could form up to 10 3+1 G-quadruplexes. The oligonucleotides were based on telomeric sequence ((TTAGGG)<sub>n</sub>). This sequence is present at the end of human chromosomes and is responsible for maintaining the length of the chromosome. In some cells (stem and cancer cells) is present enzyme telomerase which is able to elongate the telomeres. Since this enzyme is only present in stem cells and cancer cells it is believed to be a cancer marker. All systems were examined using circular dichroism spectrometry. This technique allows on determination of G-quadruplex topology (parallel, antiparallel or hybrid). Using this technique we were able to determine the formation of 3+1 G-quadruplexes by changes in the spectra between probe alone and probe with target. We also observed that probes alone can form intermolecular G-quadruplexes. The next stage of the research focused on determination of melting temperatures of studied systems. Melting temperature provides the information on stability of the G-quadruplex. For this purpose we used CD spectrometry and UV-Vis spectrophotometry. Melting temperature were determined from melting profiles obtained by measuring CD or absorbance changes during temperature change (10-90°C). Melting temperatures proved that 3+1 G-quadruplexes possessed higher stability than G-quadruplexes formed by probes alone. The addition of hemin also increased stability of the studied systems.

The presented results are the first stage of the project aiming to develop new signal enhancement method. The

results proved that designed systems are able to form 3+1 G-quadruplexes. In the next stage the activity of DNazymes formed by 3+1 G-quadruplexes will be tested. The final stage of the study will include the development of assays for telomerase and other biologically significant analytes.

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### ANTIOXIDANT ACTIVITY OF FULLERENOL IN IRRADIATED ERYTHROCYTE MEMBRANES AND ITS COOPERATION WITH L-ASCORBIC ACID AND AN ANALOGUE OF $\alpha$ -TOCOPHEROL

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There are many reports that fullerenols  $C_{60}(OH)_x$ ,  $x > 24$  could be applied in biomedical applications to protect cells against oxidative stress generated by chemical or physical factors i.e. ionizing radiation. Biological antioxidants such as ascorbic acid and alpha-tocopherol are also capable of inhibiting oxidative damage. Our group demonstrated that highly hydroxylated fullereneol  $C_{60}(OH)_{36}$  is non-toxic to human erythrocytes, however, can adsorb to plasma membrane proteins, especially to ion-dependent ATPases and the band 3 protein. Fullereneol  $C_{60}(OH)_{36}$  at relatively high concentration of 120  $\mu$ M protected the erythrocytes against the radiation-induced hemolysis. In this work the antioxidant properties of lower concentration of fullereneol  $C_{60}(OH)_{36}$  combined with L-ascorbic acid or with an analogue of  $\alpha$ -tocopherol (2,2,5,7,8-pentamethyl-6-hydroxychroman, PMHC) were assessed under oxidative stress induced by ionizing radiation in erythrocyte membranes.

Erythrocyte plasma membranes (1 mg of membrane protein per mL) in PBS were incubated with fullereneol (16  $\mu$ M), or fullereneol and ascorbic acid (20  $\mu$ M), or PMHC (1  $\mu$ M) for 1 h at room temperature and exposed under air to high energy electrons from the 6 MeV ELU-6 linear accelerator. The absorbed dose was 325 Gy as evaluated by a Fricke dosimeter.

Lipid peroxidation was quantified by measuring the formation of thiobarbituric acid reactive substances (TBARS) after extraction of TBARS from an aqueous phase by 1-butanol.

SDS-PAGE was performed according to Laemmli (1970) using Bio-Rad system. The gels were digitalized and analyzed using GelScan software (Kucharczyk TE).

Our results showed that irradiation of erythrocyte membranes caused lipid peroxidation and degradation of band 3 protein. All antioxidants and their systems used in this study suppressed oxidative damage in the membrane.

However, mixtures of fullereneol with ascorbic acid or PMHC did not enhance the protection of membranes as compared to the each antioxidant used alone.

#### ACKNOWLEDGMENTS

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### STRUCTURE – ACTIVITY RELATIONSHIP APPROACH IN THE RATIONAL DESIGN OF cNIIIB ENZYME INHIBITORS

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Human cytosolic 5' nucleotidase cNIIIB, belongs to the family of eight enzymes catalyzing the hydrolytic dephosphorylation of nucleoside 5'-monophosphates to nucleosides and orthophosphate. As a one of catabolic enzymes, it contributes to the regulation of nucleotide levels in living cells, but its exact role in the cell has not been established so far. Due to the distinctive activity towards  $m^7$ GMP, it has been proposed that cNIIIB participates in mRNA cap turnover and protects cells against undesired salvage of  $m^7$ GMP that could lead to its incorporation into nucleic acids. [1] We envisaged that properly designed inhibitors or chemical probes could aid in the elucidation of biological roles of cNIIIB. Rational design of compounds suitable for cNIIIB activity modulation or monitoring is crucial to ensure selectivity, especially in biological samples where additional, interfering 5' nucleotidase activities are present. Considering  $m^7$ GMP as the hallmark of substrate specificity for cNIIIB, we prepared a synthetic library of nucleoside monophosphates, analogs of  $m^7$ GMP, to investigate their inhibitory properties towards cNIIIB. This allowed us to identify a set of modifications of  $m^7$ GMP that ensured both hydrolytic resistance and inhibitory properties. The identified inhibitors were then used as leads to design second-generation library of inhibitors and for crystallization trials to determine detailed structure-activity relationship for cNIIIB. The most potent inhibitors were also investigated in more detail to verify their selectivity in the context of other  $m^7$ GMP binding proteins, including eIF4E and DcpS. Finally, the activity of the identified inhibitors was confirmed on endogenous cNIIIB activity present in HEK293 lysate using LC-MS/MS method, thereby placing the compounds as new molecular tools for studies on mRNA cap metabolism.

#### ACKNOWLEDGMENTS

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## QUANTUM-CLASSICAL MOLECULAR DYNAMICS. THEORETICAL FOUNDATIONS AND APPLICATIONS IN BIOMOLECULAR SCIENCES

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A deeper understanding of biological systems and processes requires a multi-disciplinary approach employing methods of biology, chemistry, as well as computational sciences and physics, with the leading role of the latter. Quantum-classical molecular dynamics (QCMD) combines quantum and classical MD algorithms, and is capable to describe atomic motions as well as electron and proton transfer processes in biomolecules. There are two basic problems to be solved: how to generate effective and reliable potential energy functions and how to run the dynamics. In practical applications, the separation of the phase space into quantum and classical domains is dictated by the problem under study, as well as by the required accuracy of the time-dependent solutions. Typically, the dynamics of a quantum subsystem is described by the time-dependent Schroedinger equation, while the rest of the system is described by the Newtonian equations of motion. The coupling between the quantum  $\{x\}$  and classical,  $\{\bar{R}_\alpha(t)\}$ , domains is described by the time-dependent potential function  $V=V(x,\{\bar{R}_\alpha(t)\})$  in the Schroedinger equation and by Hellmann-Feynman forces  $\bar{F}_\beta = \left\langle \psi \left| \frac{\partial H}{\partial R_\beta} \right| \psi \right\rangle$  modifying the classical forces in the Newtonian equations of motion. Other models and theories, in particular Car-Parrinello molecular dynamics (CPMD), will also be described. For more information see e.g. [1-3].

Selected applications in the studies of (bio)molecular systems and processes will be reported. In particular:

- CPMD simulation study of intramolecular vibrational mode-sensitive double proton-transfer in porphycene [3],
- QCMD study of an enzymatic process catalysed by phospholipase A<sub>2</sub>, [4], and a
- QCMD simulation study of the enzymatic process involving KPC  $\beta$ -lactamase and a model ligand for a novel class of boron-based antibiotics [5].

Finally, the basic principles of causal analysis of dynamical structural changes in (bio)molecules, in particular those observed in MD simulations, will be presented - see also [6]. Practical applications of this methodology for the study of correlated intramolecular atomic motions in HIV-1 protease will be shown.

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## CHEMICAL ANALYSIS WITH INFRARED SPECTROSCOPY COUPLED WITH CHEMOMETRICS

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Infrared spectroscopy is characterized by the ability to recognize the tested organic compounds. It is said, it reveals so-called molecular fingerprint - each compound has its own unique spectrum. Moreover, this technique is quite simple to use - minimal sample preparation, relatively inexpensive apparatus.

These features are complemented by a specific method of data processing, known as chemometrics. Based on the spectra sets, appropriate correlations of spectral variations and interesting physicochemical parameters of samples are found. It is also possible to study several parameters simultaneously or to reveal subtle material properties.

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## BISPHENOLS EXPOSURE AND HUMAN HEALTH RISKS

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Bisphenols (BPs) are chemical substances used in massive amounts in the synthesis of polymers (polycarbonates, epoxy resins, polysulfones) and thermal paper, which are utilized in the production of numerous every day products including food containers, drinking bottles, toys, medical equipment, electronic devices, register receipts, books, newspapers, and food cartons.

The main representative of BPs is bisphenol A (BPA) with annual production exceeded 7 million tons but also other BPs (BPA analogs) including bisphenol F (BPF), bisphenol S (BPS) and bisphenol AF (BPAF) are commonly used in the industry.

It has been proven that BPs as a result of diffusion and hydrolysis of polymers, can migrate into the environment (the atmosphere, surface waters) and human surrounding (water, food, dust, etc.), and then accumulate in the human body (blood, urine, adipose tissue). Food has been considered to be the most important source of the exposure of the general population to BPs; nevertheless drinking water consumption, dust inhalation and dermal contact with thermal paper must be taken into consideration to estimate human exposure to these substances.

It has been observed that BPs may influence animal and human organisms by interactions with estrogen, androgen, aryl hydrocarbon and peroxisome proliferator-activated receptors; therefore they can disturb function not only endocrine system (changes in sex hormones, insulin, leptin or thyroxin levels) but also impacts other systems of the body including the immune or nervous ones.

The results of the investigations have shown that BPs exert multidirectional effects in living organisms by affecting various receptors, ROS level, cell signaling as well as genotoxic and epigenetic modifications. Epidemiological studies have found that the exposure of the general human population to BPA and some of its analogs may increase risk of coronary heart disease and metabolic disorders including obesity and diabetes; nevertheless further investigations must be conducted in order to confirm these findings.

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### NON-COVALENT INTERACTIONS BETWEEN BIOLOGICALLY ACTIVE COMPOUNDS AND THEIR POSSIBLE ROLE IN MODULATING DRUG ACTIVITY

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Keywords: methylxanthines, biologically active compounds, non-covalent interactions, nanoparticles, drug action modulation

Methylxanthines (MTX) are probably one of the most commonly consumed alkaloids worldwide. Carbon and metal nanoparticles (NPs) are another group of increasingly used substances, especially as drug delivery system agents. Both MTX and NPs may affect the activity of other biologically active compounds (BACs). Direct non-covalent interactions between MTX or NPs and other BACs is one of possible explanation of this phenomenon. For MTX, the mechanism of such interactions is based on stacking mixed aggregates formation with BACs. For NPs, physical van der Waals and electrostatic interactions between surface of NPs and BACs are probably responsible for formation of mixed aggregates. The interactions of MTX or NPs with BACs may be analyzed with several statistical-thermodynamical models. This allows to determine association constants and concentrations for all mixture components. Based on determined appropriate thermodynamic parameters, it is possible to investigate correlation between concentration of free active, form of BACs and their biological activity, measured with e.g., mutagenicity Ames assay. Additionally, confocal microscopy may be applied to observe accumulation of fluorescent drugs in the cells and to assess possible impact of MTX and NPs on this process. Summarizing, biophysical methods provide useful tool to analyze interactions of MTX/NPs with other BACs and to assess possible modulatory effects. Many of BACs that exhibit cytostatic properties are being used in anticancer chemotherapy.

## THEORETICAL INVESTIGATIONS OF ALTERNATIVE RIBOSYLATION PROCESS OF SELECTED 8-AZAPURINES BY PURINE NUCLEOSIDE PHOSPHORYLASE

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Protein Nucleoside Phosphorylase (PNP) is an enzyme, which catalyzes reversible conversion process (ribosylation and phosphorolysis) between nucleobases (purines) and their nucleosides. PNP plays important role in nucleotide metabolism, because it participates in a salvage metabolic pathway of nucleotide synthesis, which utilizes nucleobases and nucleosides available in the cell. This is an alternative pathway to more common, but energetically more expensive, *de novo* synthesis process [1]. Biochemical properties of PNP can be utilized in pharmacological, medical and practical processes. One of the negative consequences of PNP activity can be phosphorolysis of nucleoside drugs and therefore appropriate inhibitors must be applied to attenuate this process [2]. On the other hand, PNP can activate prodrugs, which can be either nucleosides or nucleobases. Some of the PNP enzymes, e.g. from *E. Coli*, can be used in gene therapy of cancer, in which cytotoxic nucleic acids are released as a result of phosphorolysis of non-toxic nucleosides [3]. It has been shown, that deficiency or lack of PNP activity leads to dysfunction of T-cells and it causes decreased cell immunity. PNP can also be used as immunosuppressive drug for transplant rejection, drug for cancers causing overproduction of T-cells and drug for autoimmune diseases such as gout, rheumatoid arthritis, psoriasis, multiple sclerosis [4].

Experimental studies showed that calf PNP ribosylates purine analogs in specific positions – diamino-aza-purine (DaaPur) in positions 7 or 8 (1 to 1 ratio) and 8azaGuanine (azaGua) in position 9 of the triazole ring.[5] The reason of this phenomena can be a different exposition of purine substrates to the channel leading to the binding site. This hypothesis was verified by application of molecular modelling techniques to two complexes of purine analogs DaaPur-calfPNP(pdb-code: 1LVU) and azaGua-calfPNP(pdb-code: 2A11). Docking and molecular dynamics simulations of these complexes were carried out in order to select the most probable binding poses and examine their exposition to the binding channel of calf PNP. Only the most populated tautomers, obtained from quantum chemistry computations of DaaPur (H9, H8 protons) and azaGua (H1-H9, H1-H7, H1-H8 protons), were selected for docking procedure, which led to the selection of 11 and 15 possible binding poses for DaaPur and azaGua, respectively. Results of docking procedure do not resolve validity of our hypothesis, because of close proximity of scoring functions obtained for different poses. Therefore, molecular dynamics simulations combined with MM-PBSA solvation free energy computations and normal modes analysis were performed on selected binding poses obtained from docking procedure. The final binding free energies showed that most probable binding poses expose N8 nitrogen for

DaaPur and N9 or N8 nitrogens for azaGua into the binding channel and ruled out exposition of N9 for DaaPur and N7 for azaGua, partially in agreement with the experimental data.

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## SPECTROSCOPIC CHARACTERIZATION OF BIRD CHERRY FRUITS EXTRACTS AND ITS ANTIOXIDANT POTENTIAL

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Recently much attention is paid to the influence of natural compounds present in every-day diet on human health. Much emphasis is put on non-medical or semi-medical cosmetics, food and drink products, like herbs (fresh and dried), teas and infusions, as well as juices and extracts and that their antioxidant performance is one of crucial factors.

One of possible antioxidants source are fruits of European bird cherry (*prunus padus*) which is European and Asian native tree of a rose family. The bark, leaves and fruits has been known in folk medicine, considering their antibacterial, diuretic, antirheumatic, stypitic and other applications. Nevertheless, properties of any part of the tree, including fruits and fruit extracts are poorly known, and only a few reports on the topic are yet available [1]. They show that bird cherry fruits contain a number of compounds including polyphenols and bioactive compounds, especially vitamins and where many of above poses antioxidant activities.

In this study we have examined the extracts from bird cherry fruits pulp (stones were removed manually), obtained by elution and from partially dried, squeezed fruits to obtain pure native juice. The stock samples of fruit extracts were prepared from water, ethanol and organic solvents with different hydrophobicity. Two fruits and 3 ml of solvent were used to prepare each stock sample. Stock samples were diluted directly before measurements. The dilution rate was adjusted as required for absorption and fluorescence spectroscopy measurements.

To estimate composition of obtained extracts for each sample we measured absorption spectra in the UV-VIS

range, the fluorescence spectra at different excitation wavelengths and additionally the fluorescence lifetime of each sample was recorded.

The results of our studies on the extracts indicated the presence of vitamin E, strong antioxidant, or its derivatives. Moreover, using solvents with hydrophilic and hydrophobic we were able to observe presence of different types of polyphenols, including anthocyanin and flavones, compounds with strong antioxidant properties. The above detected compounds were present in both, a native juice squeezed from fruit and in extracts.

Considering presence of antioxidant compounds in obtained samples, we carried out additional tests to estimate antioxidant potential of water and ethanol extracts of bird cherry fruits. For this purpose we have used DPPH method, and obtained results have shown that both aqueous and alcoholic extracts poses high antioxidant potential, were DL-alpha-tocopherol and ascorbic acid, were used as standards.

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#### THE ENERGY TRANSFER IN BIONANOHYBRID NETS

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Here we are showing the energy transfer in a net, composed of nanoparticles and fluorescent or redox-active proteins. We used two types of nanoparticles: colloidal quantum dots (QD) and carbon nanodots (CND). QDs are a semiconductor, quasi-spherical fluorescent nanoparticles, with a diameter of a few to several nanometers. Here we used QDs composed of cadmium telluride, varied in size and, at the same time, emission maximum (510-750 nm). CNDs are also fluorescent nanoparticles but produced as a result of carbohydrates (here, glucose) carbonization [1]. The bio-part of our nanohybrid nets were redox-active, non fluorescent cytochrome c (Cyt c), its fluorescent derivative with iron substituted by zinc, and fluorescent proteins (green fluorescent protein GFP, mCHERRY, mBANANA and phycocyanine). We already characterized electron transfer between CdTe QDs and Cyt c [2,3]. Here we analysed competition between photoinduced electron transfer (PET) from CdTe (but not CNDs) to Cyt c and fluorescent energy transfer (FRET) to Cyt c fluorescent derivatives. For other fluorescent proteins, we showed

FRET occurrence in solution, between QD-protein pairs and in bigger complexes (especially for phycocyanine), with QDs as donor, mediator and acceptor. We also characterised energy transfer between sequential monolayers, composed of QDs or CNDs, fluorescent proteins and optional spacers. The possible consequences and applications of such systems will be discussed.

#### ACKNOWLEDGMENTS

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#### THE "PATCH-CLAMP" STUDIES ON THE INFLUENCE OF SELECTED POLYCYCLIC COMPOUNDS ON VOLTAGE-GATED POTASSIUM CHANNELS Kv1.3 IN NORMAL AND CANCER CELLS

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Voltage-gated potassium channels of the Kv1.3 type are widely expressed in many cells, both normal and cancer. Kv1.3 channels participate in several processes including proliferation and apoptosis of Kv1.3-channels' expressing normal and cancer cells. Kv1.3 channels were discovered both in the plasma membrane and in the inner mitochondrial membrane (mito Kv1.3 channels). For some years, both plasma membrane and mito Kv1.3 channels are considered as a potentially new molecular target in several pathologies including some cancer disorders [1].

It is known that some small-molecule organic inhibitors of the channels including biologically active plant-derived polycyclic compounds may selectively induce apoptosis of Kv1.3 channels' expressing cancer cells, while sparing normal ones. These compounds may be promising candidates for a putative application in a therapy of some cancer disorders, characterized by an over-expression of Kv1.3 channels, such as breast, colon and lymph node cancer, melanoma or B-type chronic lymphocytic leukaemia (B-CLL) [1].

Electrophysiological studies on the influence of selected plant-derived polycyclic compounds on the activity of Kv1.3 channels are carried out in the Laboratory of



Bioelectricity at the Department of Biophysics at Wrocław Medical University. The whole-cell „patch-clamp” technique is applied in these studies [2]. Studies are carried out on Kv1.3 channels endogenously expressed both in normal human T lymphocytes isolated from peripheral blood of healthy donors and on cancer cells – human leukemic T cell line Jurkat T [1].

This presentation shows a summary of results of our studies on the influence of selected plant-derived polycyclic compounds, and their selected derivatives, from the groups of flavonoids, stilbenes and chalcones on the activity of Kv1.3 channels expressed both in normal and in cancer cells. It is pointed out that some of the selected compounds inhibit Kv1.3 channels in normal and in cancer cells. Ability for Kv1.3 channels’ inhibition is not a general property of examined compounds [1]. Differences in a chemical structure between the channels’ inhibitors and non-inhibitors are subtle. The presence of a prenyl group is a factor that facilitates the ability of flavonoids and chalcones to inhibit Kv1.3 channels [1]. The inhibition of Kv1.3 channels may contribute to the total anti-proliferative and pro-apoptotic effects of these compounds on cancer cells, however, the mechanism of this contribution remains to be elucidated [1]. Finally, it is mentioned that statins represent a new group of potentially effective inhibitors of Kv1.3 channels in cancer cells. These compounds known as inhibitors of biosynthesis of cholesterol and isoprenoid metabolites, are widely applied in a treatment of hypercholesterolemia and atherosclerosis [3]. It was shown that statins – mevastatin and simvastatin exert antiproliferative, pro-apoptotic and reversing drug resistance effect in human colon adenocarcinoma cell line LoVo and its doxorubicin-resistant subline LoVo/Dx [3]. Preliminary results of electrophysiological studies, presented separately on a poster, show that three selected statins: mevastatin, simvastatin and pravastatin are all effective inhibitors of Kv1.3 channels in cancer cells.

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#### ANTIFREEZING GLYCOPEPTIDES (AFGP) – STRUCTURE AND PROPERTIES

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Antifreeze glycoproteins are a class of biological agents which enable living at temperatures below the freezing point of the body fluids. Antifreeze glycopeptides usually consist of repeating tripeptide unit (-Ala-Ala-Thr\*-), glycosylated at the threonine side chain. However, on the microscopic level, the mechanism of action of these compounds remains unclear. As previous research has shown, antifreeze activity of antifreeze glycopeptides strongly relies on the overall conformation of the molecule as well on stereochemistry of amino acid residues. The desired monoglycosylated analogues with acetylated amino termini and the carboxy termini in a form of N-methylamide have been synthesized. Conformational nuclear magnetic resonance (NMR) studies of the designed analogues have shown a strong influence of the stereochemistry of amino acid residues on the peptide chain stability, which could be connected to antifreeze activity of these compounds. A better understanding of the mechanism of action of antifreeze glycopeptides would allow applying these materials e.g. in food industry and biomedicine.

#### ACKNOWLEDGMENTS

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#### THE DYNAMICS OF ATP-SENSITIVE POTASSIUM CHANNELS

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ATP-sensitive potassium channels (KATP) play a key role in insulin secretion from pancreatic beta-cells. They close in response to a change in the ATP/ADP ratio stopping the K<sup>+</sup> outflow, which leads to insulin release. In normal conditions this happens when the blood glucose level rises. Malfunctions in the dynamics of KATP may lead to diabetes.

Despite its enormous physiological role, the mechanism of closing/opening of KATP is not known yet. Fortunately, since 2017 the KATP structure is known (Lee, 2017; Martin, 2017; Wu, 2018). It is a huge complex (~8000aa) composed of four Kir6.2 subunits and four sulfonylurea receptor moieties. This discovery opens a way to model the KATP channel gating. The complexity of KATP system calls for methods able to monitor structural changes. One of them is molecular dynamics. By performing extensive computer modeling of the whole KATP complex we hope



to move towards understanding mechanisms of the KATP channel gating.

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#### BINDING OF N-ACETYLCHITOTRIOSE BY WILD TYPE LYSOZYME AND ITS MUTANT WITH CHANGED DIPOLE MOMENT AS A FUNCTION OF IONIC STRENGTH

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Here, we report preliminary results of investigation of binding kinetics of tri-N-acetylglucosamine (NAG3) to wild type chicken egg lysozyme and to its mutant (D48N/K116Q), in 20 mM glycine-HCl buffer, pH 4.0, at 20°C. At acidic pHs, both proteins have similar average charge, close to +14.0e, and similar magnitudes of the electric dipole moment, around 200 Debyes. However, both dipoles are oriented with respect to each other at an angle of about 150°. Therefore, one expects substantial differences in the electrostatic steering of polar NAG3 ligand (19 Debyes) towards the binding sites of these proteins. We intend to detect these differences by following ionic strength dependence of NAG3 binding by both proteins.

Binding of NAG3 to respective proteins was followed by tryptophyl fluorescence observation of the transients in a stopped-flow spectrofluorimeter, using a 320 nm cut-off filter and a LED light source excitation of 295 nm. The ionic strength of the solution was changed by adding KCl in the range 0-500 mM. The registered progress curves were analyzed with the DynaFit program. The model discrimination procedure, implemented in the program, indicates as the best, the two-step binding model in the case of wild-type lysozyme, which is consistent with earlier work on the binding of NAG3 to this protein. Analysis of residual variations shows that in the case of

the mutant, the two-stage model is at least as good as the one-step model.

Our results show that the association rate constants of the protein-ligand complex,  $k_a$ , have similar values for both proteins, for all ionic strengths. However, they differ with respect to the dissociation rate constant,  $k_d$ . The equilibrium dissociation constant ( $K_D = k_d/k_a$ ) assumes similar values for both proteins at salt concentration of 0 mM. With increasing concentration of KCl, the difference between the values of this constant increases to reach the highest value for 200 mM KCl, and then decreases with further increase of salt concentration. These results confirm that stopped-flow fluorimetry and investigation of ionic strength dependence of the kinetics of ligand binding give a useful tool for studying electrostatic effects in biomolecular association processes.

#### ACKNOWLEDGMENTS

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#### BENDING THE RULES – PLASTOGLOBULES OF SEVERAL MUTANTS OF ARABIDOPSIS

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Plastoglobules (PGLs) are lipoprotein structures – suborganelle compartment of the chloroplast. Very closely related to thylakoids membranes in most cases in physical touch with them [1]. Their numbers increase during the upregulation of plastid lipid metabolism in response to oxidative stress and during senescence. It has been observed that the size and number of these small structures are regulated in correlation with the fitness of the thylakoid membranes. Therefore, appears to be essential to characterize their lipid composition in correlation to their topography and physical properties.

We have chosen for this study *Arabidopsis thaliana* mutants of three groups: chilling sensitive ones (cs, chs5 and chs6), with a different arrangement of main membrane lipids (mgd1, dgd1) and with different saturation levels of lipids' acyl chains (fad3, fad5, fad7-1-8) with appropriate backgrounds of Columbia accessions (Col0 and Col1). We used AFM and TEM measurements as well as HPLC/MS chromatography for polar lipid composition of PGLs and thylakoids, and FTIR spectroscopy for possible lipid-protein interactions within the membranes.

PGLs, as imaged with AFM, are spherical, soft structures. The elasticity of all tested PGLs measured by Young's modulus (E), was close to 1 MPa. This value is in the range of fibroblasts as well as structures like gelatine and significantly lower than the elasticity of collagen [2]. The specific E, however, differs between *A. thaliana* mutants. Interestingly, we observe using TEM a broad distribution of PGLs in terms of size between all analyzed

plants with diameters ranging from 20 nm to approximately 700 nm. We tried to correlate size distribution and physical properties of PGLs with their polar lipid composition.

and water are transported as an isosmotic solution of 145mM of NaCl.

#### ACKNOWLEDGMENTS

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### **ISE-BASED APPARATUS FOR Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, pH, delta V, REAL-TIME SIMULTANEOUS MEASUREMENTS OF ION TRANSPORT ACROSS EPITHELIAL CELLS MONOLAYER**

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Cystic Fibrosis (CF) is the most common fatal human genetic disease, which is caused by a defect in an anion channel protein (CFTR) affecting ion and water transport across the epithelium. We devised an apparatus to enable the measurement of concentration changes of sodium, potassium, chloride, pH, and transepithelial potential difference by means of ion-selective electrodes, which were placed on both sides of a 16HBE14 $\sigma$  human bronchial epithelial cell line grown on a porous support. Using of flat miniaturized ISE electrodes allows reducing the medium volume adjacent to cells to approximately 20  $\mu$ l and detecting changes in ion concentrations caused by transport through the cell layer (Zajac et al., 2019). In contrast to classic electrochemical measurements, in our experiments neither the calibration of electrodes nor the interpretation of results is simple. The calibration solutions might affect cell physiology, the medium composition might change the direction of actions of the membrane channels and transporters, and the transport of ions is accompanied by water flow that might trigger or cut off the transport pathways. We found that, in the isosmotic transepithelial concentration gradient of sodium or chloride ions, there is an electroneutral transport of sodium chloride in both directions of the cell monolayer. The ions

# Posters

## SPECTROSCOPIC STUDIES OF INTERACTIONS BETWEEN ORTHO DERIVATIVES OF *p*-DIMETHYLAMINO BENZOATE AND BOVINE SERUM ALBUMIN

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Understanding the interaction between organic molecule (potential drug) and the proteins is fundamentally essential, especially for medical diagnostics [1]. In this report, the interaction between bovine serum albumin (BSA) and two ortho derivatives of *p*-methylaminobenzoate (methyl *o*-methoxy *p*-methylaminobenzoate (**I**) and methyl *o*-hydroxy *p*-methylaminobenzoate (**II**)) have been studied using steady-state spectroscopic technique. The molecule **I** dissolved in aprotic solvent exhibits only locally excited fluorescence, whereas the molecule **II** exhibits dual fluorescence i.e., emission from the locally excited state and the intramolecular proton transfer state [2]. In the first step of our studies, spectroscopic measurements were employed to investigate the nature of interactions of three biochemically important aromatic amino acids residues viz., tryptophan, tyrosine and phenylalanine (which are constituents of protein) with studied dyes [3-6]. The presence of isosbestic point in absorption and fluorescence spectra of **II** obtained in phosphate buffer, in the presence of tryptophan at its various concentrations, suggests the formation of 1:1 complex between molecule **II** and tryptophan. Similarly, **II** was found to strongly interact (specifically and universally) also with proteins (potential drug related with bovine serum albumin) by fluorescence quenching. The quenching mechanism between **I** and **II** bovine serum albumin was determined as mainly dynamic quenching, combined with static quenching.

### ACKNOWLEDGMENTS

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## PRIMARY REACTIONS IN BACTERIORHODOPSIN PHOTOCYCLE – REVISITED

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Bacteriorhodopsin (BR) is a protein and retinal complex found in purple membranes (PM) that acts as a light-driven proton pump. Under the influence of BR lighting, it is subject to cyclic reactions. It is generally accepted that the primary reaction (the first step of the photocycle), as a result of which energy is accumulated for further transformation of BR is the trans-cis isomerisation of the chromophore taking place without the "communication" of the chromophore with its immediate environment. There are suggestions, however, that another process (for example, the redistribution of electric charge along the chromophore) is the first step in the transformation of BR and that the closest surroundings of the chromophore, e.g. water molecules, can influence this step.

In order to explain both controversial issues, femtosecond absorption spectroscopy was applied and three types of samples were used: native PM, PM with fluorinated bacteriorhodopsin and PM deposited electrophoretically on SnO<sub>2</sub>. The water content in the samples was regulated by reducing the pressure in a special cryostat. Because "dry" samples can be easily destroyed by irradiation with laser radiation, a special, very precise device was constructed that moved the cryostat with the sample in x-y direction.

It was noted that the kinetics and yields of femtosecond changes of native and fluorinated BR are different. The changes were strongly dependent on the water content in BP. The obtained results suggest that the redistribution of charges along the chromophore is a step earlier than its trans-cis isomerization. In addition, it can be stated (contrary to earlier publications) that the "communication" of the chromophore with the closest surroundings (eg. through water molecules) affects the original BR reactions. It is suggested that similar "electrostatic communication" between chromophore and opsin may take place in rhodopsins, visual complexes.

## EVALUATION OF THE EFFECT OF ORGANOPHOSPHORUS FLAME RETARDANTS ON HUMAN ERYTHROCYTES

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Intensive growth of manufacturing of synthetic polymers present in our life increases risk of fire. That is why various methods are used in order to reduce flammability of daily use products. One of them is the usage of flame retardants, which are designed to slow down the combustion process, and thus affect the emission of smoke. This group of chemical compounds includes organophosphorus flame retardants. So far, there is insufficient data for evaluation of the toxic effects of these chemicals on the environment and living organisms.

The aim of this study was to determine hemolytic and oxidative properties of two selected phosphorus flame retardants – tris(2-chloroethyl) phosphate, and (2-chloroisopropyl) phosphate. The study assessed changes in cell viability and morphology (flow cytometric analysis of cell size and granulation) as well as alterations in methemoglobin and reactive oxygen species (ROS) levels in human erythrocytes. The erythrocytes were separated from blood (leucocyte-buffy coat) from healthy donors. Blood was obtained from the Regional Blood Donation and Blood Treatment Center in Łódź.

Hemolysis and methemoglobin content showed a tendency to increase along with the increasing concentrations of the compounds studied. Similarly, the level of ROS determined on the basis of the dichlorofluorescein fluorescence raised along with the increasing concentrations of the substances studied, but it did not reach high value.

The results of this study have shown that organophosphorus flame retardants are characterized by relatively low toxicity in comparison to the most commonly used brominated flame retardants (BFRs), because the majority of changes have been observed only at their highest concentrations, which may penetrate into the human body as a result of acute poisoning. The lowest concentrations of the tested compounds did not cause any statistically significant changes in the parameters analyzed.

## FAST FIELD-CYCLING NMR RELAXOMETRY CHARACTERIZATION OF HYDROCOLLOIDAL SYSTEMS

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Hydrocolloids are polymers of biological or synthetic origin with of a large number of hydroxyl groups, widely used in food processing technologies as gelling agents, thickeners or fat and saccharose replacers. Water binding

affects texture and processing characteristics, which is why knowledge of the state of water in such biopolymer suspensions is essential to understand and predict their behaviour during production, storage and thermal processing. A useful technique to study the state of water in foods is nuclear magnetic resonance (NMR); the usual way of probing the dynamics using NMR is to examine relaxation at different temperatures and assume a function for the temperature dependence of the correlation times. However, in such a way large temperature range needs to be covered, which can be problematic in foods, as its structure and properties are temperature dependent. The alternative is to determine so-called spectral density function of the substance by measuring spin-lattice relaxation time,  $T_1$ , over a wide range of Larmor frequencies. By using this so-called field-cycling (FC) technique one can probe the dynamical processes in the system [1].

The aim of the study was to acquire Nuclear Magnetic Relaxation Dispersion (NMRD) profiles of several binary systems based on agar, gelatin and carrageenan varying in concentration and temperature. Relaxation data complemented with viscosimetry measurements allowed to draw basic conclusions on the dynamics of water present in the systems and proved a potential of FC NMR relaxometry as tool to characterize food products.

## ACKNOWLEDGEMENTS

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## THE ROLE OF PICEATANNOL IN COUNTERACTING GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE AGGREGATION AND NUCLEAR TRANSLOCATION IN HIPPOCAMPAL CELLS

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The primary aim of modern neurobiology/science is to prevent or slow down the progression of neurodegenerative diseases. One available solution is supplementation with superfoods. To widen the knowledge

about compounds that are contained in various fruits and vegetables, we examined one naturally occurring stilbene derivative - piceatannol and its effect on glyceraldehyde-3-phosphate dehydrogenase (GAPDH). This enzyme is one of the most susceptible to oxidative modifications. Further, GAPDH changes, under certain conditions, promote and accelerate neurodegenerative processes [1]. In this study, we demonstrated how piceatannol influences on these processes.

The objective of the presented study was to determine whether piceatannol inhibits unfavourable GAPDH nuclear translocation in hippocampal cells as well as protein aggregation induced by excessive oxidative stress. For this purpose we applied following methods:

MTT assay (cell viability), immunostaining and confocal microscopy, immunoprecipitation and Western Blot and flow cytometry analysis.

We found that piceatannol significantly suppresses GAPDH nuclear translocation as well as protein aggregation induced by excessive stress.

The piceatannol anti-aggregation activity and ability to counteract GAPDH nuclear translocation place this compound as a new drug candidate for *in vivo* tests.

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#### EFFECT OF CARDIOPROTECTIVE FLAVONOIDS ON THE ACTIVITY OF THE MITOCHONDRIAL BK<sub>Ca</sub> CHANNEL

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Flavonoids belong to a large group of polyphenolic compounds that are widely present in plants. Some of them, including luteolin, quercetin or cyanidin, have been shown to be cardioprotective. Although the antioxidant effect of flavonoids has been long thought to be a crucial factor accounting for cellular cardioprotection [1,2]. Also, mitochondrial pathways (including mitochondrial large-conductance Ca<sup>2+</sup>-regulated (mitoBK<sub>Ca</sub> channel) are presently emerging potential targets for a specific pharmacological action of flavonoids in the anti-ischemic strategies [3].

The aim of these studies is the characterization of interactions between cardioprotective flavonoids and the mitoBK<sub>Ca</sub> channel present in the inner mitochondrial membrane of the endothelial cells.

Single channel activity of the mitoBK<sub>Ca</sub> was measured

with patch-clamp of the mitoplasts isolated from endothelial cells (EA.hy926). Application of 3 μM cyanidin has an inhibitory effect. In the presence of luteolin, changes of open probability of the mitoBK<sub>Ca</sub> channel were not observed. Furthermore, regulation of the mitoBK<sub>Ca</sub> channel by flavonoids were studied in the presence of 0.5 mM dithiothreitol. Changes in the redox state causes that luteolin and cyanidin have activatory properties. Open probability of the mitoBK<sub>Ca</sub> channel increase from 0.02 to 0.36 at -40 mV in the presence 10 μM cyanidin. However, quercetin has strong activating properties both under control conditions and reduced by DTT. Additionally, possible cytoprotective properties of quercetin with using apoptosis/necrosis assays were also studied.

We expect that our studies describing the regulation of mitochondrial potassium channels by the natural substances of plant origin will bring us closer to a better understanding of flavonoid-induced cytoprotective mechanisms.

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#### BIOLOGICAL PROPERTIES OF CHITOSAN-GRAPHENE NANOCOMPOSITES

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Chitosan is an amino-carbohydrate obtained from incomplete deacetylation of chitin. It is biocompatible, fully degradable, water-soluble and can be used as a colloidal solution, handled as a solvogel, triggered as a pH-responsive physical or chemical hydrogel, cast as thinner or thicker films, and shaped as self-standing microspheres to provide highly porous CO<sub>2</sub>-dried monolithic aerogels or lyophilized cryogel scaffolds. These features account for implementing chitosan scaffolds in various fields, including scavenging

chemicals, tissue-engineering, wound-dressing, drug-release and food-packaging.

Graphene oxide is an increasingly studied nanomaterial that has recently been used as nanosized filler to build novel exfoliated nanocomposites. However, few functionalized graphene (oxide) derivatives are known, and informative studies dealing with the biological effects of graphene surface functionalization are currently missing in the open literature.

The aim of the study was to evaluate the effect of chitosan-graphene nanocomposites on human erythrocytes and hemoglobin.

Results shows the hemolytic activity after incubation time of 1, 3 and 24 h. All chitosan-reinforced graphene nanocomposite films induced hemolysis. After incubation for 1 and 3 h, the hemolysis of erythrocytes was approximately 6.5% with no statistically significant differences between composites. After 24 h of incubation, the changes are not statistically significant compared to the hemolysis obtained after shorter incubation times. As hemolysis was not dependent on incubation time, we investigated possible hemoglobin adsorption on the surface of chitosan-reinforced graphene films. After 3 h incubation of hemolysate with graphene composites, a negligible adsorption of hemoglobin was experienced. However, hemoglobin adsorption reached 22-29% after 24 h of incubation. These results suggest that hemoglobin released from erythrocytes remains adsorbed to chitosan-graphene films after 24 h, which causes a decrease in the hemoglobin content in the solution and was misread as a lack of hemolysis increase after 24 h incubation. Thus, the percentage of hemolysis after 24 h does not reflect real hemolytic activity but is rather associated with the accumulation of hemoglobin (released from erythrocytes) on the surface of graphene composites.

All chitosan-graphene films caused the oxidation of hemoglobin after 3 h of incubation with the erythrocytes. For the control, the percentage of methemoglobin after 3 h of incubation was only 1.8%, and after 24 h, the percentage increased to 4%. Statistically significant changes in the percentage of met-Hb content were observed for all graphene composites after 3 and 24 h incubation.

### MOLECULAR MECHANISMS OF PHOTOPROTECTION IN THE PHOTOSYNTHETIC APPARATUS OF PLANTS

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Life on Earth is powered by the energy of light reaching our planet from the Sun, but utilization of this energy by living organisms is only possible thanks to the process of photosynthesis that converts the energy of electromagnetic radiation to the forms which can be directly used to drive biochemical reactions [1]. Photosynthesis in plants

operates under conditions characterized by severe risk factors associated with the exposure to high light. Under excess light, violaxanthin (Vio) is converted rapidly to zeaxanthin (Zea), and this reaction is reversed under low light levels. Efficient and safe operation of the photosynthetic reactions is vital to plants and is assured by the activity of numerous regulatory processes functioning to increase excitations under low light and to quench excessive, potentially harmful excitations, under high light conditions. For many decades there has been a debate on the role of zeaxanthin, synthesized in the xanthophyll cycle, in photoprotective excitation quenching and conclusions from various studies are often contradictory [2,3].

Molecular spectroscopy techniques such as steady-state, time-resolved fluorescence and resonance Raman scattering were used in this work. Action of the xanthophyll cycle and chlorophyll excitation quenching were analyzed in *Arabidopsis thaliana*, the wild-type and two mutants, npq1 (lacking Vio de-epoxidase) and npq4 (lacking the PsbS protein demonstrated to be essential for an efficient Zea-dependent photoprotective excitation quenching). The results of the experiments show that zeaxanthin can account for ca. 50 % of the photoprotective quenching of chlorophyll excitations.

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### INVESTIGATION OF DRUGS MOLECULES RELEASE FROM POLYURETHANE HYDROGELS CONTAINING CLAY NANOPARTICLES

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Polyurethane hydrogels due to their unique swelling properties are very versatile in case of possible applications in many fields, especially in biomedicine [1]. The ability to maintain a hydrated environment, high capacity to absorb the solution and the ability of the polymer to release active substances made them good candidates for biomedical applications [2]. These features enable the design of a moist hydrogel dressing to facilitate wound healing as well as relieve pain, releasing the drug

into the skin [3]. Improvement of material properties is possible by adding nanoparticles that expand the intermolecular spaces in the polyurethane matrix and increase the swelling capacity of the polymer matrix [4].

The description of swelling and release of active substances is crucial aspect examined in terms of the applicability of hydrogel materials. The transport of solutes in swollen gel membranes is subject to two mechanisms: dissolved substances penetrate the membrane through the pores filled with solvent (diffusion) and the reaction of the polymer to the stresses exerted by the attack of solvent molecules occurs (relaxation) [5].

The main purpose of our research is to achieved material with predetermined and well defined hardness, elasticity, and with appropriate swelling and release profiles. In previous research we described method of synthesis and studies of basic mechanical properties and structural properties [6-8].

In the present studies, we examined polyurethane nanocomposite hydrogels doped with various amount of and nanofiller – Cloisite® 30B. In particular, we investigated the influence of Cloisite® 30B on the swelling and release of active substances: naproxen sodium and paracetamol. The presence of clay mineral plates in hydrogels remarkably improves the swelling capability, but on the other hand slows down the release. We also performed an accurate theoretical analysis in different theoretical and semi-empirical models [8-9].

#### ACKNOWLEDGMENTS

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#### ONE-TRYPTOPHAN MUTANTS AS MARKERS OF TRIMERIC MAMMALIAN PURINE NUCLEOSIDE PHOSPHORYLASE UNFOLDING

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The folding and unfolding of oligomeric protein are not well explored. To this group belongs homotrimeric purine

nucleoside phosphorylase (PNP) – the enzyme that plays a key role in the nucleoside and nucleotide metabolic salvage pathway, and is a target for anti-cancer and immune system suppressing therapies [1]. Our studies have shown that although the enzyme exists in a trimeric form, each subunit functions independently [2], and monomers, if exist, are unstable and prone to aggregation [3].

To answer the question how the unfolding of PNP proceeds – during one step, without presence of monomers, or in two steps where trimer first dissociates to unstable monomers, three one-tryptophan mutants were obtained (W16-PNP, W94-PNP and W178-PNP). All these mutants have catalytic properties similar to that of the wild type PNP. Their fluorescence spectra show a clear difference between folded and unfolded forms making them a good tool for characterizing PNP folding/unfolding processes.

The stopped-flow unfolding measurements initialized by mixing of folded protein with buffer containing high concentration of denaturant - guanidinium hydrochloride show that the tryptophan environment changes the fastest for the W94-PNP mutant, in which Trp is located closest to the symmetry axis of the protein. It suggests that during unfolding, PNP trimer first dissociates into unstable monomers.

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**CHARACTERISTIC OF SPECTROSCOPIC PROPERTIES AND ANTIOXIDANT ACTIVITY OF NEW SYNTHESIZED ALPHA-TOCOPHEROL DERIVATIVE**

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This work concerns spectroscopic, DLS and antioxidant studies of alpha-tocopherol (Toc) analog modified at the O-1 position, named 1-carba-alpha-tocopherol (1CT). The studied vitamin E derivative contains the 1,2,3,4-tetrahydronaphthalene skeleton instead of the chroman ring. This modification should significantly change its physico-chemical and spectroscopic properties compared to the parent tocopherol.

In this study, spectroscopic properties (absorption, fluorescence and fluorescence lifetime) of 1CT in homogeneous environments and in liposomes composed of dipalmitoylphosphatidyl choline (DPPC) were measured. In order to estimate the influence of 1CT on the properties of model membranes dynamic light scattering (DLS) technique was used. For this derivative, antioxidative activity tests using the DPPH radicals were also performed.

In organic solvents with different physical properties, the absorption maxima for 1CT was located at similar positions (283-286nm), with extinction coefficients ranging from 1200M<sup>-1</sup>cm<sup>-1</sup> in octanol to 8000M<sup>-1</sup>cm<sup>-1</sup> in hexane. The investigated Toc analog exhibited a blue shift of 9-12nm compared to Toc. The fluorescence maximum of 1CT in the investigated solvents was found at the wavelengths ranging from 303 to 311nm, and is blue shifted at about 18nm compared to Toc.

In a model lipid membrane Toc exhibited emission spectra which consisted of an unstructured band with maximum at 325nm. The fluorescence maximum of 1CT in DPPC was found at 306nm and this position was held within a wide fluorophore concentration range. For 1CT the linear fluorescence increase was observed with increasing concentration of this derivative what suggests that the observed emission arises from a monomeric form of 1CT. In liposomes the emission maximum and fluorescence lifetime of Toc analog were similar to those observed in methanol, which suggests medium value of dielectric constant and low viscosity environment. Simultaneously, the fluorescence lifetime of 1CT (3,5ns) incorporated into DPPC is longer that observed for Toc (1,2ns).

The particle size distribution in the DPPC suspension was determined using DLS method. The mean values of liposome sizes (110nm) were determined from the analysis of number of peaks and was not changed significantly in the presence of different amounts of 1CT.

The antioxidant activity of 1CT was determined by the method of quenching DPPH radicals, which relies on measuring absorbance intensity at the characteristic for DPPH wavelength equal to 517nm. The antioxidant

properties of 1CT was compared with that of the parent Toc sample. The obtained results confirmed that 1CT reveals antiradical properties and quenches DPPH radicals. However, its antioxidant efficiency was much lower that observed for free Toc. This phenomena results from deprivation of heterocyclic oxygen, which plays a key role in the antioxidant activity of vitamin E.

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**COMPUTATIONAL STUDY OF SELECTED 4-HYDROXYMETHYL-3-AMINOACRIDINE DERIVATIVES WITH ANTICANCER ACTIVITY P**

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4-hydroxymethyl-3-aminoacridine (4-HM-3-AA) derivatives were synthesized [1] and evaluated for anticancer activity [2] in laboratories in France. It is interesting that the most cytotoxic compounds (i) intercalate to DNA but do not inhibit DNA topoisomerases activity, and (ii) differ in cell distribution (Peixoto *et al.*, 2009). In this research the molecular properties of selected 4-HM-3-AA derivatives were studied using quantum mechanical calculations methods and then the results were discussed to explain the difference in their biological activity. It has been found that there are some differences in both structural parameters and electronic properties of molecules, which may explain their different biological behavior.

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The calculations were performed on a computer and software in the Laboratory of Computer and Analytical Techniques, University of Lodz, Poland.

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## EXAMINATION OF THE CADMIUM-CHLOROPHYLL COMPLEX: SPECTRAL PROPERTIES, KINETIC AND REASONS FOR INHIBITING PHOTOSYNTHESIS

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Heavy metals can be taken up by plants from the environment and transported with water to stems and leaves [1] thus causing plant growth inhibition [2], formation of reactive oxygen species [3], and inhibition of photosynthesis [1]. In order to reveal the mechanism of cadmium-induced chlorophyll degradation, spectroscopic analyses were carried out, using a series of chlorophyll ( $C=1 \times 10^{-5} \text{M}$ ) solutions with  $\text{CdCl}_2$  (from  $C=1 \times 10^{-5} \text{M}$  to  $9 \times 10^{-3} \text{M}$ ) in methanol. With increasing  $\text{Cd}^{2+}$  concentration, both,  $Q_y$  and the Soret chlorophyll bands were shifted by 9 nm towards the short-wave range. New absorption bands for the reaction products were formed at 656nm and 420nm. The fluorescence spectra were shifted hypsochromically by 11 nm (677 nm to 666 nm) relative to the chlorophyll fluorescence band. The final absorption and fluorescence spectra of the pure complex were recorded after 240h for the  $C_{\text{Cd}}=1 \times 10^{-5} \text{M}$  and for the  $C_{\text{Cd}}=9 \times 10^{-3} \text{M}$  after 17h.. The reaction rate constants were increased in samples from  $k=1.510 \times 10^{-5} \text{M}^{-1} \text{min}^{-1}$  for  $C_{\text{Cd}}=1 \times 10^{-5} \text{M}$  to  $k=13.350 \times 10^{-4} \text{M}^{-1} \text{min}^{-1}$  for  $C_{\text{Cd}}=9 \times 10^{-3} \text{M}$ . The experiments show that cadmium is bound into the chlorophyll molecule substituting its magnesium. In plants intoxicated with cadmium, taken up from contaminated soil, the energy transfer between Chl and Cd-Chl will be impaired, which may be one of the reasons for the inhibition of photosynthesis. This is indicated by two times smaller overlap integrals of the Cd-Chl absorption spectrum with the Chl fluorescence spectrum,  $I_{\text{Chl,CdChl}}=2.4223 \times 10^{-13} \text{cm}^3/\text{M}$  (twice lower probability of transfer) in comparison with overlap integral for Chl→Chl transfer:  $I_{\text{Chl,Chl}}=4.6210 \times 10^{-13} \text{cm}^3/\text{M}$ , and lower Förster critical distance for resonance energy transfer:  $R_{o\text{Chl} \rightarrow \text{CdChl}}=46.773 \text{Å}$ ,  $R_{o\text{Chl} \rightarrow \text{Chl}}=52.086 \text{Å}$ .

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## RESONANCE RAMAN SPECTROSCOPY STUDY ON LOCALIZATION AND ORIENTATION OF LUTEIN IN A LIPID BILAYER

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Lutein, together with zeaxanthin and meso-zeaxanthin, are xanthophyll pigments with special significance for humans. In the human body they accumulate selectively in the retina of the eye and thus protect the retina from damage [1]. These compounds are antioxidants scavenging efficiently free radicals and besides they act as a light filter absorbing harmful to the eye shortwave radiation, hence their presence in the yellow spot of the retina is essential for maintaining the proper functioning of the vision organ. However, they not only affect the eye, but also the various tissues of living organisms.

In biological membranes xanthophylls are present as components of lipid phase or in the form of a protein complex [2]. In the presented work, giant unilamellar vesicles (GUVs) were used as a model of biological membranes to verify the response of Raman spectroscopy to the interaction occurring between lutein and dipalmitoylphosphatidylcholine (DPPC). It seems to be helpful for understanding the processes taking place inside the living organisms.

Lutein-containing GUVs were formed at 0.5 mol % xanthophyll concentration with respect to DPPC lipid (Avanti Polar Lipids). Before liposomes preparation, crystalline lutein (Extrasynthese) was repurified by using HPLC technique and then has been added to a lipid solution in ethanol. Obtained mixture were deposited to two platinum electrodes fixed in the Teflon holder at a distance of 4 mm, placed for 1h in a vacuum (to remove organic residues) and next in a cuvette which contained the buffer solution (1.4 mL, 20 mM Tricine, 10 mM KCl, pH 7.6). Finally, electric connections were attached to the AC field supply (DF 1641A). Electroformation process was carried out over 2 h with an applied AC sinusoidal field with 10 Hz frequency and voltage 3 V (peak-to-peak). The temperature was stabilized at 45°C.

The obtained results confirm that application of the resonance Raman technique enables to determine the orientation of the transition dipoles of xanthophylls molecules due to the photoselection process. Analysis of Raman images of individual liposomes shows that lutein can adopt two orientations: perpendicular and parallel with respect to the membrane plane. In case of using the lowest possible laser power, the preferred molecules orientation is vertical and at these points, spectroscopic analysis indicates the presence of the trans-xanthophyll forms in the unilamellar vesicle. On the other hand, the increase in laser power causes the formation of more distorted structures of lutein, which are oriented horizontally in relation to the membrane plane (signal in the upper and lower sector of liposome).

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**CHANGES IN ANTIOXIDANT ENZYMES  
ACTIVITIES AND REACTIVE OXYGEN SPECIES  
LEVEL IN HUMAN ERYTHROCYTES EXPOSED  
TO SELECTED PHTHALATES**

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Phthalates have been extensively used as plasticizers in various branches of industry including food, cosmetic and pharmaceutical. Phthalates do not form covalent bonds with other compounds, thus they can easily migrate from various products, and then reach the body with air, food and water. Significant concentrations of phthalates and their metabolites have been determined in urine, breast milk, blood serum, venous blood, and cord blood.

It has been shown that phthalates like di-n-butyl phthalate (DBP), butylbenzyl phthalate (BBP) as well as their metabolites including mono-n-butylphthalate (MBP) and mono-benzylphthalate (MBzP) can induce oxidative stress. Therefore, the aim of our work was to evaluate the effect of selected phthalates on the activities of antioxidant enzymes, i.e. superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) and the level of reactive oxygen species (ROS) in human erythrocytes.

The erythrocytes were incubated with the compounds studied in the concentrations ranging from 0.5 to 500 µg/ml for 24 h. It has been found that DBP, BBP and their metabolites: MBP, MBzP induced ROS (including  $\cdot\text{OH}$ ) formation, increased CAT activity and decreased the activities of SOD and GSH-Px.

It has been noted that the strongest alterations in ROS formation, and antioxidant enzymes activities were induced by DBP and BBP in the concentration of 2.5 µg/mL.

**SPECTROSCOPY OF TRI-CYCLIC GUANINE AND  
ISOGUANINE DERIVATIVES AND THEIR  
RIBOSIDES**

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Tri-cyclic analogs of natural purines and their derivatives are known to react with many enzymes of purine metabolism [1], and are important intermediates of the chemical mutagenesis.

The purine-nucleoside phosphorylase enzyme (PNP, E.C.2.4.2.1) is responsible for the regulation of the various nucleoside concentrations within the living cells, and a target of many types of pharmaceutical interventions [2]. PNP isolated from *E. coli* is active towards tri-cyclic  $\epsilon\text{Ado}$  and its 2-aza analog [3]. In the absence of phosphate ions, it is possible to observe the reverse reaction - attachment of the sugar moiety to the tri-cyclic base, where the second substrate is a phosphorylated sugar ( $\alpha\text{-D-ribose-1-phosphate}$ , R1P).

Our investigations have shown that 1,N<sup>2</sup>-ethenoguanine is an excellent substrate for PNP from *E. coli*, with catalytic and Michaelis' constants comparable to that for ribosylation of the parent guanine. The reverse reaction (phosphorolysis of the nucleoside) is also easily observed in the presence of phosphate ions. These facts may be important in view of a significant mutagenic role of 1,N<sup>2</sup>-ethenoguanine lesion in many organisms. The isomeric N<sup>2</sup>,3-ethenoguanine is not a substrate for PNP from *E. coli* and calf spleen [4].

Spectrophotometric titrations of the 1,N<sup>6</sup>-ethenoisoguanine ( $\epsilon\text{isoGua}$ ) indicate that this compound exists as a neutral species at pH 4.5–7, and above pH 8 undergoes deprotonation. The anionic forms are virtually nonfluorescent, while the neutral form and the cation are strongly fluorescent, with maxima at ~400 nm.

Ribosylation of  $\epsilon\text{isoGua}$ , catalyzed by PNP from *E. coli* gave two products: The highly fluorescent N9-riboside, and N7-riboside with less intense fluorescence shifted to ~355 nm. The analogous reaction catalyzed by the calf PNP gave one main product, very intensely fluorescent, but with UV absorption spectrum markedly shifted to the longer wavelengths, identified as N<sup>6</sup>-riboside. All ribosides may be useful as fluorescent probes in enzymology.

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## STRUCTURAL CHANGES OF COAL CAUSED BY AUTOCHTHONIC MICROBIOTA - FTIR STUDIES

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The aim of the research was recognition of structural changes of coals as an effect of activity of autochthonous microorganisms. FTIR (Fourier Transformed Infrared Spectroscopy) was applied to analyze pristine samples of polish hard coals and lignites and same materials subjected to long-term anaerobic microcosm incubations. Microbial activity and community structure were studied using gas chromatography and next generation sequencing.

Stimulation of microbiota resulted in a decrease of free C=O ( $>1740\text{cm}^{-1}$ ), probably as an effect of activity of species that utilize the Wood–Ljungdahl pathway which enable some anaerobic *Bacteria* and *Archaea* both energy and biomass production [1]. The surface area of the peak characteristic of a -COOH stretching vibration decreased upon incubation, indicating the possibility of usage of this group by the acetotrophic methanogens [2].

The lignites revealed a significant reorganization of the structure concerning aromatic/aliphatic character revealed by the change in the regions representing aromatic CHx stretching ( $3000\text{-}3100\text{ cm}^{-1}$ ), aromatic C=C ring stretching ( $1550\text{-}1650\text{ cm}^{-1}$ ), aromatics' CHx out of plane deformation ( $650\text{-}900\text{ cm}^{-1}$ ), aliphatic CHx stretching ( $2800\text{-}3000\text{ cm}^{-1}$ ) and aliphatic CHx bending ( $1300\text{-}1550\text{cm}^{-1}$ ). When considering hard coals, in samples analyzed, the decrease in aromaticity was accompanied by an increase in aliphaticity and CH<sub>2</sub>/CH<sub>3</sub> ratio. The released -CH<sub>3</sub> and -OCH<sub>3</sub> groups comprise a readily available substrate for methylotrophic microorganisms.

In the microbiota composition of hard coals as well as lignites *Bacteria* comprised 97-99% of the community. Among them, the major phylum was *Proteobacteria* (43-61%). In the pristine communities *Archaea* constituted only 0.03-1.51% and increased several times during anaerobic incubation. The structural changes of lignites and hard coals indicate that these materials harbor microbial communities capable of anaerobic degradation of the organic matter and by providing fermentation substrates may support methanogenesis.

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## REGULATION OF MITOCHONDRIAL POTASSIUM CHANNELS BY FLAVONOIDS P

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Rapid, electrogenic transport through cell membranes is mediated by many different types of potassium channels. Recently, many studies focus on the intracellular potassium transport. The protection of cardiac cells against ischemia/reperfusion injury by activators of the mitochondrial K<sub>ATP</sub> channel and the mitochondrial BK<sub>Ca</sub> channel is now widely accepted. Mitochondrial potassium transport-dependent cytoprotection against ischemia/reperfusion and oxidative stress induced injury has also been demonstrated in other numerous tissues [1].

In recent years, the subject of many studies are chemical compounds found in plants. Due to the numerous biological effects, a particularly interesting group are flavonoids. Interest in health benefits of flavonoids has increased due to their potent antioxidant and free-radical scavenging activities. The biological activity, bioavailability and low toxicity set broad prospects of the usage of some of these substances as potential therapeutics for a number of human diseases. Some flavonoids have also been shown to be cardioprotective. Although the antioxidant effect of flavonoids has been long thought to be a crucial factor accounting for cellular cardioprotection, mitochondrial pathways (including mitochondrial ion channels) are presently emerging potential targets for a specific pharmacological action of some flavonoids in the anti-ischemic strategies [2,3].

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**THE INHIBITORY EFFECT OF STATINS ON  
VOLTAGE-GATED POTASSIUM CHANNELS Kv1.3  
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Voltage-gated potassium channels of the Kv1.3 type are widely expressed in many cells, both normal and cancer. Kv1.3 channels participate in several processes including proliferation and apoptosis of Kv1.3-channels' expressing normal and cancer cells. It is known that some small-molecule organic inhibitors of the channels including biologically active plant-derived polycyclic compounds may selectively induce apoptosis of Kv1.3 channels' expressing cancer cells, while sparing normal ones. These compounds may be promising candidates for a putative application in a therapy of some cancer disorders, characterized by an over-expression of Kv1.3 channels, such as breast, colon and lymph node cancer, melanoma or B-type chronic lymphocytic leukaemia (B-CLL) [1].

Statins are compounds known as inhibitors of 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. This leads to an inhibition of biosynthesis of cholesterol and isoprenoid metabolites. Therefore, statins are widely applied in a treatment of hypercholesterolemia and atherosclerosis [2]. It was shown that statins – mevastatin and simvastatin exert antiproliferative, pro-apoptotic and reversing drug resistance effect in human colon adenocarcinoma cell line LoVo and its doxorubicin-resistant subline LoVo/Dx [2]. Studies performed in our electrophysiological laboratory applying the whole-cell patch-clamp technique showed that statins: mevastatin and simvastatin are effective inhibitors of Kv1.3 channels in cancer cells – human T cell line Jurkat. It was shown that an application of mevastatin and simvastatin in the concentration range from 7.5  $\mu\text{M}$  to 30  $\mu\text{M}$  inhibited the channels in a concentration-dependent manner. The inhibitory effect was partially reversible. The inhibition was accompanied by a significant acceleration of the currents' inactivation without any significant change of the activation rate. In the case of an application of another statin: pravastatin – an inhibitory effect on Kv1.3 channels was observed only at the concentration of 50  $\mu\text{M}$ , whereas

at lower concentrations no significant inhibition was observed. A mechanism of the channels' inhibition and its contribution to a regulation of cancer cells' proliferation and apoptosis by the statins is discussed.

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**THE EFFECT OF BROMOPHENOLIC FLAME  
RETARDANTS ON DNA DAMAGE IN HUMAN  
PERIPHERAL BLOOD MONONUCLEAR CELLS P**

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Bromophenolic flame retardants (BFRs) are synthetic substances widely used in the industry (manufacture of electrical and electronic equipment, textiles, furniture and other everyday products) [1,2]. Products containing BFRs protect property; however there are fears about harmful impact of these substances on human health.

In 2012, the European Food Safety Authority concluded that it is not possible to determine the health risk posed by BFRs due to insufficient data on the presence of these compounds in edibles and the food chain, and the negligible number of toxicological data.

Damage to DNA of human peripheral blood mononuclear cells (PBMCs) may contribute to impaired immune response, which may lead to autoimmune diseases or cancer development. That is why in this study, we have assessed the effect of selected BFRs, i.e. tetrabromobisphenol A (TBBPA), tetrabromobisphenol S (TBBPS), 2,4,6-tribromophenol (2,4,6-TBP) and pentabromo-phenol (PBP) on double-strand breaks creation and hydroxyl radical formation in human PBMCs.

The cells were incubated with the compounds studied in the concentrations ranging from 0.01 to 10  $\mu\text{g/ml}$  for 1 or 24 h. DNA damage was assessed using neutral version of the comet assay [3], while hydroxyl radical formation was determined by flow cytometry using fluorescent probe – hydroxyphenyl fluorescein.

The results of this study have shown that TBBPA at 1 and 10  $\mu\text{g/ml}$  caused statistically significant increase in DNA double strand-breaks (DSBs) formation, while other compounds studied did not induce DNA damage. It is well-known that highly reactive oxygen species (mainly hydroxyl radical) are involved in DSBs formation [4]. We have observed that only TBBPA at 1 and 10  $\mu\text{g/ml}$  increased hydroxyl radical level in human PBMCs.

In conclusion, TBBPA caused low level of DNA damage in human PBMCS, which was mainly due to hydroxyl radical formation in this cell type.

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