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PREFACE

Dear Colleagues,

This year's 18th Congress of the Polish Biophysical Society PTBF2022 is organized and hosted by the Polish Biophysical Society, Institute of Biology Warsaw University of Life Sciences, Faculty of Physics, the University of Warsaw and Polish Academy of Science celebrating the 50th birthday of PTBF.

The congress aims to promote research, to develop collaboration between congress participants, and representatives of different scientific disciplines ranging from basic sciences, such as physics, chemistry, and biology to appl, such as laboratory diagnostics, pharmacy or medicine, and to encourage students and early career investigators to stay involved and build connections with colleagues and leading experts in biophysics.

PTBF2022 will be attended by approximately 140 delegates. With 10 organized sessions, there will be 55 lectures, 3 memories talks, and 2 workshops. Plenty of time will be allocated for viewing the over 50 posters presented by the delegates.

We are grateful to our sponsors and supporting organizations for their generous financial contribution. Last but not least, we are indebted to all of you, who bring the latest and most exciting science to PTBF2022! It only remains for us to wish you a successful congress and a memorable stay in Warsaw.

Piotr Bednarczyk
PTBF President

Beata Wielgus-Kutrowska
PTBF Vice-President

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Plenary lectures

ON ORIENTING ELECTROSTATIC AND HYDRODYNAMIC STEERING EFFECTS IN THE KINETICS OF RECEPTOR-LIGAND ASSOCIATION

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The formation of a diffusional encounter complex by receptor and ligand molecules is a preliminary step in many processes taking place in biological systems [1]. Diffusional encounter is thought to influence or limit the rate of these processes. The traditional Smoluchowski theory, which assumes spherical reaction partners with uniformly reactive surfaces, yields the bimolecular diffusional encounter rate constant $k=4\pi(D_A+D_B)(r_A+r_B)$, where D_x and r_x are the translational diffusion coefficient and the radius of the reaction partner X.

The use of the Smoluchowski equation to determine the rate of formation of encounter complexes by biological molecules is limited due to several factors. First, the receptor and ligand molecules encountered in biological systems are usually not spherical. Secondly, the rate of productive diffusional encounter is modulated by orientational requirements for complex formation – i.e. surfaces of the receptor and ligand molecules are not uniformly reactive. Finally, and third, the speed of the encounter complex formation also depends on the long-range interactions between the reaction partners. The importance of two long-range interactions in the encounter processes has been recognized for a long time. These are electrostatic interactions and hydrodynamic interactions.

The electrostatic attraction between a receptor and its ligand can multiply the rate constant of formation of their encounter complex. This has been termed electrostatic steering of the ligand to the receptor binding site [2]. This electrostatic steering can be tested experimentally by changing the ionic strength of the solvent or modifying the reactants leading to the reduction of their electric charge.

Theoretical considerations lead to the conclusion that the existence of hydrodynamic interactions reduces the speed of the formation of encounter complexes, but the experimental study of these effects is not possible as we do not have the tools to regulate the magnitude of hydrodynamic interactions.

An interesting aspect of the influence of electrostatic and hydrodynamic interactions on molecular association arises in situations where the molecules not only must be brought into close proximity, but also must be oriented correctly in space for a successful encounter (e.g., binding or reaction) to occur. The first papers, the authors of which discuss the orienting effects of electrostatic [3,4] and hydrodynamic [5] interactions accelerating the proper orientation of the ligand towards the receptor binding site during the formation of the encounter complex, appeared nearly three decades ago.

Here, I discuss these issues in the light of more recent works. In particular, I am considering the possibility of an experimental demonstration of the orienting influence of hydrodynamic interactions in the association of molecules.

ACKNOWLEDGMENTS

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LASER INTERFEROMETRY SYSTEM FOR BACTERIAL BIOFILM STUDIES

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Optical properties of chemical substances are widely used at present for assays thereof in biological studies. Instrumental optical methods constitute the canon of standard analytical techniques, used in biological sciences. One of the measurement techniques used in physical sciences, with a potential for new applications in biology, is laser interferometry. This method, based on the phenomenon of wave interference, enables quantitative substance assays by means of measurement of the difference between light refractive indexes for the studied and control substances. Moreover, properties of laser-generated radiation, i.e. low beam divergence, narrow spectral band, and a high degree of coherence all have a positive influence on assay sensitivity.

In biological studies, a laser interferometry system constructed at Jan Kochanowski University is used. A beam of monochromatic light is emitted by a He-Ne laser and weakened as a result of passage through the polariser. Next, upon transformation into a coherent 80 mm-diameter plane wave, it falls on the beam splitter cube and is separated into two beams. One of them passes through a membrane system dedicated to biological studies, with temperature control equipment. The other beam is directed by the mirror onto a compensator plate. Because the beam splitter cube is used, both beams interfere. The resulting interference images are recorded by a CCD camera and presented on a graphic screen. A computer image-processing system, complete with dedicated software, enables mathematical analysis of interferograms shown on the system screen. Moreover, taking a series of pictures of interference images in time and conducting a mathematical analysis thereof enables quantitative analysis of real-time substance release kinetics and substance concentration distribution in near-biofilm surface fields. The result of light beam interference when passing through materials with varying refractive indexes is the phase difference between them, which causes a shift of interference stripes. The computer software scans areas within any distance from the biofilm surface with the resolution of the used CCD camera and specifies the deviation of a stripe from its straight-line path at all points. While analyzing their curves, it is possible to determine substance concentration distribution at any distance from the biofilm surface. On the basis of obtained experimental data, the amount of transported substance through the biofilm matrix is calculated.

The laser interferometry system was used to measure the growth medium diffusion through *Pseudomonas aeruginosa* PAO1 biofilm incubated with bacteriophages. This method is able to determine the biofilm matrix-degrading agents activity, as bacteriophages [1,2]. In this method, the biofilm degradation degree is positively correlated with TSB diffusion through the matrix.

Moreover, the laser interferometry system might be used to determine the diffusion properties of biologically active agents transported through bacterial biofilm. [3].

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FLUORESCENCE CORRELATION SPECTROSCOPY STUDIES OF PROPERTIES AND INTERACTIONS OF INTRINSICALLY DISORDERED PROTEINS INVOLVED IN REGULATION OF GENE EXPRESSION

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Gene expression in living organisms is a fundamental cellular process controlled in a complex manner at many different levels. One of them is microRNA-dependent gene silencing by translation repression and mRNA 3' deadenylation. In this silencing pathway, the specific miRNAs bind to the targeted mRNAs and recruit a multiprotein machinery. One of the pivotal components of this huge complex is the glycine and tryptophan rich protein of 182 kDa (GW182) [1] that binds subsequently the scaffolding CNOT1 subunit of 3' deadenylase complex CCR4-NOT [2] through its silencing domain (SD) [3]. While CNOT1 is mostly an α-helical protein, the striking feature of the GW182 SD is the structural intrinsic disorder [4]. The intermolecular interactions of this fuzzy molecular complex [5] are yet not fully characterised.

In this work, we performed fluorescence correlation spectroscopy (FCS) studies of the hydrodynamics and intermolecular interactions of a large set of proteins. We discuss the molecular properties of the intrinsically disordered *vs.* folded proteins in the context of protein dynasome [6], as well as the binding affinity of the key components of the miRNA-dependent gene silencing machinery, *i.e.* different mutants of GW182 SD and CNOT1, compared to protein standards. The results provide biophysical insights into how local structural features of GW182 SD might play a role in regulating its interaction with CNOT1(800-999) fragment.

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MOLECULAR INTERACTION OF SARS-CoV-2 SPIKE PROTEIN WITH HUMAN VIMENTIN

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Vimentin (Vim) is a cytoskeleton protein involved in various cellular functions, including proliferation, cell signaling, maintenance of cellular architecture, and mechanical resistance to external mechanical stress [1-3]. Extracellular Vim has recently been identified as a host cell attachment site for various viral and bacterial pathogens, including SARS-CoV-2 virus [4-6].

This study examined the physicochemical nature of the binding between the SARS-CoV-2 S1 (S1 RBD) glycoprotein receptor binding domain and human Vim. The interaction between S1 RBD and Vim was assessed using AFM-based single molecule force spectroscopy (AFM-SMFS). In order to confirm the experimental results, simulations of the molecular dynamics of Vim-S1 RBD docking were performed. AFM was used to test the interaction between S1 RBD and Vim using two models - Vim molecules immobilized on the mica surface and extracellular Vim present on the surface of a confluent fibroblast culture. In addition to the fibroblasts expressing Vim (mEF +/+), fibroblasts not expressing this protein (mEF -/-) were used as control. The AFM-tips were functionalized with the RBD S1 subunit protein using a heterobifunctional maleimidopropionyl-PEG-NHS linker. A set of force-distance maps for different AFM probe approach/retract rates was collected to investigate the interaction between S1 RBD and Vim. Based on the recorded force curves, the breaking forces between S1 RBD and Vim were calculated. For the AFM approach/retraction speed of 5 μm/s, the mean fracture force of S1 RBD and Vim immobilized on the mica surface was 59 pN. The mean fracture force for the S1 RBD and ACE2 receptor was 33% lower. The bond kinetics was estimated on the basis of the breaking force distribution using Bell-Evans model. The experiment was repeated for the wild S1 RBD strain and 3 variants considered (VOC) and mEF +/+ cells. For an AFM approach/retraction rate

of 5 μm/s, the mean disruption force of wild S1 RBD and Vim cell area was 28 pN. The Vim binding results for the S1 RBD from the UK, Brazil and South African variants were also evaluated. Interactions between Vim and S1 RBD were additionally confirmed using in silico studies.

Our biomechanical studies of the interaction between S1 RBD and Vim provided new evidence of a specific interaction between these molecules. This supports the results of previous biological studies in which Vim was identified as a coreceptor for virus entry.

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REVIEW OF QUANTUM-CASSICAL MOLECULAR DYNAMICS METHODS AND THEIR SELECTED APPLICATIONS IN THE STUDY OF COVALENT DOCKING PROCESSES OF BORATE INHIBITORS

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Quantum-classical molecular dynamics (QM/MM) combines quantum and classical MD algorithms and is able to describe the motions of whole atoms, the electron and/or proton transfer processes in biomolecular structures, as well as the processes of creating and/or breaking chemical bonds. There are two basic problems to be solved: how to generate efficient and reliable potential energy functions, and how to manage dynamics. In practical applications, the division of the configuration space into quantum and classical domains is dictated by the problem under study and the required accuracy of time-dependent solutions. Typically, the dynamics of a quantum subsystem is either described by a stationary, time-independent Schroedinger equation (adiabatic QD) or by an explicit time-dependent Schroedinger equation (nonadiabatic QD), while the rest of the system is usually described by the Newton's time-dependent equations of motion. The coupling between the quantum $\{x\}$ and classical, $\{\vec{R}_\alpha(t)\}$, domains is described by the time-dependent potential function $V=V(x,\{\vec{R}_\alpha(t)\})$ in the Schroedinger equation, and also uses Hellmann-Feynman forces $\vec{F}_\beta = \left\langle \psi \left| \frac{\partial H}{\partial \vec{R}_\beta} \right| \psi \right\rangle$ modifying classical forces in the Newton's equations of motion.

For an overview of the approaches to quantum biology, as well as to a number of QM/MM models and theories, see [1-5]. It should also be emphasized that research into complex contemporary biological problems requires a network of interrelated disciplines, including bioinformatics, quantum computing and machine learning, see the commentary of the global forum on synthetic biology [6].

We have recently developed molecular covalent docking QM/MM protocols to design new classes of inhibitors that form chemical bonds with their biological targets. In particular, the strategy for the design of boron-based inhibitors, holds great promise for enzymes produced, among others, by antibiotic-resistant gram-negative bacteria. We simulated the covalent docking process of inhibitors based on boronic acid and bicyclic boronate scaffolds for β-lactamases belonging to the A, C and D classes. Molecular fragments containing boron can be

transformed from the neutral sp^2 state to the anionic, tetrahedral sp^3 state. Time-dependent QM/MM simulations indicated several significant geometric preferences leading to covalent docking processes - visible on simulation movies [7]. Our simulation methodologies can support the rational design of boron-based covalent inhibitors for many other enzyme systems of clinical relevance.

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TWO-STEP BINDING OF NEOMYCIN TO AN RNA APTAMER

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A synthetic RNA aptamer, forming a riboswitch named N1, regulates gene expression by binding aminoglycoside antibiotics, such as neomycin. Experiments showed that some mutations hinder the riboswitch activity, particularly the A17G mutation reduces the translation regulation efficiency 6-fold [1]. Recently, we elucidated how single-point mutations in the N1 riboswitch affect the internal dynamics of this aptamer and its complexes with aminoglycosides [2]. The dynamical picture conforms with the experimentally determined aminoglycoside dissociation constants [1,3]. In the current study, we focus on the association of neomycin to the N1 riboswitch in order to elucidate the binding process. We apply two-dimensional replica-exchange molecular dynamics to enhance conformational sampling by varying both the temperature and RNA-neomycin distance.

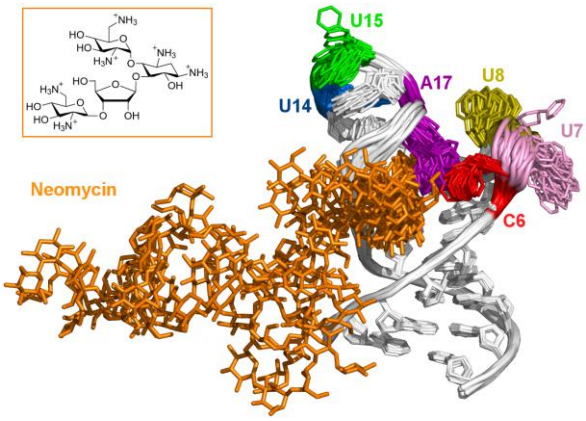

Fig.1. Superposition of replica structures with different RNA-neomycin distances.

The simulations indicate that neomycin binding occurs via one predominant direction even though the shape of the RNA aptamer allows for other binding paths. The two-dimensional free-energy surfaces show two stable low-energy minima along the pathway, suggesting a two-step binding mechanism. The energetically most

favorable minimum corresponds to the neomycin-bound state [2]. The slightly less favorable one likely corresponds to an intermediate state detected experimentally [4]. In the latter local minimum, the G17 nucleobase of the A17G mutant interacts with U7 in a different way than A17 in the N1 riboswitch. We propose that this difference reduces the binding affinity of neomycin to the A17G mutant compared to the wild-type.

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PERMEABILIZATION OF OUTER BACTERIA MEMBRANE BY NANOPARTICLES TO ENHANCE ANTIMICROBIAL EFFECT OF ANTIMICROBIAL PROTEINS-LYSOZYME AND ENDOLYSIN

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Antibacterial resistance has become a global threat, in particular, multidrug-resistant (MDR) bacteria with a broad spectrum of virulence factors. The main problem with bacteria resistance is that they can acquire or develop resistance to commonly used antibiotics. The antibacterial agents currently in clinical development are mostly derivatives of well-established antibiotic and they are affected by pre-existing cross-resistance, which may reduce their efficiency in critical ill patients. Nowadays, several strategies have been studied to overcome multi drug resistance including bacteriophages, antimicrobial proteins (lysins), stimulators of immune system or membrane permeabilizator (eg. antimicrobial peptides and nanoparticles). Among various permeabilizer candidates, cationic nanoparticles are of particular interest because they showed potent antibacterial activity. This may not only lead to bacteria death but also create a route for additional antibacterial compounds eg. phage lysins for peptidoglycan degradation. Bacteriophage-encoded endolysins have emerged as a novel class of antibacterial agents to combat the surging antibiotic resistance. Lysins act as efficient antimicrobials with economical potential. The PG degrading effect of lysins can be seen as osmotic lysis of targeted cell, making these enzymes a desirable and efficient antibacterial agent. However, its use against G-ve bacteria is limited because the outer membrane (OM) of Gve bacteria hinders the permeation of exogenously applied lysins. Therefore, the complexation of nanoparticles with phage-derived endolysin and lysozyme can improve their antibacterial properties against gram-negative bacteria [1-4]. The nanoparticles can be complexed with endolysin or lysozyme, where nanoparticles act as permeabilizers of the bacterial outer membrane (OM) and thus can lead to strengthening bactericidal activity of antimicrobial protein responsible for the degradation of peptidoglycan PG. This helps to create a new tool to fight with multi drug resistance bacteria.

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SUPER-RESOLUTION FLUORESCENCE MICROSCOPY

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New microscopic techniques make it possible to overcome the limitations of traditional optical microscopy. These techniques include: SR-SIM (Super-Resolution Structured Illumination Microscopy), photolocalization techniques: PALM (Photoactivated Localization Microscopy), STORM (Stochastic Optical Reconstruction Microscopy) and others. On the other hand, traditional techniques of fluorescence microscopy can be extended to FLIM (Fluorescence Lifetime Imaging Microscopy) technique.

The presented system is based on a classic wide-field fluorescence microscope, which also enables observations in transmitted light. Most of all, however, what distinguishes this microscope from traditional ones are the modules responsible for super-resolution techniques and fluorescence lifetime imaging.

The SR-SIM and photolocalization techniques use lasers with wavelengths of 405, 488, 561 and 639 nm, and in the FLIM technique - pulsed lasers with wavelengths of 375, 405 and 488 nm. SR-SIM enables observation with a resolution of approx. 100 nm in the XY plane and less than 300 nm in the Z axis, which is two times better than in classic optical microscopes. This improvement in resolution is obtained for standard samples using classic fluorescent markers (Fig. 1). On the other hand, in the PALM technique, the use of special photoconvertible or photoactivatable fluorescent probes allows the observation of objects with sizes well below 100 nm. The TIRF (Total Internal Reflection Fluorescence) technique allows to observe the fluorescence signal coming only from a thin layer with a thickness of 100-200 nm, which removes the blurry background, improving the contrast.

The FLIM technique uses a confocal system mounted in the side port of the microscope and enables point-by-point scanning of the sample, not only to visualize the intensity of the emitted light, but above all to visualize the lifetime of fluorescence and phosphorescence.

In all of the techniques mentioned, 3D imaging is possible, the maximum depth from which information can be gathered depends on the wavelength and the transmittance of the sample. Such a combination of super-resolution and FILM techniques gives the possibility of many unique, previously unavailable observations.

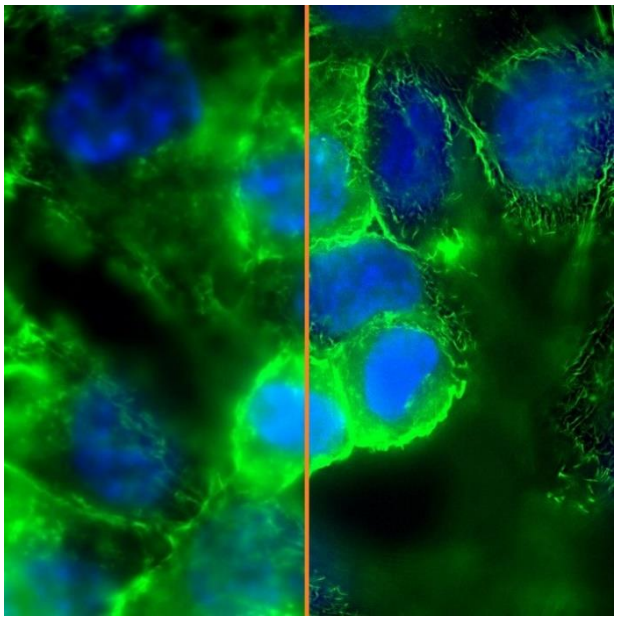

Fig. 1. The image of the tumor cell line obtained by classical fluorescence microscopy (left) and SR-SIM super-resolution microscopy (right). The blue and green colors represent the selectively stained cell structures visible when excited with light at 405 and 488 nm, respectively. The imaged sample area is 125 x 125 μm^2. Source of the sample - Department of Histology and Embryology, CM UMK.

MULTIPLE ASPECTS OF PROTEIN CONFORMATIONAL DYNAMICS REVEALED BY HYDROGEN DEUTERIUM EXCHANGE

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Discoveries of the last years showed that a large portion of the proteome consists of proteins which are not able to form a rigid, well-defined three dimensional structure, constituting the realm of intrinsically disordered proteins/regions (IDP/IDR), the presence of which is widely accepted now. The initial skepticism on their functional role in biology has also diminished due to flow of data on IDRs and their biological activity. Multiple cases of biologically important proteins have been found to contain large IDR's and studied, leading to the inventory of new functional classes that in some cases require disorder. Multiple functional advantages of the dynamics are also mentioned allowing for multivalency, moonlighting, mimicry, plasticity,

dynamic allostery, ultrasensitive signal integration, threshold PTM triggering, fuzzy molecular recognition, entropic chains/bristles, liquid–liquid-demixing phase separations, functional amyloids and alike. New techniques have enriched the portfolio of protein structural studies that enable to tackle the dynamic character of these subjects. The old approach to monitor the kinetics of exchange of backbone amide hydrogens to deuteria (HDX), and therefore to map the entanglement of these hydrogens in secondary or tertiary structure, gained new momentum as an ideal tool to get unique insight into protein regions characterised by different levels of dynamics, the timeframes of which in proteins may span several orders of magnitude. Selected cases from more than 40 protein systems studied by HDX in our lab will be presented and the dynamics-function relationships emerging from these studies will be discussed. These cases include intermediate filament proteins, centriole proteins and translation elongation protein complexes to illustrate the progress in understanding the dynamic aspects of protein function, still not appreciated due to domination of crystallocentric paradigm, as we witness the change in this paradigm and fall of the dogma that function requires structure.

GLUCOCORTICOIDS AND NATURAL KILLER CELLS: A SUPPRESSIVE RELATIONSHIP

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Glucocorticoids exert their pharmacological actions by mimicking and amplifying the function of the endogenous glucocorticoid system's canonical physiological stress response. They affect the immune system at the levels of inflammation and adaptive and innate immunity. These effects are the basis for therapeutic use of glucocorticoids. Innate immunity is the body's first line of defense against disease conditions. It is relatively nonspecific and, among its mediators, natural killer (NK) cells link innate and acquired immunity. NK cell numbers are altered in patients with autoimmune diseases, and research suggests that interactions between glucocorticoids and natural killer cells are critical for successful glucocorticoid therapy. Production and release in the blood of endogenous glucocorticoids are strictly regulated by the hypothalamus-pituitary adrenal axis. A self-regulatory mechanism prevents excessive plasma levels of these hormones. However, exogenous stimuli such as stress, inflammation, infections, cancer, and autoimmune disease can trigger the hypothalamus-pituitary-adrenal axis response and lead to excessive systemic release of glucocorticoids. Thus, stress stimuli, such as sleep deprivation, intense exercise, depression, viral infections, and cancer, can result in release of glucocorticoids and associated immunosuppressant effects. Among these effects are decreases in the numbers and activities of NK cells in inflammatory and autoimmune diseases (e.g., giant cell arteritis, polymyalgia rheumatica, and familial hypogammaglobulinemia).

BIOPHYSICAL HALLMARKS OF TUMOR GROWTH AND INFLAMMATION

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Histopathological examination is a very important stage in the diagnosis of diseases. By means of microscopic techniques, it enables the visualization of morphological changes in tissue structures. Individual cells and their structural assembly into tissues can also be characterized using biophysical techniques to describe their rheological properties, which are "invisible" during histopathological examination, but may be helpful in clinical diagnosis.
Nanoscale changes in the rheological properties of cells / tissues can be observed earlier than microscale histopathological changes [1-7]. Overall, the rheological manifestations of inflamed tissues are less understood than those of neoplastic tissues, while the possibility of differentiating inflammatory processes and carcinogenesis is essential for the development of specific tumor mechano-markers.
In this study, the rheological properties of neoplastic and inflammatory tissues, represented by colon cancer and appendicitis biopsy samples, respectively, were assessed. Collected specimens were tested using a HAAKE Rheostress 6000 rheometer. To determine the rheological characteristics, the elastic modulus (G') and loss modulus (G") as a function of shear strain at different tissue compression states were determined. Additionally, to confirm changes in the mechanical properties of inflamed tissues, examination of biopsy specimens was performed using a NanoWizard 4 BioScience atomic force microscope (AFM), BRUKER JPK. The elastic modulus of the tissues (Young's modulus) was determined from the force-distance curves recorded on the tissues. Force indentation curves,

collected using a silicon nitride cantilever with a 4.5 μm diameter polystyrene bead attached, were analyzed based on the Hertz/Sneddon model. The mechanical characteristics of the tumor or inflamed tissues were determined in comparison to the control normal tissue margins. Properties that distinguish neoplastic or inflamed tissue from healthy tissue have been identified. A comparison of the mechanical properties of the healthy and diseased tissues indicates that the inflamed tissues are softer compared to the healthy ones. The obtained results suggest that changes in the rheological parameters during tissue inflammatory processes, which induced cell infiltration and modifications in the tissue stroma, can be considered as the novel mechano-markers. Rheological examination of tissues involved in pathological processes may enhance standard histopathological diagnosis.

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FROM IDEA TO A PRODUCT FOR MEDICAL DIAGNOSTICS - PCR|ONE AND BACTEROMIC

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Academic research and scientific discoveries create opportunities for breakthrough technologies. I will use the examples of two systems for medical diagnostics developed by the Scope Fluidics group - PCR|ONE offering rapid, fully automated genetic detection of infectious agents, and BacterOMIC offering comprehensive characterisation of antibiotic susceptibility of bacterial pathogens. I will describe the path from the idea to the product and the challenges we faced along the way.

INTRINSIC DISORDER AND PHASE SEPARATION OF THE bHLH-PAS TRANSCRIPTION FACTORS

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The basic helix–loop–helix-Per-ARNT-SIM (bHLH–PAS) proteins represent important class of transcription factors (TFs) which expression is specifically regulated by physiological states and/or environmental signals [1]. Representatives of this family perform a wide spectrum of functions, like Aryl hydrocarbon receptor (AHR) acting as receptor for highly toxic dioxins [2], Clock and Aryl hydrocarbon receptor nuclear translocator-like protein 1 (ARNTL1) regulating circadian rhythms of the organism [3] or Hypoxia inducible factor 1α (Hif-1α) [4], acting as a specific oxygen sensor in cells. Lack of balance in these processes are often linked with the genesis of various diseases, including cancer, stroke, or heart diseases [4]. bHLH-PAS proteins are commonly divided into two classes based on their dimerization pattern, with proteins assigned to class I unable to form homodimers and dependent on the dimerization with class II partners which can also form homodimers [5].
The N-terminal part is structurally conserved and contain bHLH domain responsible for dimerization and DNA binding, followed by PAS domains important for the specificity of DNA binding and signal reception. In contrast to defined domains located within the N-terminal part of bHLH–PAS proteins, their C-termini are highly variable. They usually comprise specific

regions responsible for protein–protein interaction (PPI) known as transcription activation/repression domains (TADs/RPDs) [6,7,8] responsible for the specific modulation of the bHLH–PAS TFs action [9]. Importantly, C-termini of the bHLH-PAS proteins were predicted as intrinsically disordered regions (IDRs) [10]. The experimental verification and characterization of the long C-terminal IDRs of bHLH-PAS representatives was performed by our group for Methoprene tolerant (MET) [11] and germ cell-expressed (GCE) proteins [12]. Both proteins were documented as juvenile hormone (JH) receptors in the model organism *Drosophila melanogaster* [13].

The lack of a defined structure is critical for IDR functionalities in hub proteins enabling different signal pathways crossing [14,15]. IDRs found in bHLH TFs were proposed to contribute directly to the evolution of complex multicellularity [16]. The ability of IDR-containing proteins to form multivalent, weak, and transient interactions enable of proteins to undergo liquid–liquid phase separation (LLPS). Interestingly, although in some cases PPI could lead to LLPS formation, there are also instances where LLPS may prevent protein interactions [17,18,19]. In the context of TFs, it is very interesting to consider the putative role of LLPS in fast cellular responses to external stimuli [20,21]. The ability of protein to undergo the LLPS process may be regulated by a wide spectrum of post-translational modifications (PTMs) and alternative splicing [22]. The extended conformation and low compactness result in IDRs as especially good targets for PTMs and proteolytic degradation, which are typical ways of activity regulation in proteins [23], for example Hypoxia Inducible protein (HIF) [24]. Recently, we discussed the importance of disordered character of bHLH proteins and their propensities to LLPS for functioning as TFs [25].

Neuronal PAS Domain-Containing Protein (NPAS4) belonging to the bHLH-PAS family is one of the immediate early genes (IEGs) that can activate mechanisms related to the first defense against many cellular stresses [26]. Importantly, IEGs are regulated by a specific stimulus with no need for a de novo protein synthesis [27]. NPAS4 has been proposed as a novel therapeutic target for depression and neurodegenerative diseases [28] and as a component of new stroke therapies [29]. Despite discovering in neurons, Npas4 was shown to be multifunctional protein expressed in many types of cells, including β pancreatic [30] and endothelial cells [31]. To date, no structural or biochemical characterization of this protein was performed. In the presentation, we aim to discuss NPAS4 in silico analysis [32] and preliminary results of experimental work in the context of IDR, LLPS and putative ways of this protein functioning as cytoprotective protein.

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EXPRESSION OF PROTEINS CONTROLLING THE MITOCHONDRIAL DYNAMICS IN SH-SY5Y CELLS, IN RESPONSE TO STIMULATION WITH DRUG-MIMETIC COMPOUNDS IN NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases (ND) belong to a group of acquired diseases that are genetically coded and result in the destruction of central and peripheral nervous systems. Recently, it has been suggested that mitochondria (MT) may have a great impact on the development of neurodegenerative diseases. In particular, the imbalance of the fusion/fission events of these organelles is observed [1], favoring the MT division mechanism.

To better understand the mechanism of these processes, we have examined the expression of mitochondrial proteins involved in the fusion/fission dynamics, including DRP1 and its phospho-modifications (S616 and S637), as well as Mfn1 [2]. To perform the experiments, we used 24 h incubated SH-SY5Y cells in Mdivi-1, the mitochondrial fission inhibitor, and a potential drug for neurodegenerative diseases, that is proven to break the MT splitting events [3]. Two concentrations of Mdivi-1 in DMSO were used: 0 and 50 μM. After a 50 μM treatment, discernible morphological changes were observed. Subsequently, we have investigated the expression of fission (DRP1, p-DRP1(S616), p–DRP1(S637) and fusion (Mfn1) proteins to understand how the neuroblastoma cells react to the compound used. The Western blotting with densitometric analysis was then performed on the cytosolic fraction and isolated mitochondria. To normalize the results, β-actin and VDAC proteins were used as the loading control, for the described fractions, respectively.

The acquired images and their saturation statistical analysis strongly suggest that DRP1 and phosphorylated DRP1 (Ser616) protein expression levels in mitochondrial fraction decrease with increasing concentration of Mdivi-1.

At the same time, the mitochondrial p-DRP1 (S637) and Mfn1 and all cytosolic proteins remain unchanged. It implies that Mdivi-1 significantly reduced MT fission by decreasing the recruitment of DRP1 to the outer MT membrane and lowering S616 phosphorylation. However, it has not concurrently enhanced the MT fusion. The morphological changes in neuroblastoma cells, such as the decreased body projections, were also observed. This may be associated with the decreased MT fission and the obstructed translocation of mitochondria to distant cell regions. The obtained results show that Mdivi-1 acts as the potent drug-mimetic compound in restraining the MT divisions. This feature could be utilized in studies of neurodegeneration. Further investigations, using SH-SY5Y cells, differentiated into neuronal phenotype, are needed.

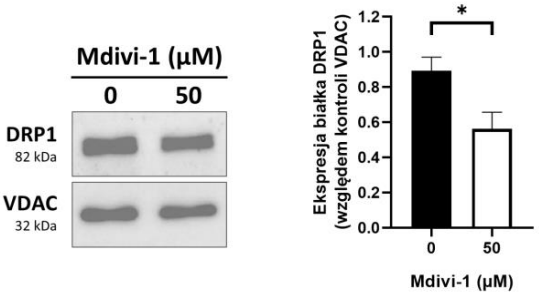

Fig.1. DRP1 protein (82 kDa) expression, relative to VDAC (32 kDa) expression, for 0 and 50 μM Mdivi-1 in MT fraction, using Western blot. Statistical difference marked as * ($p<0.1$).

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ENHANCED FLUORESCENCE DETECTION FOR BIOMEDICAL DIAGNOSTICS AND IMAGING

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Fluorescence-based detection plays a prominent role in modern medical diagnostics and biomedical imaging. Recent progress at the interface of chemistry, physics, micro and nanoscale engineering, nanomaterials, and detectors theoretically enabled ultimate detection sensitivity down to a single molecule level. As single molecule studies in ultra-purified systems and radically limited volume become common the practical biomedical applications are still drastically limited by the background signal that is many orders of magnitude stronger than any single molecule response. Effectively, the broad range of practical applications that include biomarkers detection (e.g. cancer markers or cardiac markers), diagnostics tests (e.g. DNA detection or antibody based assays), imaging and monitoring of intra- and extra-cellular environment to practical forensic applications (like sample collection, processing, and amplification) cannot benefit from single molecule sensitivity.

In this presentation, we will discuss recently developed detection technology that utilizes the difference in fluorescence lifetimes to highly reject background signal and increase detection sensitivity. Taking advantage of longer fluorescence lifetime (10 ns and longer) the excitation pulse sequence highly improves sensitivity for detection, diagnostics, and imaging.

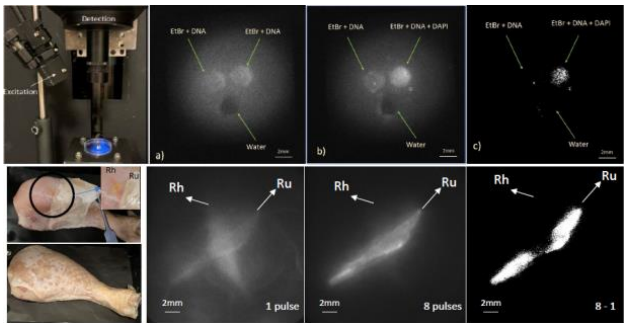

Fig.1. Schematic setup and representative data.

N2-MODIFIED CAP ANALOGUES – A VERY POTENT TOOL FOR mRNA ENGINEERING

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The use of nucleic acids in therapeutics has been intensively researched for many years. The year 2020 proved to be a breakthrough, when the first drug based on mRNA molecule, the COVID-19 vaccine, was introduced to the pharmaceutical market. It was estimated that the COVID-19 vaccine, whose two most commonly used products are formulations containing the mRNA molecule, saved the lives of nearly 20 million people in the first year of the vaccine use.

The mRNA molecule is extremely interesting as a drug. Once developed, the procedure for its synthesis can be applied to obtain many different therapeutic proteins that can be encoded in mRNA sequence. This allows the production process to be quickly adapted to current societal needs.

Intensive research is underway to give mRNA new and even better properties to fully exploit the potential inherent in this molecule. It can be used not only to fight viral diseases, but also in protein supplementation therapy or regenerative medicine[1,2]. One of the components of mRNA that is being intensively studied and modified is the cap structure. This is the element located at the 5' end of the mRNA that provides stability and high translation efficiency. Modification to this region makes mRNA more competitive than native molecules in the patient's cells.

Here, we present biochemical characterization of the new class of RNA capped with newly synthesized dinucleotide cap analogues containing a single aromatic substituent at the N2 position of 7-methylguanosine. We tested the new compounds both alone as inhibitors of the translation process and after incorporation into mRNA. The results showed that the new compounds are 5 to 16 times more potent inhibitors of translation than the standard m^7GpppG cap. These data indicate that the investigated group of analogues has extremely favorable inhibitory properties compared to other compounds studied so far[3].

To characterize the affinity of the newly synthesized compounds for the translation initiation factor eIF4E, we analyzed the thermal stability of murine eIF4E in their presence using DSF. Estimated apparent affinity based on the change in Tm in response to increasing concentrations of the ligand showed that the most potent interacting compounds have approximately 5x higher affinity for eIF4E than m^7GpppG.

We further demonstrated that the new cap analogues are efficiently incorporated into mRNA by RNA polymerases. Interestingly, modifications introduced at N2 guanosine increased the probability of cap incorporation, and for some substituents this modification was sufficient for the analog to be incorporated only in the correct orientation. Thus, the newly synthesized compounds provide an alternative to ARCA-type cap analogues or trinucleotides that ensure correct incorporation into mRNA.

Finally, RNA capped with the newly synthesized compounds showed improved translational properties both in the cell-free translational system of rabbit reticulocytes and in HEK293 cells. In summary, dinucleotide analogs with substituents at the N2 position show much better properties than those commonly used for mRNA preparation, namely m^7GpppG or ARCA.

ACKNOWLEDGMENTS

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LASER EMISSION OF THIOFLAVIN T TO UNCOVER THE EARLY SYMPTOMS OF NEURODEGENERATIVE DISEASES

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Neurodegeneration becomes one of the most commonly diagnosed dysfunction with no certain cure, which has a greater chance of affecting individuals with the age of 65 years or older [1]. Neurodegenerative diseases are commonly associated with neuropathologically distinct amyloid plaques, neurofibrillary tangles and intracellular inclusions in the brain.

Nowadays, the main perpetrators are believed to be small mobile aggregate forms called oligomers, for example misfolded amyloid beta in the case of Alzheimer disease or α-synuclein (α-syn) in the case of Parkinson disease. Oligomers possess the potential to subvert several aggregation pathways and overwhelm cellular functions causing toxicity. Thus it is important to find the way for detecting the toxic oligomer species at the very early stage of their formation, so that patients can receive rapid information about their health condition and have a better outcome in the therapy [2].
A common and widespread method to detect protein aggregates is fluorescence. For that purpose the aggregates are stained with organic molecule named Thioflavin T (ThT) dye which is a gold standard in imaging of neurodegeneration. But fluorescence of ThT lacks the sensitivity to oligomer species.

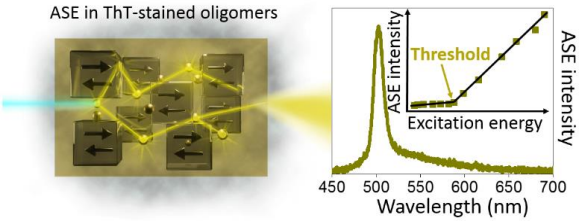

Fig. 1. The amplified spontaneous emission (ASE) of Thioflavin T dye in solid films prepared of early stage protein aggregates. The ASE depends on structural motif, yield of scattering arising from the growing aggregates and seeds of patient's cerebrospinal fluids.

To boost ThT sensitivity fluorescence can be amplified in the process of stimulated emission (Fig. 1). In the amplified spontaneous emission (ASE) process photons emitted spontaneously by excited molecules are multiplied in the stimulated emission process when they interact with other excited molecules during their propagation through the medium (it is the physical mechanism that underlies the operation of lasers). The result is a directional emission of high intensity light with its spectrum significantly narrower than that of fluorescence [3].
ASE was shown to detect aggregation in vitro and in various tissues including the cerebrospinal fluid (CSF), whereby the disease-related protein recombinant is seeded with the patient's fluid. By monitoring the ASE a remarkable recognition sensitivity to pre-fibrillar oligomeric forms can be achieved. Thus, in contrast to fluorescence, ASE, can be used to detect and differentiate amyloid oligomers and evaluate the risk levels of neurodegenerative diseases to potential patients before the clinical symptoms occur [4].

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ANALYSIS OF FUNCTIONAL DIFFERENCES BETWEEN HUMAN VDAC3 ISOFORMS

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The outer mitochondrial membrane is permeable to ions and small molecules due to the presence of VDAC (voltage-dependent anion-selective channel) proteins. Gene duplication and differentiation during evolution resulted in synthesis of distinct VDAC protein paralogs in almost all organism. In mammals three different VDAC paralogs (VDAC1, VDAC2, and VDAC3) have been identified and among them the biological function of VDAC3 is the least understood [1, 2].
It is known that VDAC3 gene-depleted male mice are infertile due to sperm dysfunction. Moreover two VDAC3 isoforms are known, i.e. canonical and alternative. The alternative VDAC3 isoform results from alternative RNA splicing including additional three-nucleotide (ATG) microexon. Consequently, the VDAC3 isoform contains additional methionine at position 39 [3]. Moreover, the isoform is expressed only in tissues with high energy consumption such as brain, heart and skeletal muscles.
The canonical VDAC3 isoform, but not the alternative

VDAC3 isoform, has been proved to be present in the testes [3]. This suggests that the alternative VDAC3 isoform must play an important role in processes other than reproduction. To explain the alternative VDAC3 isoform function we decided to investigate channel electrophysiological properties and the ability to transport metabolites such as NADH and ADP by the two VDAC3 isoforms.
The results indicate differences in metabolite transport between the two isoforms of the VDAC channel, which may have important implications for clarifying the physiological role of the alternative isoform.

ACKNOWLEDGMENTS

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SEARCH AND PROCESSING OF HOLLIDAY JUNCTIONS WITHIN LONG DNA BY JUNCTION-RESOLVING ENZYMES

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Resolution of four-way Holliday junctions (HJs) is a critical intermediate step of Homologous Recombination. Failure of this process can leave unresolved junctions that covalently link chromosomes together, making cells unable to divide. HJs are resolved by abundant junction-resolving endonucleases that introduce two hydrolytic cleavages. Although their catalytic activity and interaction mode with HJ-DNA have been characterized in great detail by biochemical and structural studies, it remains unclear how the endonucleases find their substrate located in kilobase pairs of duplex DNA.
Here, we studied the interaction of the T7 endonuclease I with a long dsDNA molecule that has a junction located at its center. We employed correlative optical tweezers with confocal fluorescence microscopy to track a single molecule of endonuclease I on the DNA template in real-time. We observed that the enzyme binds remotely to the dsDNA and then undergoes 1D diffusion. Upon encountering the four-way junction, a catalytically impaired endonuclease I mutant remains bound at that point for long periods. If an active enzyme is used, after a few seconds we observe a cleavage event.
We can rationalize all our new data in terms of our earlier crystal structure of T7 endonuclease bound to a DNA junction [1]. We propose that the duplex DNA binds in one of the two DNA binding channels within the dimeric complex. The floor of the channel ha a non-uniform electrostatic potential, and we propose that the DNA duplex will be “floating” in the channel. This model is supported by experiments with an N-terminal 16 amino acid truncation mutant that fails to diffuse on the DNA, indicating the DNA is able to “hover” only because the DNA in the protein channel is encircled by the N-terminal peptide. Such observations would not be possible with any other ensemble or structural approach due to the flexibility of terminal peptides and transient interactions of the “tail-less” endonuclease with DNA duplex.
In this work, we present a robust, quantitative framework to characterize target search by enzymes that recognize DNA secondary structure. Our single-molecule imaging and analysis revealed a comprehensive description of the facilitated diffusion mechanism and the complete resolution trajectory that is likely to be applicable to most junction resolving enzymes.

ACKNOWLEDGMENTS

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MECHANOSENSITIVE CHANNELS - FROM BACTERIA TO MITOCHONDRIA

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All cells sense and respond to mechanical stimuli. Among several molecular machines that react to such cues are mechanosensitive (MS) channels, which are present in the membranes of organisms from all three domains of life: bacteria, archaea, and eukarya. Mechanosensitivity evolved multiple times and MS channels are an extremely heterogeneous group. However, biophysical principles underlying their functioning seem to be common and there are currently two main models of how MS channels are activated. In the first "force-from-lipid" model, MS channels sense directly mechanical deformations of the membrane including changes in tension, thickness, and curvature, and as a result, alter their conformation between closed and open states. In the second model, a spring-like tether links the channel to either the cytoskeleton or extracellular matrix, and displacement of it during stimuli leads to channel opening. Most of the MS channels are still mechanosensitive after reconstitution into pure lipid bilayers supporting the universality of the "force-from-lipid" model.

The physiological roles of MS channels are diverse. Bacteria contain two major classes of MS channels - MscL (mechanosensitive channel of large conductance) and MscS (mechanosensitive channel of small conductance) with typically few MscS paralogs present in a given species. The role of MS channels is insignificant in an osmotically stable setting but is pivotal during osmotic downshocks. Indeed, most bacteria live in osmotically unstable environments, and *Escherichia coli* cells deleted with MS channels do not survive osmotic downshocks. Interestingly, bacterial MS channels have different characteristics including single-channel conductance or the threshold of activation. Some MscS paralogs exhibit intrinsic inactivation (channels close despite constant stimulus) [1]. All this variability seems to be required for fine-tuning cell response to osmotic challenges. In addition to bacteria, MscS-like channels are present also in fungi and plants. A common denominator of these organisms is the cell wall and it was shown that MscS activity might be related to cell wall metabolism in bacteria [2].

Remarkably, one of the MscS-like channels MSL1 in the plant *Arabidopsis thaliana* localizes to mitochondria [3]. Loss of MSL1 resulted in increased mitochondrial membrane potential and a higher oxidation state of the mitochondrial glutathione pool under abiotic stress. This suggests that MS channels might play a regulatory role in mitochondria. MscS-like channels are not present in animals but mitochondrial metabolism seems to be affected by mechanical stimuli in their cells. It might be that other channels exhibiting mechanosensitivity present in their mitochondria like mitoBK$_{Ca}$ [4] fulfill the functions of mechanical sensors. Nevertheless, mitochondrial mechanosensitivity is an uncharted field requiring further exploration.

ACKNOWLEDGMENTS

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KINETIC ASPECTS OF EXTRACELLULAR COPPER TRANSPORT

R. Kotuniak[1], P. Szczerba[1], M.J.F. Strampraad[2], D. Sudzik[1], I. Ufnalska[1], P.-L. Hagedoorn[2], W. Bal[1]

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Extracellular transport of copper ions and their acquisition by cells is arguably the least understood aspect of copper physiology. There is no consensus as to which molecules carry copper in blood serum and

deliver them to cells, and the knowledge of copper transport in other body fluids is even less advanced [1]. Furthermore, the identified and putative copper transport sites in blood serum proteins and peptides are very inert in exchange reactions, aggravating the enigma. These sites, present in human serum albumin, hCtr1 cellular transporter and some other proteins and peptides belong to the ATCUN/NTS family, characterized by N-terminal Xaa-Zaa-His sequences. They coordinate a Cu(II) ion in a cooperative fashion, saturating its coordination sphere (4N complex, Fig. 1). Previous experiments showed that Cu(II) exchange half-times in these motifs ranges from minutes to days, casting doubt on their physiological role [2,3]. This is because physiological processes have specific timeframes which must be matched by the kinetics of a given chemical reaction to make it biologically useful. Specifically, both the Cu(II) uptake by hCtr1 and Cu(II) release and reuptake in the synaptic cleft apparently occur within a ca. 100 ms time window.

We performed stopped-flow investigations with UV-vis spectral detection on Cu(II) binding to a number of ATCUN/NTS peptides, including simple chemical models, N-termini of identified actors in Cu(II) transport and other relevant biological Cu(II) ligands [4]. The Cu(II) dissociation from some of them was studied using the pH jump approach [5]. In all cases a partially coordinated reaction intermediate was found (2N complex, Fig. 1). It was formed within a millisecond and lasted between ca. 100 ms and several s, depending on a peptide. It was also detected as a dissociation intermediate. Unlike the 4N species, the 2N complex is labile in exchange reactions and can be easily reduced to a Cu(I) species, therefore meeting all criteria for a real physiological Cu(II) transport and exchange species. Our research demonstrated how kinetic studies can reveal actual active species in the biology of metal ions.

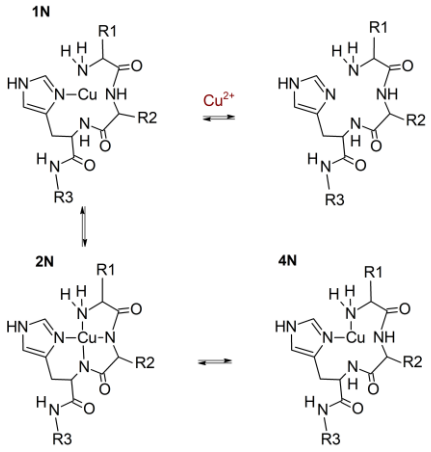

Fig.1. A scheme for Cu(II) binding to ATCUN/NTS peptides. The most reactive conformers for 1N and 2N species are shown [1,4].

ACKNOWLEDGMENTS

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NEW REGULATORY MECHANISMS OF MITOCHONDRIAL POTASSIUM CHANNELS

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Mitochondrial potassium (mitoK) channels play an important role in mitochondrial physiology. Activation of mitoK channels has beneficial effects and can protect brain and cardiac tissue against injury induced by ischemia reperfusion [1]. Mitochondrial, large conductance calcium activated potassium ($mitoBK_{Ca}$) channel was found both in brain and cardiac tissue. Activation of the channel leads to influx of potassium ions into mitochondrial matrix. This induces depolarization of the inner mitochondrial membrane. This simple phenomenon of K^+ influx has multiple consequences for mitochondrial physiology. It was shown that activation of mitoK channels influences oxygen consumption or synthesis of reactive oxygen species in mitochondria [2].

The basic properties of mitochondrial potassium channels, including the $mitoBK_{Ca}$ channel, are very

similar to those of the plasma membrane channels. Interestingly, recent years have shown that these proteins can be specifically regulated due to their location in the mitochondria. Moreover, there are many indications that the activity of mitochondrial potassium channels is regulated by reactive oxygen species synthesized in mitochondria, which is of key importance for the cytoprotection mechanism [3].

The activity of the mitochondrial respiratory chain has been shown to regulate the activity of the mitoBK$_{Ca}$ channel. This suggests a functional and perhaps structural interaction between complexes of respiratory chain and the channel [4]. However, our recent observations put the direct interaction of these proteins into question.

In addition, recent studies indicate the regulation of the mitoBK$_{Ca}$ channel by gasotransmitters such as carbon monoxide or hydrogen sulfide. Electrophysiological studies have shown that the regulation of a channel by carbon monoxide requires the presence of heme bound to the pore-forming subunit of the channel [5].

The presentation will focus on the latest data on the regulation of mitochondrial potassium channels with an emphasis on specific regulation resulting from the localization of these proteins in the mitochondria.

ACKNOWLEDGMENTS

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NANOIR3 - A COMBINED ATOMIC FORCE MICROSCOPE AND NEAR FIELD IR MICROSCOPY (AFM-IR)

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The nanoIR3 is the latest generation system from long history of technology leadership and innovation in AFM-based nano-optical characterization instruments. Designed with an unmatched level of performance, integration, automation, and flexibility, the nanoIR3 sets a new standard for research productivity and ease of use.

The NanoIR3 is a combined Atomic Force Microscope and Near Field IR Microscopy (AFM-IR) system capable of providing nanoscale infrared spectroscopy and chemical imaging, with 10 nm spatial resolution. The system also provides AFM topographic imaging and material property mapping with nanometre-scale resolution [1].

The instrument also integrates an atomic force microscope designed for seamless integration for AFM-IR operation with support for a variety of laser sources. Laser modules allow integration with a user definable number of lasers. It provides nanoscale IR spectroscopy and chemical imaging as well as hyperspectral imaging.

Key Laser Options:

The nanoIR3 supports wide range of lasers for high resolution Tapping AFM-IR spectroscopy and imaging and patented contact mode-based Resonance Enhanced AFM-IR spectroscopy and FASTmapping Imaging. Spectral ranges vary by laser type.

The FASTspectra QCL is a 4 chip QCL laser providing a spectral range of 950-1900cm^{-1}. The laser includes hyperspectral imaging at speeds of approx. <2 second/spectra and FASTspectra spectroscopy of approx. 15 secs for a full spectral sweep.

AFM-IR achieves this goal by using the tip of an atomic force microscope to locally detect thermal expansion of a sample resulting from local absorption of IR radiation. The AFM tip itself thus acts as the IR detector. Because the AFM tip can detect the thermal expansion with spatial resolution approaching the AFM tip radius, the AFM-IR technique can overcome the spatial resolution limits of conventional IR microspectroscopy.

AFM-IR technique has found many diverse applications, including those in materials and life sciences, polymers sciences and technology, including applications on polymer blends, composites, multilayer films, fibers, and conducting polymers. AFM-IR is also finding exciting applications in the life sciences, including subcellular spectroscopy and chemical imaging, nanoscale chemical analysis of tissue, protein secondary structure analysis, including research into protein misfolding related neurogenerative diseases. Additional

applications including photonics, pharmaceutical sciences, perovskites (solar energy), and semiconductors may be also presented.

Total reflection X-ray fluorescence spectroscopy (TXRF) as promising method in analytical chemistry since 1971.

When atoms are irradiated with high energy X-rays, they give off secondary X-rays in the form of fluorescence radiation. The wavelength and energy of the fluorescence radiation is specific for each element, and the intensity of the fluorescence radiation is proportional to the concentration of the each element present in a sample.

In TXRF, the sample is deposited on a reflective disc as a very thin film, and a monochromatic X-ray beam is used to irradiate the sample holder containing the sample at a very small angle (0.3^0–0.6^0) resulting in the total reflection of the X-ray beam. This total reflection reduces the absorption and the scattering of the X-ray beam in the sample and its matrix resulting in greatly reduced background noise, higher sensitivities, and the reduction or elimination of matrix effects [2, 3].

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THE CELL-IN REAGENT AS A METHOD FOR MACROMOLECULES DELIVERY INTO MAMMALIAN CELLS

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Crossing the cell membrane barrier is a critical step in many biochemical and biomedical studies. The great majority of them rely on the use of biomacromolecules that are not spontaneously taken up by the cell due to the selective permeability of the cell membrane. One method of intracellular delivery relies on the disintegration of intracellular vesicles under osmotic shock. During disintegration, probes within the vesicles are released into the cell. This process is used in the new Cell-IN reagent, a product of long-term laboratory research. Cell-IN supports the process of vesicle disintegration - the nanostructure of the working solution proved to be crucial for efficiency.

Cell-IN allows the introduction of a broad spectrum of cargos (dyes, polymers, proteins, nucleic acids, nanoparticles) into different types of mammalian cells (normal, cancer, epithelial, mesenchymal cells). Cell-IN enables the delivery of cargos with sizes ranging from single nanometers to more than 200 nm (diameter).

The effectiveness of cargo delivery for all tested probes and all tested cell lines was assessed quantitatively by measuring the efficiency coefficient, Q (Fig. 1) [1]. Q is the ratio of the cargo concentration inside the tested cells to the cargo concentration in the Cell-IN solution.

High viability (> 80%) of cells undergoing the Cell-IN procedure was confirmed. The percentage of viable cells of the HeLa (cervical cancer) and MDA-MB-231 (triple-negative breast cancer) lines were determined 1h, 1.5h, 2h, and 3 and 4 days after the macromolecules delivery procedure by osmotic shock. Cell viability was examined by two independent cytotoxicity assays: MTT and alamarBlue® (Fig. 2).

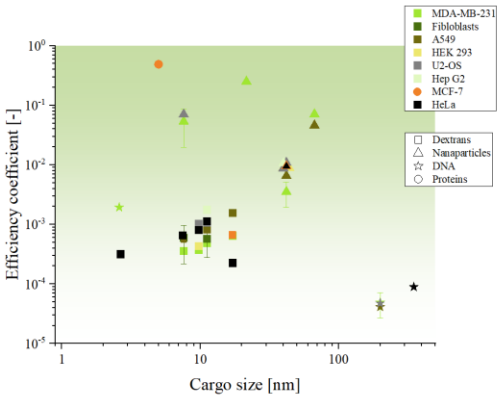

Fig. 1. Efficiency coefficient dependence on a cargo's size, including all tested cell lines and cargo type.

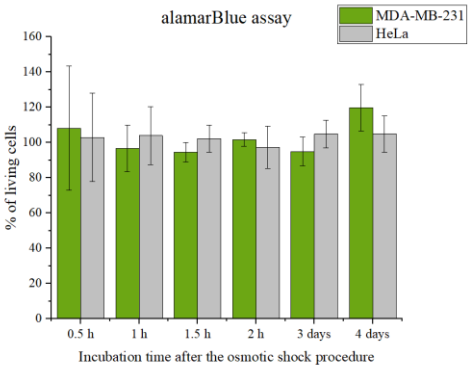

Fig. 2. Comparison of Cell-IN and InfluxTM efficacy for EGFP plasmid delivery into HeLa cells. (A) HeLa cells after EGFP plasmid introduction (24h before imaging) with Cell-IN

product. (B) HeLa cells after EGFP plasmid introduction (24h before imaging) with Influx™ product.

ACKNOWLEDGMENTS

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EFFECT PATERN OF AMINO ACIDS AND NMDA ON ACTION POTENTIALS OF GLR GENES-LACKING MACROALGAE

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Electrical signaling is an integral part of plant physiology. Propagation of action potentials (APs) transduce information to distant parts of the plant body and leads to modulation of vital physiological stimuli [1]. In higher plants, various external stimuli elicit
effect of amino acids and NMDA on the APs of electrical signaling, which a significant role is played by receptor-like homologs of mammalian glutamate receptors (GLR). GLR-mediated responses are calcium-based and have plant-specific physiological functions ranging from carbon/nitrogen metabolism to wound-induced leaf-to-leaf electrical signaling [2]. Despite the wide range of GLR-binding amino acids, classical synthetic NMDA-type iGluR agonist NMDA (binding to the Glu site in the iGluR) was demonstrated to be inactive in higher plants [3]. However, NMDA and amino acids did induce APs in the liverworts – a GLR genes possessing plants [4].

Aquatic Characeanen macroalgae are closely related to land plants, yet they do not passess genes of GLR [5], however, we demonstrated the modulating *Nitellopsis obtusa* – a classical model system of plant electrophysiology [6]. Standardized parameters of action potentials (AP) and excitation current transients were studied in intact internodal cell *via* current clamp and two-electrode voltage clamp modes.

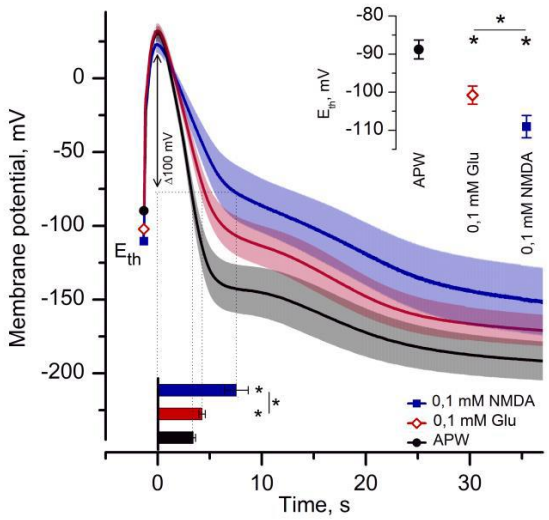

Fig.1. Averaged APs after exposure to 0.1 mM Glu or 0.1 mM NMDA. Solid lines represent mean MP with transparent SE values. Note the differences of AP threshold (E_{th}) and relatively unchanged values of peaks. Dot lines and arrow indicate evaluated repolarization duration (presented in the lower insert). Corresponding E_{th} values are presented in the upper insert. n=8-9.

Results indicate that Glu, Asn and NMDA (0.01-1 mM) increase AP amplitude by hyperpolarizing excitation threshold potential (E_{th}) and prolong AP repolarization phase (Fig. 1). These effects, together with alterations of excitation transient parameters, exhibit dose dependency and a specific effect pattern shared by amino acids and NMDA.Yet, the effect of NMDA exceeds that of Glu. Further, we demonstrated that NMDA-induced alterations are inhibited by ionotropic glutamate receptor inhibitors AP-5 (NMDA-type receptors) and DNQX (AMPA/Kainate-type).

This research presents unpublished correlations between excitation parameters obtained by current-clamp and voltage-clamp approaches and introduces the quantified chloride efflux temporal dynamics during excitation as viable electrophysiological parameters altered by glutamate receptor-like channels' agonists. It finally presents a detailed quantitative description of amino acids and NMDA excitatory effect pattern on APs in a single GLR genes-lacking algal cell.

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EFFECTS OF QUERCETIN AGAINST PARTICULATE MATTER INDUCED IMPAIRMENT OF HBE CELL FUNCTION

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Air pollution and airborne particulate matter (PM) is omnipresent risk factor in modern world. It affects health and it is related to many diseases of respiratory and cardiovascular system. The toxic effects of particulate matter have been linked to many adverse effects within the cell, such as reactive oxygen species (ROS) overproduction leading to e.g. inflammatory response. There is constant search for the new methods reversing the harmful effects of particulate matter induced toxicity.

For more than decade potassium channel openers are known for their protective effects. Moreover it was well documented that mitochondrial large conductance potassium channel (BK_{Ca}) plays crucial role in cytoprotective effect against ischemia-reperfusion events [1].

Cytoprotective and antioxidant effects are well known for many natural components such as flavonoids. However, the exact mode of action is still under investigation. It was previously shown that one of the flavonoids, namely quercetin, can activate mitoBK_{Ca} [2]. Here, we would like to show that quercetin, manifests its fuction in the HBE cells via mitochondrial pathway and mitochondrial BK_{Ca} activation in PM induced toxicity.

The research was conducted on human bronchial epithelial cell line (HBE). The methods incorporated in the study included patch-clamp technique of the inner mitochondrial membrane, transepithelial electrical resistance assessment, mitochondrial respiration measurements with the use oxygen electrode, fluorescence methods for the ROS level and mitochondrial membrane potential assessment, and cell viability measurements using trypan blue staining.

It was observed that PM (<4μm diameter) decreased the transepithelial electrical resistance in HBE cells in dose dependent manner. The effect was partially abolished by quercetin but not by its analog, isorhamnetin. Penitrem A (BK_{Ca} channel inhibitor) reversed the effect of quercetin. The patch-clamp findings confirmed that the effect is associated with channels. Quercetin activated mitoBK_{Ca} channel and the effect was abolished by penitrem A. Isorhamnetin did not affect the channel activity. The results were compatible with mitochondrial membrane and respiration measurements. Quercetin, but not isorhamnetin, decreased the mitochondrial membrane potential and increased mitochondrial respiration. The effect was abolished by penitrem A only in whole cell respiration measurements. Both quercetin and isorhamnetin reveal antioxidant properties. The reduction of PM-induced ROS level occurs both on cellular and mitochondrial level. It correlates with cell viability results for quercetin which increases HBE cell viability after PM administration, whereas isorhamnetin has no effect on cell survival. The toxic effect was also shown on mitochondrial level. The PM incubation with the cells substantially reduced the mitochondrial function measured as respiration control with fully uncoupled mitochondria compared to inhibited electron transport chain. However, neither quercetin nor isorhamnetin could reverse the effect.

The results indicate that PM influences the function of HBE cells on cellular and mitochondrial level. Quercetin is capable to improve the function of HBE cells after PM administration. We show that the effect in HBE cells is connected with its ability to activate mitochondrial BK_{Ca} channel. However, the mechanism of action of quercetin is not exclusively determined by modulation of the channel activity.

ACKNOWLEDGMENTS

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GROUND STATE TAUTOMERIC EQUILIBRIUM AND EXCITED STATE PROTON TRANSFER IN SELECTED PURINE NUCLEOSIDE PHOSPHORYLASE LIGNADS DETERMINED BY QUANTUM CHEMISTRY METHODS

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8-azaGuanine (8AG) and 8-azaisoGuanine (8AIG) are Guanine derivatives in which carbon at position 8 is replaced with nitrogen (Fig. 1). This modification significantly increase intrinsic fluorescence properties of these molecules, which can be utilized for monitoring their interactions with biological polymers like proteins or nucleic acids. In particular these molecules are substrates for ribosylation processes catalyzed by Purine Nucleoside Phosphorylase protein (PNP). In order to better understand protein-ligand interactions it is important to understand the ground-state tautomeric equilibrium as well as possible excited state proton transfer processes, which can take place in these compounds.

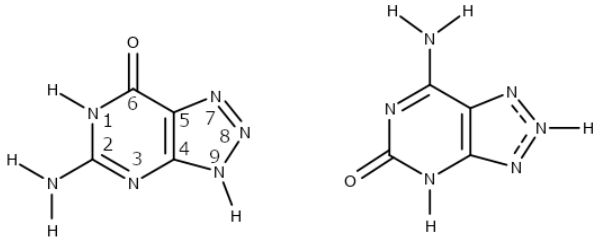

Fig.1. 8-azaGuanine and 8-azaisoGuanine molecules

Ground state tautomeric equilibrium of 8AIG was revealed in our previous publications [1,2]. It was shown that besides the dominant tautomer protonated at positions 3 and 8 some minor tautomers might be present in water solution. Moreover methylation of 8AIG at positions 9 surprisingly significantly increase population of enol forms of the molecule. For 8AG molecule tautomeric equilibrium seems to be much simpler as tautomers protonated at positions 1 and 9 dominates both in water and in the gas phase. Methylation of the molecule at position 9 does not lead to any important shifts in tautomeric equilibrium with dominant form protonated at position 1. The results obtained from population analysis are also supported by vertical absorption energies, which are in a very good agreement with the experimental data.

Calculation of vertical emission energies with TDDFT method sheds light on possible ESPT processes which can take place in both investigated molecules. In particular, vertical emission energies determined for all neutral tautomers of 8AIG strongly support ESPT mechanism proposed by Wierzchowski et al [3]. Therefore, based on the agreement of experimental fluorescence peaks and calculated vertical emission energies, most probable tautomeric species responsible for fluorescence process are suggested for ionic and methylated forms of both molecules.

Moreover, based on our results, we recommend B3LYP and PBE0 functionals combined with aug-cc-pvdz basis set for calculation of vertical absorption and emission energies, respectively for purine analogs.

ACKNOWLEDGMENTS

Most of computations were performed in the Wrocław Centre for Networking and Supercomputing (http://wcss.pl, grant number 349).

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PEPTIDE STAPLING AS A WAY TO ENHANCE ANTIBACTERIAL PROPERTIES OF ANOPLIN

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The emergence of multi-drug resistant bacteria together with the overuse of antibiotics shows an increased need to develop antimicrobial peptides (AMP). One of AMP is anoplin, a linear 10-amino acid peptide extracted from the venom of a soliary wasp [1]. In spite of its weak antimicrobial activity, anoplin has an important property of adopting an amphipathic helix structure near the lipid environment [2]. We thus supposed that stabilizing the active α-helical structure of anoplin would enhance its antimicrobial activity. To test our conjecture,

we introduced hydrocarbon staples into the anoplin sequence.

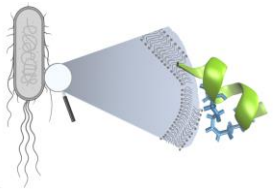

Fig.1. The proposed destructive effect of the stapled α-helical anoplin analog on the cell wall of *E.coli* strain.

Firstly, we designed and synthesized two anoplin derivatives: anoplin[2-6] and anoplin[5-9] by introducing two non-natural amino acids with olefinic side chains into the peptide sequence. The staple insertion sites were chosen so that the charge and hydrophobic-hydrophilic nature of anoplin was unchanged. Secondly, using the Grubbs catalyst, the analogs were covalently linked, i.e., stapled. Thirdly, by circular dichroism spectroscopy, the helical secondary structure was confirmed. The designed stapled peptides exhibited higher antibacterial activity against various bacterial strains, also the resistant ones [3]. Moreover, to investigate if the antibacterial mechanism of analogs is related to their destructive effect on the bacterial cell wall we performed the propidium iodide uptake assay. According to the results, anoplin[2-6] effectively passed through the membrane of *E. coli* K12 so had higher activity against Gram-negative bacteria. However, anoplin[5-9] had a stronger destructive effect on the *S. aureus* Gram-positive cells. Finally, we confirmed that by using the hydrocarbon stapling technique we obtained peptides with stable secondary structures and they did not show toxicity and hemolytic activity [3].
In the future, we plan to conjugate the stapled anoplin to a conventional antibiotic to tackle the problem of bacterial resistance arising due to antibiotic modification.

ACKNOWLEDGMENTS

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RESHAPING MITOCHONDRIAL NETWORK AS A PART OF CELLULAR ADAPTATION IN PRIMARY HUMAN FIBROBLASTS FROM HEALTHY DONORS AND FROM ALZHEIRMER'S DISEASE PATIENTS.

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Mitochondria form a dynamic network, shaped by the processes of fusion and fission, which play an important role in optimizing mitochondrial function. Changes of mitochondrial network morphology impact mitochondrial bioenergetic efficiency and ROS production, they also enable mitochondrial renewal via the processes of biogenesis and mitophagy. Apart from adaptation to changing physiological conditions, alterations in mitochondrial network dynamics accompany various disease states.
We have investigated mitochondrial dynamics in primary fibroblasts derived from patients with Alzheimer's Disease and observed in these cells altered mitochondrial network morphology, as well as a general decrease in the intensity of dynamic processes shaping mitochondrial network: mitochondria fusion, fission, motility and turnover [1,2]. We have also compared the observed changes with the ones characteristics for cellular aging, and with the responses to the physiological insults such as limited nutrient availability [3].

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FEDERATED EUROPEAN GENOME ARCHIVE – POLISH NODE

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The availability of data decreases with the amount of time passed from publication [1]. Such a situation is associated with the "reproducibility crisis" in science [2]. Probably that's why founding organizations like NIH, ERC or NCN, started to require DMPs when submitting grant application. This was also noticed by key players and there were set DbGap [3] by NIH in USA and in Europe - European Genome-phenome Archive [4] by EMBL and Elixir Consortium. Both are online repositories for storing and sharing scientific data on human genetics.

Let's focus on the second imitative since 2013 until 2021 there were sub-mitted more than 12,5 PB of data from about 3 000 studies and more than 30 PB of data were re-used in research. Data from 624 studies were reused at least once. The most re-used data set was used 25 times since publication in the archive. [5]

From 2018, the GDPR came into force in the EU. Member State implementations differed in their approach to sharing human genetic data. To avoid the turbulence associated with the storage of this type of data, the idea of the Federative EGA was born. The idea of distributed archive where data from a given area is stored in a local national node under the jurisdiction of a given country. The Polish node is being created by the University of Lodz based on the government cloud and is intended to be launched fully operationally in the first quarter of 2023.

Free storage and sharing are not the only advantages of FEGA. The functionalities of the repository ensure full control over the deposited datasets thanks to the use of Data Access Committee (DAC) mechanisms and data encryption with the PKI infrastructure.

This tool seems to be the right choice for scientists who want to comply with the requirements of open data policies, DMPs, FAIRness of data, and to increase the chance of theirs work to be seen and reused in international scientific collaborations.

The final argument in favor of a Federated EGA is the savings. When using existing data, costs are significantly lower when compared to a sample acquisition and data generation or even when compared to re-generating data from an already existing sample. With savings in mind, we should not limit ourselves to the economic dimension only. Each time a biological sample is used, its volume is reduced. However, in the case of re-use of existing data, there is no such loss – there appear a saving in biological material.

ACKNOWLEDGMENTS

Founded by Digital Poland Operational Programme Grant Polish Genomic Map in open access - digitization of biomolecular resources of the Biobank Lab POPC.02.03.01-00-0097/19.

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RHEOLOGICAL ASPECT OF BIOPHYSICS OF CIRCULATORY SYSTEM

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Biophysics of circulatory system is an important aspect of medical sciences. Medicine as science of human health and disease treatment and prevention uses results of research within various disciplines. Due to the natural liquid consistence of living organisms, rheology is often applied as a field of science related to the study of liquids, their flow, deformation, and phenomena accompanying this flow. The objects of rheological studies in medicine are primarily blood and body fluids such as joint ooze or saliva, however equally important are the viscoelastic properties of other tissues, like muscles or fat tissue, as well as the micro-viscosity understood as viscous properties in the sub-micron scale, natural for motion inside individual cells and in

the intercellular environment. Rheological tests are also essential for many studies in pharmacy or dentistry. In this work rheological aspects of biophysics of circulatory system are considered

The phenomenon of liquid flow in blood vessels is a very complex process. Blood flow creates friction between both blood components and the blood against the blood vessel wall. According to the Hagen-Poiseuille law, blood flow stream (Q) increases with increasing vessel radius (r) and with increasing pressure difference (Δp) causing flow and decreases with increasing blood viscosity (η) as well as vessel length l (eq. 1) [1,2].

$$Q = \frac{\pi r^4}{8\eta l}\Delta p \qquad (1)$$

Hemorheological studies focus primarily on the measurement of blood viscosity as a function of shear rate, plasma viscosity and red cells aggregability and deformability. Blood, as a suspension of morphological elements in the plasma is a non-Newtonian liquid [1]. Blood viscosity is a strong function of shear rate and it also depends on the sammple history.

Blood viscosity has been studied in many research centers and among many different groups of patients. The main disorders related to the hemorheological properties are: coronary insufficiency, vascular congestion, myocardial infarction, cerebral circulation disorder, Reynaud disease, ischemic limbs, diabetes, anemia, tumors [1-3].

The flow curve measurement involves determining the shear rate dependence of blood viscosity. The viscoelastic properties of blood were investigated in oscillatory measurements. The data obtained from the flow curve measurements can be analyzed using mathematical rheological models like Quemada model in which we use the measured hematoctrite value and plasma viscosity [4].

Blood flow can also be modeled. Numerical fluid mechanics, computational fluid mechanics (CFD) is a tool used to create and solve mathematical models of transfer processes that are based on differential equations of conservation of mass, momentum, and heat [2].

Many hemorheological aspects of living organism functioning are not known yet. We believe that thanks to the new research methods we should learn better the autoregulatory mechanisms aiming at improvement of blood flow. This knowledge could be used to develop alternatives in the case when a living organism cannot do it by itself.

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MECHANICAL UNFOLDING, REFOLDING AND MISFOLDING OF BIOLUMINESCENT PROTEINS: ONE MOLECULE AT A TIME

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Single-molecule force spectroscopy (SMFS)[1] allows individual molecules of biopolymers such as polysaccharides, DNA and proteins to be mechanically stretched into high-energy conformations that are not accessible to other biophysical techniques that operate near equilibrium. Such conformations can be uniquely maintained for a long time and probed. Relaxation pathways to energy minimum structures can be subsequently followed. Using atomic force microscopy (AFM), optical traps (OT) or magnetic tweezers (MT) to execute SMFS measurements it became possible to unravel individual proteins to their extended polypeptide chain conformation and to follow their relaxation pathways until they recover their characteristic 3D fold. Thus, SMFS enabled protein folding study under geometrically restrained conditions that are very different from those using typical bulk biophysical approaches involving thermal or chemical denaturation and renaturation, and arguably closer to the conditions experienced in vivo during the vectorial folding of ribosome-bound nascent polypeptide chains. In this paper, the application of AFM-based SMFS to examine nanomechanics of bioluminescent proteins such as firefly luciferase and Nanoluc is illustrated. Firefly luciferase (Fluc) has been a model, protein for studies of co-translational protein folding as well as a preferred substrate to investigate chaperone assisted refolding, due to its easy bioluminescence readout as a measure of the robustness of its fold. Nanoluc, (Nluc) is an engineered bioluminescent protein that is much smaller than Fluc (170 versus 550 amino acids) yet its light intensity is over 100-fold greater than that

of Fluc. SMFS studies of Fluc revealed that the protein is able to correctly refold to its native structure under partial mechanical unfolding as long as the N terminal domain (~200 amino acids) remains folded. Misfolding occurs when the completely unraveled polypeptide chain is allowed to relax, however Hsp70 chaperones are able to rescue Fluc's fold, apparently, by separating its N and C-terminal domains during AFM-controlled chain refolding. SMFS of Nluc demonstrated that the monomeric protein unfolds and refolds robustly without chaperones' help, however synthetic Nluc constructs composed of tandem repeats of two or three Nluc domains are prone to misfolding under mechanical or thermal denaturation conditions and become chaperones' substrates. The misfolding of Nluc repeats is possibly driven by domain swapping, as suggested by coarse grain molecular dynamics simulations. These observations illustrate unique strengths of SMFS approaches to examine protein folding and misfolding pathways and to probe the mechanism of their interactions with chaperones.

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THE INFLUENCE OF PLANT-DERIVED POLYPHENOLIC COMPOUNDS AND COMPOUNDS FROM THE GROUP OF STATINS AND PHENOTHIAZINES ON DRUG-SENSITIVE AND DRUG-RESISTANT CANCER CELLS AND ON PROPERTIES OF MODEL LIPID MEMBRANES

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Flavonoids, statins and phenothiazines belong to the heterocyclic compounds that exert numerous effects in biological systems. Statins are popular drugs widely used in medical treatment to reduce blood level of cholesterol in hypercholesterolemia. Phenothiazines are commonly used in medicine as tranquillizers. However both groups of the drugs exert also a lot of other biological effects. In many studies on cancer cells various phenothiazine derivatives were shown to act as antitumor, antiproliferative or pro-apoptotic agents. Also spectrum of biological activity of flavonoids which are plant-derived polyphenolic compounds is very broad. The structure of all these compounds enables their specific interactions with different membrane proteins and also non-specific interactions with lipid phase of membranes. Such interactions with membrane components contribute to the various biological effects observed in the presence of phenothiazines, statins and flavonoids.

Phenomenon of multidrug resistance (MDR) is considered to be a major obstacle in successful chemotherapy in tumor diseases. Studies on this phenomenon and its modulation by various groups of compounds are carried out in our laboratory from 25 years [1,2].

Among many different results of studies performed in our laboratory during last years an very important one is that one which shows that combined use of phenothiazines and statins strongly increases accumulation of anticancer drug, doxorubicin in colon cancer cells [3]. It was also observed that flavones such as baicalein and luteolin express anticancer activity [4] and that statins and statins applied in combination with flavonoids reverse drug resistance in colon cancer cells [5].

For better understanding of the mechanism of multidrug resistance and possibility of its overcoming also results of the studies concerning the influence of different group of compounds on properties of lipid membranes are important because lipid bilayer properties control membrane partitioning, transport of P-gp substrates and they may also influence activity of multidrug resistance transporters [6].

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ACTIVATION MECHANISMS OF THE GABA TYPE A RECEPTOR

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GABA type A receptor ($GABA_AR$) is a member of the pentameric ligand gated ion channels family (pLGICs). It plays a crucial role in inhibition in adult mammalian brain and its dysfunction may cause e.g. epilepsy, anxiety and depression. The $GABA_AR$ is composed of five subunits, each consists of extracellular (ECD), transmembrane (TMD) and intracellular domain. Upon the agonist binding at the ECD site, the protein undergoes a series of transitions leading to the opening of the TMD channel gate. Although structures of $GABA_ARs$ are emerging[1,2], the molecular mechanisms of the receptor activation remain elusive. The aim of the presented project was to combine various methods to shed light on these mechanisms.

First, a set of the receptor residues of key importance for the receptor function was selected on the basis of the structure analysis and molecular dynamics simulations. To assess their role, patch-clamp recordings at macroscopic and at single-channel level were conducted for currents mediated by wild type and mutated receptors. These recordings together with modeling enabled us to assess the transition rate constants and to infer the importance of specific residues in distinct conformational changes. In addition, molecular modeling and docking were utilized to further estimate the roles of considered residues.

Our results indicated, that mutations of the residues at the agonist binding site (βE155, βF200, αF64, αF45) are influencing the receptor dose-response relationship to a higher extent than those located in other regions of ECD or TMD (e.g. βF31, αH55, βP273, αL300). On the contrary, the rates of the transitions in the bound states tend to be affected by nearly all mutations, in a manner not clearly dependent on structural localization. This indicates, that the ligand binding is a local phenomenon, but gating has a widespread character. The REFER analysis based on kinetic models was performed to estimate the timeline of structural transitions associated with receptor activation. Two synchronized components of the transition timeline were revealed: the first one composed of movement of the residues located at N-terminal region (βF31, αF14), agonist binding site (βF200, aF64) and ECD/TMD domains interface (βV53, βP273, αH55, αP277) and the second one made of remaining ECD (βE153, αF45) and TMD (βE270, βH267, βL296, αL300) areas. Compared to other pLGIC (AChR)[3], $GABA_AR$ is characterized by relatively high Φ values with a smaller differences in values throughout the protein, suggesting a higher degree of functional compactness of the receptor. Experimental data and molecular modeling underscored the key importance of the interactions at receptor subunit and domain interfaces, providing possible explanation for a relatively low variability in the Φ values in $GABA_ARs$. Thus our data indicated the allosteric character of the receptor activation, characterized by a high level of cooperation and synchronization of the respective protein regions.

ACKNOWLEDGMENTS

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HOW SHEARING AND PULLING AFFECTS GLUTEN PROTEINS: A MOLECULAR DYNAMICS STUDY

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Gluten proteins are responsible for the viscoelastic properties of the wheat dough, and therefore for the bread quality [1,2]. During breadmaking, the dough undergoes various deformations, that change its structure and make it more elastic [2]. In the Institute of Physics, Polish Academy of Sciences we conducted pioneering coarse-grain molecular dynamics simulations of gluten proteins, which allowed us to recreate the effect of shear and pull deformations on gluten proteins. Simulations use implicit solvent, each amino acid is represented by a single pseudo-atom. Details of the model can be found in our articles [3,4]. Periodic

boundary conditions are used in the X and Y directions, but there are attractive solid walls in the Z direction. After preparation and equilibration, the solid walls move periodically in the Z direction (pulling mode) or in the X direction (shearing mode), then after 0, 6 or 10 full oscillations (which are are used to calculate the dynamic Young modulus) they move away from each other in the Z direction, allowing us to calculate the critical strain [4]. During this moving away phase, proteins stick to the opposite walls and rupture into two parts. We measure the force and work needed for this process.

Gluten proteins can be diveded into glutenins and gliadins. The simulations correctly recreate experimental results [2]: glutenins become more resistant to stretching after deformation (see Fig. 1). The control simulations of maize and rice proteins correctly show that they do not become more resistant after being periodically deformed [3]. The resistance rise after deformation is related to a process called strain hardening [2]. We demonstrate that on the molecular level it is based on increasing the number of entanglements and inter-chain contacts. Glutenin proteins are the longest of the proteins mentioned here (the can have over 800 residues), so naturally they can form the highest number of entanglements.

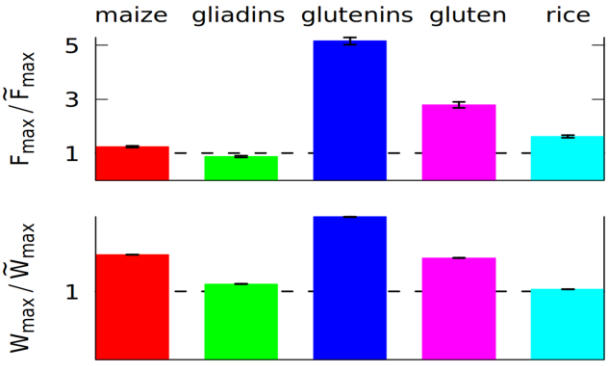

Fig.1. Maximum force and total work required to separate maize, gliadin, glutenin, gluten and rice proteins into two subsystems by pulling the walls in opposite directions. Values are divided by their counterparts measured without oscillations, so the bars show the ratio of values after 6 shear oscillations and after none.

The coordination number measures how well connected is the chain network. It is linked to the Young modulus and critical strain by the slip-bond theory [4]: the more interconnected the network is, the more force you need to rupture it, but the rupture occurs for a lower critical strain (the network breaks all at once), whereas more loosely connected networks are easier to stretch, but can withstand bigger deformations before rupturing. The simulation confirm this for gluten proteins.

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This work would not be possible without Prof. Marek Cieplak, who died recently, and his team at the Institute of Physics, Polish Academy of Sciences.

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DESIGNED RIBOZYME DIRECTED AGAINST ESSENTIAL BACTERIAL MESSENGER RNA

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The spread of antibiotic resistance is becoming a global health threat. An increasing number of bacterial infections and ineffective medicines lead to harder treatment. That problem forces scientific community to develop new efficient antibacterials. A pioneering idea for promising antimicrobial agents lies in ribozymes (RNA enzymes). The catalytic character of natural RNA has been successfully used in, inter alia, anti-viral and anti-cancer therapies [1]. Ribozymes have the capability to bind and digest a specific RNA sequence (Fig. 1). Designing a ribozyme targeting a specific mRNA transcript that encodes a crucial bacterial protein should suppress translation and inhibit microbial growth [2].

We used the hammerhead ribozyme - the best known RNA catalyzing motif. We designed the ribozyme to target the mRNA-*acpP* transcript encoding the acyl carrier protein (ACP) in *Escherichia coli* K-12 MG1655 [3]. The ACP is essential in the fatty acid biosynthesis cycle. Therefore, we expect that preclusion of ACP translation would inhibit bacterial growth.

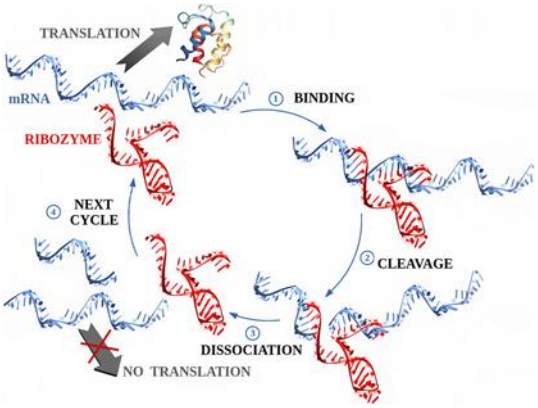

Fig.1. Scheme of cleavage of bacterial mRNA using ribozyme.

We have designed two versions of the anti-*acpP* ribozyme having short or long arms that bind the mRNA. To protect the fragile structure of the RNA enzyme from digestion by endonucleases, we hid the hammerhead ribozyme in a tRNA construct (Fig. 2). This approach should improve the lifetime of the hammerhead ribozyme, and as a consequence, enhance efficiency of cleavage of mRNA-*acpP* in bacterial cells. We have confirmed forming a binding complex of the designed ribozyme to its target in 1:1 ratio by isothermal titration calorimetry. Using gel electrophoresis, we have verified that the mRNA substrate is cleaved effectively *in vitro* by the hammerhead ribozyme. We also found promising results in antibacterial activity of the designed ribozyme.

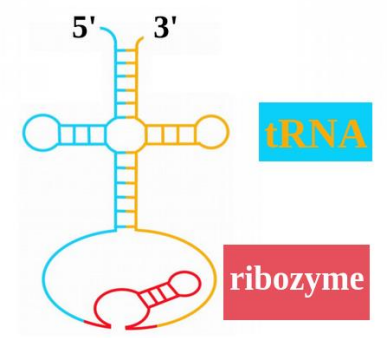

Fig.2. The ribozyme in a tRNA construct.

ACKNOWLEDGMENTS

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ASSOCIATION BETWEEN ALBUMIN AND CHIMERIC LIPID NANOPARTICLES

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Lipid nanoparticles are a well-established class of innovative excipients for the development of drug delivery systems [1]. Lately, advanced platforms that combine multiple types of biomaterials are developed, called “mixed” or “chimeric”, which present new properties and functionalities [2].
Herein, curcumin-loaded lipid/polymer nanoparticles (CLPNs) were developed and their biophysical interactions with human serum albumin (HSA) were assessed.

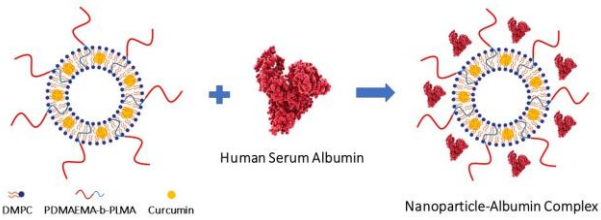

Fig.1. The interaction between HSA and chimeric nanoparticles and the formation of their complex.

Nanoparticles were prepared by thin-film hydration, followed by extrusion, by combining the phospholipid DMPC and the amphiphilic diblock copolymer PDMAEMA-b-PLMA. The particle physicochemical properties were investigated by light scattering, showing small and monodisperse size, positive zeta potential (ZP) and almost complete curcumin incorporation (10% w/w of lipids). In addition, the particles were colloidally stable for at least 15 days.

Table 2. Physicochemical properties of CLPNs.

Property	Value and unit
Hydrodynamic diameter	98.4 ± 1.9 nm
Polydispersity index	0.119 ± 0.016
Zeta potential	14.0 ± 2.1 mV
Incorporation efficiency	99.8% ± 4.1%

The morphology of the chimeric nanoparticles was studied by negative-stain transmission electron microscopy (TEM). The formation of vesicular assemblies of roughly 100nm size was confirmed.

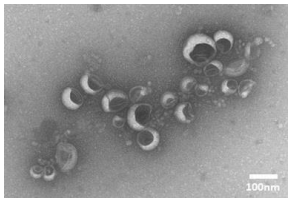

Fig.2. TEM image of the chimeric nanoparticles.

The interactions of CLPNs with HSA were investigated in phosphate buffer (pH = 7.4) by light scattering and circular dichroism (CD). The concentration-dependent effect on the properties of HSA was evident, with saturation occurring at a certain particle concentration in each case. The ZP presented an increase in value, while the system size decreased after the addition of the nanoparticles. Regarding the secondary structure of the protein, a decrease in the a-helical structure was observed, due to a decrease in the band intensity of the CD spectrum at all wavelengths.

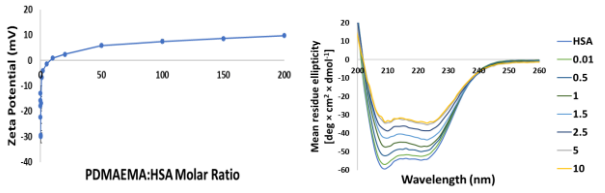

Fig.3. ZP (left) and CD spectra (right) of HSA, titrated with CLPNs at various PDMAEMA:HSA ratios.

Overall, the interactions between the developed nanoparticles (presumably through the cationic PDMAEMA groups) and HSA led to alteration of the surface properties of both elements and to reorganization of the protein domains.
Conclusively, chimeric curcumin-loaded nanoparticles were developed and their association with albumin was established. The study of the biophysical interactions of nanoparticles with physiological components is an essential element for their utilization in biomedical applications.

ACKNOWLEDGMENTS

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PEG AND FICOLL AS MODELS OF CROWDED ENVIRONMENT IN MOLECULAR DYNAMICS SIMULATIONS: TOWARDS UNDERSTANDING DIFFERENTIAL EFFECTS OF SYNTHETIC CROWDERS ON THE HYDROLYTIC ACTIVITY OF A VIRAL PROTEASE

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The highly crowded environment of the cellular cytoplasm is in stark contrast to the conditions of typical biophysical experiments. Volume exclusion and interactions with the crowder molecules can influence the diffusion of enzymes and their substrates as well conformational dynamics and activity of the proteins.
In laboratory experiments, the crowded environment is most often represented with synthetic polymers of various lengths and masses like polyethylene glycol (PEG) and branched polysucrose (Ficoll). However, these molecules are hardly ever used in atomistic molecular dynamics (MD) simulations. Instead, reduced-resolution spherical models or protein crowders are commonly used [1]. This complicates the interpretation of experiments, especially when there are distinct effects of different artificial crowder molecules. Indeed, while we investigated the effects of crowding on the hydrolytic activity of the NS3/4A protease encoded by the hepatitis C virus, we found that the rate of the substrate cleavage was oppositely affected by the presence of PEG and Ficoll [2] . Analysing the trajectories of the NS3/4A protease surrounded with all-atom models of these polymers proved very helpful in understanding the molecular mechanisms behind the effects observed in the experiments. The simulations allowed us to examine protein-crowder interactions with atomistic level of detail, and to assess diffusive

properties of the protein, its substrates and the crowders themselves. This allowed us to develop molecular-level hypotheses for the observed changes in protease activity.

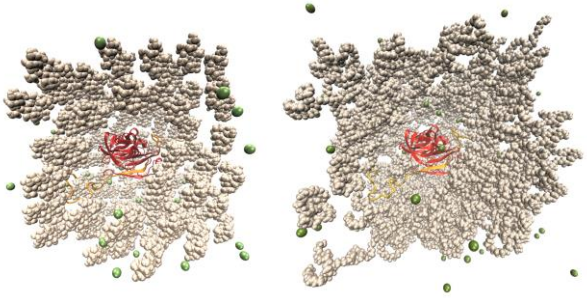

Fig.1. NS3/4A protease from the Hepatitis C Virus surrounded with polysucrose and PEG crowders

The presentation will describe the results of the protease simulations in water and in the presence of crowders, and also cover technical aspects of using non-protein polymers in MD simulations [2,3] . Those include building molecular models of the crowders, assembling the crowded systems, and preventing excessive aggregation in the simulations of a large number of molecules.

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INSIGHTS INTO LIGAND BINDING TO PTERIDINE REDUCTASE 1 FROM MOLECULAR DYNAMICS SIMULATIONS

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Pteridine reductase 1 (PTR1) is a key trypanosomatid-specific enzyme of the folate pathway that is a validated drug target. The roles of the PTR1 dynamics in its function and inhibitor binding are poorly understood. Our preliminary studies indicated that the substrate loop near the PTR1 active site is flexible, which may affect ligand binding [1]. Moreover, crystallographic data suggest that ligand binding may be regulated by the long-distance coupling between four binding sites in the PTR1 homotetramer.

Therefore, we applied molecular dynamics (MD) simulations to characterize the enzyme dynamical properties. The MD results provided insights into the differing interactions of substrates, products and the model inhibitor methotrexate with PTR1. This knowledge may help in designing more effective PTR1 inhibitors. Moreover, simulations with a non-equilibrium method, Rotamerically Induced Perturbations [2], revealed different levels of dynamical coupling between the ligands bound to different pairs of subunits in the PTR1 homotetramer [3], extending our knowledge about the long-range inter-subunit communication in the PTR1 enzyme.

ACKNOWLEDGMENTS

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INTERACTIONS OF PEPTIDE NUCLEIC ACIDS WITH RNA AND BACTERIAL TRANSPORTER PROTEIN BtuB

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Peptide nucleic acid (PNA) is a synthetic DNA analog with a neutral backbone that contains N-(2-aminoethyl)-glycine units instead of the sugar-phosphate ones [1]. A PNA oligomer forms complementary duplexes with itself and natural nucleic acids, and may also form triplexes. The complexes with PNA have higher melting temperatures than those formed by natural nucleic acids [1]. Higher thermal stabilities of short PNA−PNA duplexes over PNA−RNA and RNA−RNA were also confirmed by molecular dynamics simulations at ambient and elevated temperatures [2].

Moreover, PNA is biostable as it is not degraded by neither proteases nor nucleases. Thus, short PNA oligomers are useful in many applications including molecular biology, biotechnology and diagnostics. For example, antisense PNA complementary to mRNA transcripts encoding essential proteins have been useful to inhibit cellular growth, including the growth of bacterial cells (reviewed in [3]).

We have been investigating the interactions of PNA oligomers with bacterial ribosomal RNA and mRNA [2] with the aim to use PNA oligomers as antibacterial compounds. Unfortunately, bacteria do not uptake PNA oligonucleotides and efficient methods for PNA delivery to cells are still being searched for [3]. To deliver PNA to bacterial cytoplasm, we make use of molecules that bacteria need to uptake for growth. Such scarce metabolites enter bacterial cells via energy and receptor-dependent pathway. One of such molecules is vitamin B_{12}. Free vitamin B_{12} enters bacteria via the TonB-dependent transport system and is recognized by the outer-membrane vitamin B_{12}-specific BtuB receptor.

We have previously shown that vitamin B_{12} conjugated to a PNA oligomer, delivers PNA to *E. coli* and *S.* Typhimurium cells [4]. By engineering the *E. coli* $\Delta btuB$ mutant we have confirmed that the BtuB receptor protein is required for uptake of vitamin B_{12}-PNA through the *E. coli* outer membrane [5]. To elucidate atomistic details of this transport we simulated the passage of vitamin B_{12} and the PNA conjugate through the outer-membrane protein BtuB using molecular dynamics simulations. In order to enhance conformational sampling, we developed Gaussian-force simulated annealing method and combined it with umbrella sampling [5,6].

BtuB is a β-barrel protein occluded by a luminal domain. We found that partial unfolding of this domain makes the passage of ligands mechanically feasible [5,6]. PNA movement into the β-barrel is energetically favorable because inside the BtuB protein the hydrophobic PNA extends. In addition, we found that BtuB extracellular loops are actively involved in transport through an induced-fit mechanism.

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NANOPARTICLES AS POSSIBLE ANTICANCER DRUGS ACTIVITY MODULATORS

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Nowadays, the treatment of many cancers is still based on chemotherapy. Commonly used in chemotherapy classical anticancer drugs are often effective against cancer, however, they trigger many adverse side effects. Moreover, many of them cause cell resistance during treatment. The problems associated with the delivery of active drug molecules to cancer cells are another important difficulty in their stable activity against carcinoma.

On the other hand, it is known that many nanoparticles can influence the activity of several antineoplastic drugs. There are many hypotheses that are trying to elucidate this phenomenon. Nanoparticles of various nature may act as efficient drug carriers, directly interact with drug molecules, modify cell receptors and membrane permeability, or even influence various cell metabolism trails.

Fullerene C60 (FC60) is a carbon-based nanoparticle composed of 32 rings. Due to its small size, postulated lack of toxicity, antioxidant activity, ability to non-covalently interact with biomolecules, and capability to penetrate into cells, fullerene became an object of intense research aimed at its application in nanomedicine (drug delivery, protection against free radicals, anti-cancer properties). Fullerene C60 seems to be a great candidate for combination chemotherapy due to its capability to increase the efficiency of anticancer drugs while demonstrating a protective effect on non-cancerous cells [1,2]. Direct interactions of FC60 with various anticancer drug molecules may be one of the most relevant explanations for this phenomenon. Such interactions were examined and demonstrated for FC60 and well-known antineoplastic drugs: doxorubicin and cisplatin [2-5].

Noble metal nanoparticles, like silver- (AgNPs) and platinum nanoparticles (PtNPs), are also interesting candidates for the modulation of anticancer drug activity. It has been shown that AgNPs and PtNPs also can directly interact with antineoplastic drug molecules modulating their biological activity this way [6,7].

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THE NEW MELPHALAN ANALOG (EM–T–MEL) ALTERS THE CONFORMATION OF B-DNA AND INDUCES DNA DAMAGE IN HEMATOLOGICAL MALIGNANCY CELLS

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The second most common hematologic malignancy is multiple myeloma (MM). The treatment program according to a schema (HDM-ASCT) includes high-dose of cytotostatic drug melphalan followed by autologous stem cell transplantation. Melphalan is a bifunctional alkylating agent that covalently binds to nucleophilic sites in the DNA, effective in the treatment but with high systemic toxicity. Therefore, new approaches in drug development are necessary to reduce the side effects, while preserving the high antitumor activity.

Within the scientific framework (Lodz-Warsaw-Erlangen) we synthesized the novel melphalan derivate: [2–(thiomorpholin-methylideneamino)–3–[4–[bis(2–chloroethyl)amino] phenyl] propanoic acid methyl ester hydrochloride] (EM–T–MEL) and analyzed its impact on the DNA in three cancer cell lines: multiple myeloma (RPMI8226), acute monocytic leukemia (THP1) and promyelocytic leukemia (HL60). The ability of EM–T–MEL to affect DNA secondary structure was analyzed by the circular dichroism (CD) technique on native DNA. The level of DNA damage in cells was examined with the alkaline version of comet assay and generation of yH2AX by immunostaining.

We observed the dose-dependent (5-300 μM) changing of the conformation of the B-DNA. Three types of change were seen in the CD spectra: 1) spectral maxima at 275 nm shifted to higher wavelengths, 2) the positive ellipticity decreased between 270 and 290 nm and 3) the negative ellipticity between 233 and 248 nm. At the highest concentration of EM–T–MEL, a peak minimum at λ= 268 nm and maximum at λ= 238 nm were observed. In vitro studies showed that EM–T–MEL causes DNA fragmentation and phosphorylation of the histone yH2AX in all cell lines, and the changes are much higher than in the case of the unmodified drug. The treatment with EM-T-MEL generated DNA damage and phosphorylation of the histone yH2AX time- dependently. After 24 and 48 hours, there was an increased number of cells with abundant foci in the cell and a high percentage of DNA in the comet's tail.

In conclusion, the newly synthesized drug EM-T-MEL modify the conformation of the B-DNA, cause the fragmentation of the DNA and the phosphorylation of the histone yH2AX in a more powerful way than the origin drug.

VISCOELASTICITY OF BRAIN TISSUE AND ITS IMPACT ON GLIOBLASTOMA CELLS

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The brain is one of the the most compliant organs in the human body. Despite its natural softness, brain tissue compression-stiffens and responds in the time-dependent manner under external loads. Single cells within the brain are exposed to forces and stiffnesses higher than those predicted from *ex vivo* tissue measurements. In this regard, it is important to understand how brain cells adopt to an increased stiffness of their surrounding and whether this implies changes in their fate and function. In parallel, brain is also highly viscous, and brain cells' response to viscosity is different from their response to stiffness. Recent development of soft viscoelastic materials where the elastic and viscous moduli can be independently tuned has opened up the possibility to characterize the impact of both elasticity and viscous dissipation on brain cells. The potential of mechanical stimuli to directly influence cell function is relevant to brain tumor growth and essential for understanding how cells and tissues develop under normal conditions and how they change when exposed to altered mechanical loads. Here, the measurements of the brain tissue mechanical properties will be presented and soft viscoelastic materials that mimic brain viscoelasticity will be introduced. The response of the single glioblastoma cells to changes of substrate viscoelasticity will be discussed and that view might shed light on the changes that occur during malignant transformation in brain. Increased mechanical characterization of the brain and further investigations of the mechanobiology of single brain cells under active mechanical forces is needed and have both diagnostic and therapeutic relevance.

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NOVEL BIOSENSING SYSTEMS WITH SELF-QUENCHING FLUORESCENCE NANOFLARES FOR CANCER BIOMARKERS' DETECTION

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Many studies have recently focused on finding new biomarkers, for early detection of debilitating and high-morbidity diseases, that could also serve as the therapeutic targets. Moreover, new methods enabling widespread disease screening while offering a low cost, rapid testing, and biocompatibility, are highly sought after. The studies have shown that biosensing systems based on aptamers and molecular beacons, consisting of single stranded DNA or RNA oligonucleotides in combination with fluorescence detection offer all of these features. Therefore, they can be widely used in many fields, such as the clinical diagnostics, therapeutics, molecular imaging, drug delivery, and biosensing. It has been shown that the Sur protein and its mRNA are highly expressed in most cancers and correlate with resistance to chemotherapy and radiotherapy, associated with increased aggressiveness of tumors. This is making the anti-survivin therapy an attractive cancer treatment strategy. Furthermore, it has been shown that the deviations of ATP concentration from its normal level can be attributed to diseases such as the Parkinson's, cardiovascular disease, carcinogenesis, or cancer progression [1,2]. Therefore, the development of methods that can be used to detect survivin and enable monitoring of ATP concentration level changes are of great significance in studying of the disease severity.

In our first study, we have developed a survivin molecular beacon (SurMB) probe for monitoring Sur mRNA in SW480 cancer cells [3,4]. The probe consisted of a hairpin-like structure with the fluorophore (Joe) and the quencher (Dabcyl). In the absence of the target sequence, the fluorophore and quencher were close together, preventing fluorescence. The presence of Sur mRNA changed the probe conformation, separating the fluorescent dye from the quencher and triggering a fluorescent signal. Moreover, we have demonstrated the ability to detect mismatches in the oligonucleotide sequence and in *in vitro* studies intra-cytoplasmic survivin mRNA in SW480 cells transfected with SurMB probe using graphene oxide and liposomes as nanocarriers.

In our next study, we have developed a fluorescent "ON-OFF" switching probe for monitoring of ATP level changes [5]. For this purpose, a sensor probe consisting of a single-stranded oligonucleotide aptamer, specific for the ATP molecule (Apt (ATP)), was used. In the absence of ATP, a high fluorescent emission signal ("ON") from FAM dye was observed but it was strongly quenched (signal "OFF") in the presence of ATP due to the strong interactions Apt(ATP) with ATP molecule. Furthermore, the selectivity test of the developed aptamer probe was performed over other nucleoside triphosphates CTP, GTP and UTP.

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APPLICATION OF TWO-PHASE MICROFLUIDIC TECHNOLOGY FOR VISCOSITY CHANGE DETECTION IN BIOLOGICAL MICROLABORATORIES

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For various reactions and applications, it has been shown that droplets in microfluidic devices may serve as miniaturized chemical reactors. [1] Such fully operational microreactors can be created in microfluidic systems at rates of up to several kHz, offering fast mixing with an isochronal control of composition reagents. [1,2] Furthermore, large numbers of droplets serving as microreactors can be efficiently processed, observed and analyzed either in series (one after another) or in parallel. [3] The most straightforward chips are linear, i.e., the droplets flow in a single channel. The primary effect governing droplets' motion in such systems is the additional hydrodynamic resistance introduced by each droplet flowing in a microchannel. [4] Therefore, the presence of aquatic droplets suspended in oil changes the hydrodynamic resistance of the network.

Herein, we present how to calculate the additional hydrodynamic resistance caused by a droplet entering into the microchannel. Moreover, since a single droplet can be treated as a bioreactor, we can use it for biological measurements in which the biological process changes the viscosity of the reaction environment.

Firstly, we demonstrate how the method works on the example of culturing bacteria in a droplet for several hours (Fig.1). [5] That case shows the connection between the population size and the formation of bacterial agglomerates versus the bacterial medium's viscosity change. Secondly, we will demonstrate how the difference in the shape of isolated mitochondria can be detected by screening the viscosity of the mitochondrial suspension in a droplet. In turn, that experiment shows how to study the effects of drugs on mitochondrial response expressed by morphological changes.

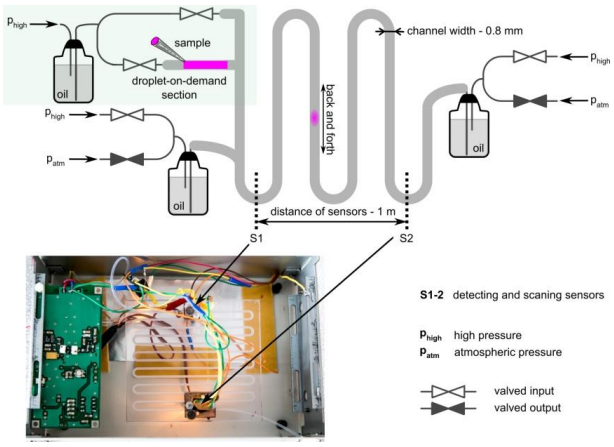

Fig. 1. Schematic of the microfluidic device used in the experiments. The photograph shows the central part of the system with marked sensors.

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ROLE OF DISULFIDE BONDS IN RIBONUCLEASE A

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Ribonucleases are enzymes that catalyze RNA degradation, necessary process for all living organisms. Ribonuclease A from bovine pancreas (RNase A) is the most studied member of ribonucleases and one of the most studied proteins in general[1]. As RNase A needs to form all four disulfide bonds to fold to a native structure, it is used as a model protein for disulfide-bond studies.

Here, we present results of our research, in which we studied formation/disruption of disulfide bonds in RNase A and their influence on protein stability in series of all-atom and coarse-grained molecular dynamics (MD) simulations. The coarse-grained UNRES force field was used to perform simulations, as it is capable of dynamic formation and breaking of disulfide bonds during the course of the simulations, which allows to study influence of disulfide bonds on stabilization of the protein. The reductive/oxidative properties of the environment was controlled by the depth of the disulfide bond formation potential depth. We verified the CG results by running all-atom simulation using AMBER force field with static treatment of disulfide bond and newly-designed pseudo-potential approximating dynamic forming/breaking of disulfide bonds.

Firstly, kinetics and thermodynamics of RNase A unfolding were studied [2] by means of a series of coarse-grained simulation with use of the UNRES force field; namely conventional molecular dynamics (cMD) simulations, run at various temperatures, and multiplexed replica-exchange molecular dynamics (MREMD) [3] simulations in 250-500K temperature range were performed.

Then, additionally, we studied mechanical stability of RNase A using steered molecular dynamics (SMD) by applying additional force to stretch N- and C-termini of the molecule during simulation. Such an approach is a computational equivalent of single-molecule atomic force microscopy (AFM) experiment [4] and allows us to study mechanical properties of the protein in atomistic resolution. SMD simulations were carried out using both all-atom and coarse-grained approaches to allow direct comparison of the data. Due to simplified representation of the polypeptide chains, UNRES force field allows using slower effective pulling speed than in all-atom simulations, which is closer to the one obtained in experimental AFM technique [5]. Both computational and experimental methods are sensitive to the timescale of the experiment as lower pulling speed allows for better equilibration of the system during simulation and may impact the unfolding pathway. Results obtained from SMD simulations were compared with the ones from cMD simulations and available experimental data. [6] This approach allowed us to explain function of each of the disulfide bond and the influence of the redox environment on their behavior.

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PROGRESS IN FLUORESCENT NUCLEOBASE/NUCLEOSIDE ANALOG DEVELOPMENT AND APPLICATIONS

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Recent decade witnessed significant progress in development of fluorescent nucleoside/nucleobase analogs, widely applied in enzymology and cell biology [1, 2]. We were particularly interested in isosteric and/or isomorphic nucleoside analogs, wich usually combine useful spectral properties with biological

activities. Now we briefly review new developments in this field, including those from our laboratory.

Introduction of an "Emissive RNA alphabet", based on thieno-pyrimidine analogs (Figure 1), by Tor and coworkers [3] resulted in many works discussing their rather surprising spectral and enzymatic substrate properties. Recently, this alphabet was extended to involve isothiazole derivatives [4].

Examples of application of this class of compounds to enzyme activity detection and other enzymological research, like RNA depurination, will be given.

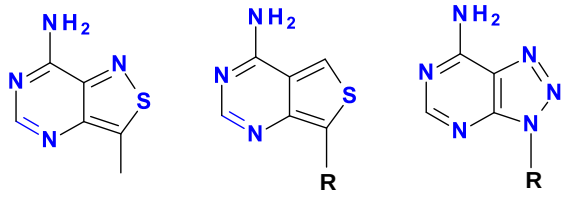

Fig.1. Examples of isosteric nucleoside analogs discussed in this work.

8-azapurines (Figure 1) and their ribosides are another set of fluorescent and isomorphic nucleobase/nucleoside analogs [5]. Their fluorescence was characterized in details in our laboratory, as well as their substrate activities toward several enzymes of purine nucleoside metasbolism. In particular, new non-typical activities of purine-nucleoside phosphorylase towards new analogs, and new methods for quantitation of this enzyme in biological material will be presented.

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NEW INSIGHTS INTO STRUCTURE AND FUNCTION OF PHOTOSYNTHETIC CYTOCHROME B_6F

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Cytochrome b_6f (cytb_6f) is a membrane-embedded homodimeric complex crucial for photosynthetic energy conversion in cyanobacteria, algae and higher plants. Cytb_6f acts on two substrates – membrane soluble plastoquinone and water soluble protein plastocyanin. Reactions of plastoquinone oxidation and plastocyanin reduction catalyzed by cytb_6f provide functional link between photosystem II and photosystem I [1]. We use optical and electron paramagnetic resonance (EPR) spectroscopies to get molecular insights into the operation of cytb_6f.

Overall action of cytb_6f is associated with its structural architecture featuring two redox-active cofactor chains, two catalytic sites (Q_p and Q_n) and cross-membrane electron transfer (ET) fostered by low-spin hemes b_p (hb_p) and b_n (hb_n). This general scheme is shared among all cytochromes *bc*, including bacterial and respiratory cytochromes bc_1 (cytbc_1). A unique feature of cytb_6f is the presence of additional heme (hc_n) at Q_n in proximity to hb_n. The role of this high-spin heme remains elusive. The structural and functional analogy between cytbc_1 and cytb_6f led to a long-standing assumption that relation between midpoint redox potential (E_m) values of hemes *b* in these two enzymes should be the same with E_m of hb_n higher than E_m of hb_p. However, while E_m values of hemes *b* are well established for cytbc_1, uncertainty in determining those values for cytb_6f prevents complete understanding of redox properties of hb_n and hb_p and, subsequently, the energy diagram for the catalytic reaction.

To address this uncertainty, we performed a large-scale equilibrium potentiometric redox titrations of cytb_6f isolated from spinach. All samples were analyzed by cryogenic: i) optical, ii) continuous wave and iii) pulse EPR spectroscopy. Extensive analysis of data obtained for cytb_6f and comparison to data obtained in the same manner for cytbc_1 revealed that in cytb_6f hb_n possesses lower E_m than hb_p. This unexpected result introduces an uphill step in the energy landscape of the enzyme. It follows that the thermodynamic state where both hc_n and hb_n are reduced at the same time is less energetically favorable than state where hc_n and hb_p are reduced. Mechanistic

consequences of this spatial separation of two electrons residing on hemes placed at the opposite sites of the membrane are discussed.

We also reflect on our findings in the context of the structure of plant cytb_6f which provided insights into potential binding mode of plastoquinone at the Q_n site [2]. We expect that a higher-resolution structure with plastoquinones bound at both catalytic sites will further advance our understanding of the mechanism of the catalytic reactions of this enzyme.

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THE INFLUENCE OF CO-APPLICATION OF STATINS AND FLAVONOIDS ON THE ACTIVITY OF VOLTAGE-GATED POTASSIUM CHANNELS kv1.3 AND APOPTOSIS OF Kv1.3 CHANNEL-EXPRESSING CANCER CELLS

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Aim of the study

Influence of co-application of the statins: simvastatin (Sim) and mevastatin (Mev) with each other and with the flavonoids: 8-prenylnaringenin (8-PR), 6 prenylnaringenin (6-PR), xanthohumol (Xanth), acacetin (Ac) and Chrysin (Chr) on the activity of voltage-gated potassium channel Kv1.3 and apoptosis of Kv1.3 channel-expressing cancer T cell line Jurkat. It is postulated that inhibition of these channels expressed in mitochondria (mito Kv1.3 channels) may selectively eliminate Kv1.3 channel-expressing cancer cells, while sparing normal ones. This may open the chance for application of these compounds in a medicinal treatment of some cancer disorders.

Experimental methods

Iinfluence of the compounds on the activity of Kv1.3 channels was examined applying the whole-cell patch-clamp technique. Cell viability upon co-application of the compounds was measured with an application of the MTT Assay. Cell apoptosis was studied by means of measurements of the activity of Caspase-3 and the expression level of Caspase-3 and cleaved Caspase-3, which was estimated applying the Western Blot. Moreover, cell apoptosis was studied by measurements of loss of the mitochondrial membrane potential (MMP).

Results

Co-application of Sim with Mev did not increase the inhibitory effect exerted on Kv1.3 channels upon application of each of the statins alone [1]. On the other hand, co-application of Mev with all the tested flavonoids produced an additive inhibitory effect on the channels. The additive inhibition was also observed upon co-application of Sim with 8-PR, 6-PR and Chr. The most significant inhibition occurred upon co-application of the statins with 8-PR, 6-PR and Chr. Stronger inhibition of the channels occurred upon co-application of Mev with the flavonoids than upon co-application of Sim with these compounds. Application of both statins reduced viability of Jurkat T cells and induced their apoptosis by means of increase of Caspase-3 expression and activity and loss of the MMP. The pro-apoptotic activity was stronger in case of application of Mev than upon application of Sim. Co-application of both statins with all the flavonoids, except for Ac, significantly reduced viability of Jurkat T cells by increasing the pro-apoptotic activity of these compounds. The most significant decrease of the viability, by means of reduction of the concentration of the flavonoid co-applied with the statin, required to diminish the viability to 50% of the control value, occurred upon co-application of Sim with Chr and Mev with 8-PR, Xanth and Chr [1].

Conclusions

1) Co-application of the statins with the flavonoids may produce an additive inhibitory effect on Kv1.3 channels in cancer cells.
2) This effect may be co-related to reduction of viability of Kv1.3 channel-expressing cancer cells due to increase of the pro-apoptotic activity of the compounds.
3) The increased pro-apoptotic activity of the statins co-applied with the flavonoids may be related to inhibition of mito Kv1.3 channels in cancer cells.
4) Obtained results may be important from the point of view of a putative application of inhibitors of Kv1.3

channels to support chemotherapy of cancer disorders, characterized by an up-regulation of Kv1.3 channels, such as melanoma, pancreatic ductal adenocarcinoma or chronic B-type lymphocytic leukemia (B-CLL) [2]. This is because co-application of the statins with the flavonoids may not only improve ant-cancer activity of the compounds, but also reduce potentially required therapeutic dose. This, in turn, may help to avoid unwanted side effects in treated patients due to potential cytotoxicity of the statins [1].

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ENSEMBLE EMPIRICAL MODE DECOMPOSITION OF BK ION CHANNELS SIGNALS

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In the current literature, we can observe some kind of disproportion between the effort put into experimental observations of BK channels and theoretical research. To fully understand the principles underlying these complex microbiological systems, a comprehensive approach, based on both experimental and theoretical results must be implemented.

In this work we decompose the signals which characterize the mitochondrial BK ion channel activity into a finite number of empirical components, using a procedure called *Ensemble Empirical Mode Decomposition (EMD)* pioneered by N. E. Huang et al. [1]. The mode extraction technique allows for a better understanding of the structure of the time series and the complex process hidden behind the data. The EMD technique has been successfully implemented for a wide range of electrophysiological time series. In the case of ionic conductivity signals, this is an innovative approach that allows for the investigation of impact of individual components with different frequency characteristics on the entire signal.

In addition, the received frequency components were carefully analyzed through the methods dedicated to nonlinear and non-stationary signals, including spectral, sample, and multiscale entropy. The investigation of signal complexity employing Entropy measures can ensure a deeper insight into the actual character of the BK channel biosystem [2]. The calculated values of EMD component features were also used for differentiating signals recorded from different types of cell lines (endothelium, fibroblast and hippocampus).

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THE CROSS-CORRELATION-BASED ANALYSIS TO DIGEST THE CONFORMATIONAL DYNAMICS OF THE BK CHANNELS IN TERMS OF THEIR MODULATION BY FLAVONOIDS

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The large conductance voltage- and Ca^{2+}-activated K^+ channels (BK) are regulated by a number of factors. Among them flavonoids, including naringenin (Nar) and quercetin (Que), arise as a promising group of the BK channel modulators from a pharmacological point of view. Although the activating effects of Nar and Que on the BK channel gating are relatively well described in the literature, the appropriate molecular picture of the corresponding channel-ligand interactions remains to be revealed.

In this work, we investigate the functional effects of the Nar and Que on the conformational dynamics of the BK channels. In that aim, we present the results of the cross-correlation-based analysis of the single-channel signals obtained by the patch-clamp method. This allows us

to assess the connectivity and occupancy of distinct conformational substates of the channel in the presence of the considered flavonoids. The obtained results in the form of phase space diagrams allow us to visually monitor the effects of the interactions between the BK channel and Nar or Que at the level of the temporal characteristics of repetitive sequences of channel conformations. The presented results enable us to indicate the ligand-specific effects on channel gating that are related to stronger channel-activation by quercetin than naringenin.

EFFECTS OF STATINS USED IN THE TREATMENT OF HYPERCHOLESTEROLEMIA ON THE OXIDATIVE METABOLISM OF MITOCHONDRIA ISOLATED FROM THE RAT BRAIN

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Atorvastatin and simvastatin are two popular hydrophobic statins that lower cholesterol blood level and are used to treat cardiovascular disease such as hypercholesterolemia. These studies, carried out on the isolated rat brain mitochondria, explain the effects of direct application of these satins on mitochondrial respiratory function, membrane potential and the formation of reactive oxygen species. Both simvastatin and calcium-containing atorvastatin influenced mitochondrial function through increased production of hydrogen peroxide, loss of outer membrane integrity, decrease in maximal respiratory rate, membrane potential, and oxidative phosphorylation efficiency (ADP/O ratio and respiratory control ratio). The action of statins indicates changes in the functioning of the brain's mitochondria by impaired functioning at the level of the respiratory chain, probably in complexes I and III, and at the level of ATP synthesis. The effect of simvastatin appears to be weaker than that of atorvastatin at a given concentration. The stronger effect of atorvastatin on the brain mitochondria was highly dependent on the calcium contained in a given statin and led to the disturbance of mitochondrial calcium homeostasis. The conclusions from this study indicate that hydrophobic statins, widely used as drugs for the treatment of hypercholesterolemia, have a direct negative effect on isolated rat brain mitochondria.

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MONITORING THE MITOCHONDRIAL NETWORK IN SH-SY5Y CELLS UNDER THE INFLUENCE OF POTENTIAL DRUGS IN NEURODEGENERATIVE DISEASES

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The motivation of this work is to explore new effective diagnostic methods in both recognition and therapy of neurodegenerative diseases, as well as to develop a new methodology for studies of drugs with selective activity against Parkinson's and Alzheimer's disorders and utilization of mitochondria as the attractive targets for mitochondrial gene therapy. Mitochondria (MT) are the main intracellular source of crucial biomolecules essential for cellular processes, such as the energy carrier adenosine triphosphate (ATP) and reactive oxygen species (ROS). Mitochondria are also involved in calcium homeostasis and in regulating and initiating cell destructive pathways. The healthy mitochondria form very efficient communicating networks. The morphological abnormalities are indicative of ongoing mitochondrial damage. [1] Therefore, understanding why mitochondrial dysfunction takes a central stage in Parkinson's disease (PD) is integral to combating this debilitating disease. It is the major challenge in developing effective treatments for neurological disorders. Successful therapeutic strategies may halt or slow disease progression instead of merely treating the symptoms.

It has been recognized that the shape and structure of mitochondria can act as functional regulatory factors. At the structural level, MTs are constantly undergoing cycles of fission and fusion. [2] Moreover, alterations in MT dynamics play a crucial part in mitochondrial diseases. [3] Therefore, we anticipate that monitoring MT dynamics (Fig. 1) in response to drugs and disease biomarkers will enable us to understand the role of MTs in various stages of disease development.

Here, we present the development of a system to track changes in the mitochondrial network in SH-SY5Y cells in a microfluidic system, where flow control and drug dosing are carefully controlled (Fig.1). Using Mito Tracker, we show how mitochondrial network dynamics change under the mitochondrial division inhibitor -

Mdivi-1 - a potential drug in neurodegenerative diseases.

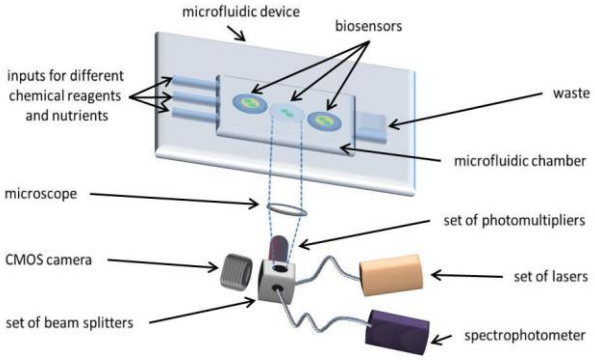

Fig. 1. The scheme of a microfluidic setup with nanogravimetric biosensors to study the response of the cells and mitochondria to chemical stimuli in the microfluidic system (drawn not to scale).

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TIME-RESOLVED FLUORESCENCE SPECTROSCOPY OF BLOOD AND PLASMA IN COVID-19 PATIENTS

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Coronavirus disease 2019 (COVID-19) was proclaimed as a critical global pandemic by the World Health Organization (WHO) in March 2020 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). There is a need to find new diagnostic and prognostic biomarkers that permit reliable and early diagnosis and risk assessment for mortality and developing persistent pulmonary fibrosis in COVID-19 patients. Oxidative stress-induced by neutrophils and hypoxia in COVID-19 pneumonia leads to albumin oxidation damages. The oxidation protein products trigger oxidative bursts of neutrophils and thus participate in “cytokine storms” to accelerate endothelial lung cell injury leading to respiratory distress and failure. This positive feedback cycle can cause the development of a critical condition or even death in some patients. We suggest that oxidized albumin would be involved in COVID-19 pathophysiology. Some possible clinical consequences of the modification of albumin are also discussed.

We present a new method of oxidative stress assessment on the basis of a small amount of blood sample (5 µl) using time-resolved fluorescence spectroscopy. The findings revealed a remarkable reduction in mean fluorescence lifetime of blood and plasma at 360 nm in patients admitted to the hospital when compared with healthy ($p<0.0001$), further decrease during 1-week hospitalization and a return after 6 months to the level of healthy donors. Moreover, significant negative correlations of mean fluorescence lifetimes with inflammatory parameters (CRP, PCT, WBC), surrogate markers of COVID-19 severity (D-dimer, LDH, troponin, AST), radiological score of computed tomography (HRCT) and positive with albumin were observed. A small amount of blood needed for the tests allows for examining the inflammation from the patient's finger without the burdensome drawing of blood.

Extension of collagen deposition in COVID-19 during injured and scar development in the early phase of wound healing may be associated with the risk of mortality. We found a new method to quickly identify the risk of developing pulmonary fibrosis from a sample of plasma on the basis of fluorescence lifetime measurements at 450 nm.

It can be presumed that fluorescence lifetime may be important in the assessment of the severity of pulmonary inflammation and the risk of death in hospitalized patients with COVID-19. Moreover, it can be used to identify individuals with the post-COVID-19 syndrome. Our results are so far the only ones that investigate the fluorescence lifetime of blood or plasma in patients with COVID-19 and pulmonary fibrosis. Although this hypothesis requires further testing, the numerous benefits of using the fluorescence lifetime measurement, encourage consideration of its application in clinical practice.

This method is non-invasive, low time-consuming, and requires only simple pre-treatment of samples without using additional reagents.

HOW IS INFORMATION DECODED IN DEVELOPMENTAL SYSTEMS?

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The development of multicellular organisms is a dynamic process in which cells divide, rearrange, and interpret molecular signals to adopt specific cell fates. Despite the intrinsic stochasticity of cellular events, the cells identify their position within the tissue with striking precision of one cell diameter in fruit fly or three cell diameters in vertebrate spinal cord. How do cells acquire this positional information? How is this information encoded and how do cells decode it to achieve the observed level of cell fate reproducibility? These are fundamental questions in biology that are still poorly understood.

In this talk, I will combine both information theory methods and mechanistic models to address these questions in the context of spinal cord development [1]. I will consider two opposing morphogen signals that are integrated to specify the arrayed pattern of neural progenitor domains that later on give raise to different type of neurons. Based on the maximum likelihood estimation rule I will define decoding map that provides predictions for shifts in the target gene domains in mutants. The predictions will be validated using experimental data obtained from naïve chick neural plate explants and from embryos with altered ventral morphogen signaling. I will present a simple model of a gene regulatory network that integrates the two morphogen signals and is sufficient to recapitulate the observed shifts in the target domains. I will investigate to what extent the level of noise in the input signals affects precision of the resulting gene expression pattern.

Interestingly the underlying interpretation strategy minimizes patterning errors in response to the joint input of noisy opposing signals. In the long-term, the identified principles of information decoding might be utilized in tissue engineering and neuroregenerative therapies.

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MEASUREMENT OF WATER TRANSPORT ACROSS WILD-TYPE AND CYSTIC FIBROSIS BRONCHIAL EPITHELIUM

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Airways are in the direct contact with the external environment and are acting as a physical barrier between external and internal environment of the human body. Lumen side of airway epithelia are lined by Airway Surface Liquid (ASL) which consists of a low viscosity periciliary liquid (PCL) and the mucus layer (ML). The composition, volume and pH of the ASL are key physiological parameters that are related to airway hydration, reactivity and antimicrobial activity and are precisely regulated [1]. Epithelial ion transport processes regulate the volume and composition of the ASL, mainly through modulation of Na^+ absorption and Cl^- secretion. The crucial role of electrolyte transport in maintaining healthy lungs is illustrated by the lung disease in cystic fibrosis (CF). CF is a life limiting disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene which lead to impairment of ion transport processes and result in abnormal ASL volume and composition [2].

Our previous studies focused of multiple ion transport showed, that the introduction of Na^+ or Cl^- gradients across the epithelium causes the flux of isosmotic NaCl. Introduction of ion transporting proteins blockers present in bronchial epithelium considerably affect the volume of transported fluid [3]. To check the water transport, we designed microfluidic platform that enables accurate measurement of its movements across the epithelium. To test the role of CFTR channel in ASL hydration, we performed the experiments on different cell lines: 16HBE14o- (WT) and CFBE41o- (CF).

Our results show that the water flow through the epithelium exposed to ionic gradients but also in symmetric solutions. The difference between water transport across WT and CF cell monolayers was noticed. Higher water fluxes were seen across WT cells when exposed to Cl^- gradients. The transport rate was higher when low Cl^- solution was introduced on the apical side of monolayer indicating the role of CFTR in this process. In Na^+ gradients, higher water transport in CF cells were seen when high Na^+ solution was introduced on apical side demonstrating the role of ENaC channel in epithelial water absorption.

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ENERGY-DRIVEN PROTEIN DISAGGREGATION: UNSCRAMBLING SCRAMBLED EGGS, ONE POLYPEPTIDE AT A TIME

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Irreversible protein aggregation has been linked to many severe diseases and is a widespread obstacle in biochemical research and biotechnology. ClpB, a heat-shock protein from the AAA+ family of ATPases associated with various cellular activities, possesses a unique capability of unraveling aggregates and reactivating aggregated proteins. ClpB cooperates with the Hsp70/Hsp40 system of molecular chaperones, and this protein-disaggregating machinery is essential for survival of bacteria, fungi, and plants under conditions of severe stress, but is absent in metazoans. A lack of human ClpB makes this chaperone an attractive target for the development of novel antimicrobials.

ClpB, like many AAA+ ATPases, dynamically assembles into ring-shaped oligomers containing either six or seven subunits. The ClpB-mediated aggregate resolubilization is linked to forced extraction of single polypeptides from aggregated particles coupled with their ATP-hydrolysis-driven translocation through a narrow channel at the center of the oligomeric ring of ClpB. Recent cryoEM image reconstructions and single-molecule studies reveal how ClpB utilizes energy from ATP hydrolysis to ratchet substrate polypeptides along the central channel.

Escherichia coli and other bacteria produce two isoforms of ClpB: the full-length ClpB95 and the truncated ClpB80 which does not contain the highly mobile substrate-interacting N-terminal domain. We found that hetero-oligomers of ClpB95 and ClpB80 form preferentially at low protein concentration with a higher affinity than homo-oligomers. Normal-mode analysis of structural oscillations in ClpB showed that the mobility of the N-terminal domain is suppressed in the oligomeric ClpB95 and it is enhanced in the ClpB95/ClpB80 hetero-oligomers. Importantly, hetero-association of ClpB95 and ClpB80 boosts the aggregate-reactivation potential of ClpB, which demonstrates that an oligomeric protein machine can achieve its full activity by assembling from two different types of monomers and underscores how a biological function relies on the modulation of stability and dynamics of an oligomeric assembly.

Within a cellular environment, ClpB must distinguish between properly folded and aggregated proteins by recognizing specific physical and/or chemical surface properties of the aggregates. However, the molecular mechanism of substrate binding to ClpB is poorly understood. We hypothesized that ClpB recognizes those polypeptide segments that promote protein aggregation because they are likely present at the surface of growing aggregates. We used an algorithm TANGO to predict the aggregation-prone segments within the model ClpB-binding peptides and investigated interactions of the FITC-labeled peptides with ClpB using fluorescence anisotropy. We found that ClpB binds the substrate-mimicking peptides with positive cooperativity, which is consistent with an allosteric linkage between substrate binding and ClpB oligomerization. The apparent affinity towards ClpB for peptides displaying different predicted aggregation propensities correlates with the peptide length. However, discrete aggregation-prone segments within the peptides are neither sufficient nor necessary for efficient interaction with ClpB. Our results suggest that the substrate recognition mechanism of ClpB may rely on global surface properties of aggregated proteins rather than on local sequence motifs.

ACKNOWLEDGMENTS

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Posters

MOLECULAR DYNAMICS SIMULATIONS OF α-SYNUCLEIN AGGREGATION

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Intrinsically disordered proteins lack a well-defined structure in extended parts of their sequences. Despite the lack of stationary structure under physiological conditions, they play important functional roles in the cell, including signalling, cell-cycle regulation, and initiation of translation. They are often involved in neurodegenerative disorders such as in Alzheimer, Huntington and Parkinson diseases. The toxicity often arises through aggregation into pore-like annular structures and amyloid fibers [1]. Here, we present results of our computational studies on α-synuclein. We performed molecular dynamics simulations within our locally developed coarse-grained Cα-based model [2,3] and an all-atom model with implicit solvent [4]. We discuss the role of transient secondary structure elements and specific contacts in the aggregation process. Moreover, we present the aggregation process dependence on the protein concentration and temperature.

ACKNOWLEDGMENTS

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DOSIMETRY OF FLAT ^{241}Am SOURCE FOR RADIOBIOLOGICAL EXPERIMENTS

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One of the most common cancer treatment methods is radiotherapy with X-ray radiation produced by electron accelerators. Recently high-energy charged particles became an innovative therapeutic option thanks to their physical and radiobiological advantages compared with X-rays. Charged particles like α particles passing through the matter lose energy in atomic and nuclear interaction. Maximum energy transfer and the highest dose deposition occur at the end of the range (Bragg peak, Fig1.)[1]. The dense ionization along the particle track induces different types of DNA damage. Radiation represented by high LET (Linear Energy Transfer) like α particles forms more difficult to repair DNA lesions than low LET radiation (X-rays and electrons) [2]. Irradiation of cell lines with alpha particles is a good utility for research on high LET-induced DNA damages and DNA repair mechanisms.

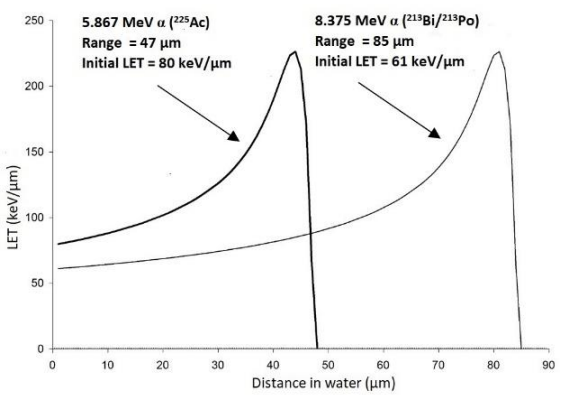

Fig.1. Changes in LET with distance in water (tissue) for 2 α-particles with different initial kinetic energies [3].

At the Heavy Ion Laboratory of the University of Warsaw (HIL), a cell irradiation system was developed in the radiobiology laboratory. This device consists of a flat Am-241 source with activity of 1.96 MBq attached by a mylar film to the inside of a Petri dish lid [4]. Cells are seeded onto 30 mm diameter coverslips and placed in a sterile Petri dish. The cell dish is covered with the top of the α-particle source dish during the irradiation.

The diameter of the active part of the source is 50 mm, and the height is 0.4 µm. The source surface is protected with a 1 µm gold layer. Am-241 emits alpha particles of energies 5388 keV, (1.7%), 5443 keV (13.1%), 5485 keV (84.8%), 5544 keV (0.4%) [5]. The source was covered with a 6 µm thick mylar foil for irradiation purposes. The experimental geometry consists of a 5.8 mm air gap between the source and the biological sample on the coverslip.

Accurate determination of the alpha particle energy loss due to the source and system geometry is crucial in radiobiological experiments. The irradiation time required to obtain the corresponding cellular α radiation doses was estimated from Monte Carlo simulations using the MCNP6.2 code [6]. Particle energy, irradiation geometry, and source activity were considered in the calculations. The simulation results were compared with experimental data.

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PLATINUM NANOPARTICLES WITH CISPLATIN – DIRECT INTERACTIONS AND MUTAGENICITY MODULATION

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Metallic nanoparticles, including platinum nanoparticles (PtNPs), attract much attention in the field of medicine [1]. Significantly, PtNPs can probably act as drug delivery platforms and possess anticancer activity on their own [1,2]. The direct interactions between PtNPs and chemotherapeutics could influence the formation of the stable complexes able to deliver drugs to the cancer cells.

Cisplatin (CDDP), as the most efficient platinum based chemotherapeutic, is widely used in treatment of many cancers including breast, ovarian, head and neck, testicular, or lung cancers [3,4,5]. CDDP acts like an alkylating agent by forming intra- or interstrand crosslinks which disrupt the DNA structure [3,4]. Unfortunately, CDDP triggers many severe side effects such as nephrotoxicity, neurotoxicity or hepatotoxicity and leads to the drug resistance [4,5].

In order to study the possible interactions between 5 nm and 50 nm PtNPs and CDDP and to investigate whether the interactions could influence the biological activity of the tested drug, we exploited various physicochemical methods: Atomic Force Microscopy (AFM), Dynamic Light Scattering (DLS), Infrared Spectroscopy (FTIR), Near Infrared Spectroscopy (NIR); and the biological Ames mutagenicity test on *Salmonella typhimurium* TA102.

The AFM results indicate that the addition of CDDP to both 5 nm and 50 nm PtNPs triggers the particles' aggregation. In case of 5 nm PtNPs, the addition of CDDP does not alter the first peak, corresponding to the smallest hydrodynamic diameter, but change the second peak and increase the polydispersity index (PdI) value to

0.855, which is characteristic for the suspensions with heterogeneity. However, in case of 50 nm PtNPs, the DLS did not revealed any changes in hydrodynamic diameter size or PdI before and after adding the CDDP. What is really important, both FTIR and NIR results confirm the presence of platinum and chloride bond which prove the complex formation. Moreover, the hydrogen bond interactions and the shift of their vibration peak was observed. The results from Ames mutagenicity test revealed that 50 nm PtNPs reduce the mutagenic activity of CDDP in the dose dependent manner. Case of 5 nm PtNPs is more complex, which relates to the DLS results, but shows that the mutagenic activity of CDDP is smaller than the positive control in the whole tested PtNPs range.

In view of the obtained results, we confirmed that the direct interactions between PtNPs and CDDP exist and can influence the biological activity of the tested chemotherapeutic.

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BISPHOSPHONATES AND ENDOTHELIAL CELL OXIDATIVE METABOLISM

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Osteoporosis is one of the most common disorders related to bone metabolism. Bisphosphonates are used as antiresorptive drugs during treatment of osteoporosis. The mechanism of their action is blocking the mevalonate pathway, which inhibits prenylation of key proteins essential for osteoclast resorptive activity. Coenzyme Q is other product of mevalonate pathway, which production is also halted during bisphosphonate administration. Coenzyme Q10 is an integral mitochondrial respiratory chain electron carrier and an important antioxidant in the cell. Endothelial cells are the lining of the entire vascular system and they are the first cells in contact with intravenously administered drugs. Endothelial dysfunction associated with mitochondrial impairment can cause cardiovascular disease. We studied the effect of chronic exposure (6-day) to two nitrogenous bisphosphonates, zoledronate and alendronate, on respiratory function of human umbilical vein endothelial cells (EA.hy926 cell line). Our results indicate that 6-day exposure to this drugs in higher concentrations induced a significant lowering of coenzyme Q10 level and cell viability. Both bisphosphonates led to an increase in mitochondria biogenesis and respiratory capacity when compared to control cells. Additionally, incubation with bisphosphonates led to increase in ROS production. However, further studies are needed to understand the role of endothelial mitochondria in metabolic adaptations associated with chronic exposure of endothelial cells to bisphosphonates.

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NASCENT FOLDING OF PROTEINS ACROSS THE THREE DOMAINS OF LIFE

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We study the nascent behavior of three model coarse-grained proteins in six rigid all-atom structures representing ribosomes that come from three domains of life. The synthesis of the proteins is implemented as a growth process [1]. The geometry of the exit tunnel is quantified and shown to differ between the domains of life: both in volume and the size of constriction sites [2]. This results in different characteristic times of capture within the tunnel and various probabilities of the escape. One of the proteins studied is the bacterial YibK which is knotted in its native state [3]. A fraction of the trajectories results in knotting and the probability of doing so is largest for the bacterial ribosomes. Relaxing the condition of the rigidness of the ribosomes should

result in a better avoidance of trapping and better proper folding.

ACKNOWLEDGMENTS

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INTERACTIONS OF PURINE BASE ANALOGS WITH ADENOSINE DEAMINASE AND PURINE 2'-DEOXYRIBOSYLTRANSFERASE – SPECTROSCOPIC STUDIES

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Biocatalytic procedures, utilizing enzymes of nucleoside metabolism, serve as alternative methods to the chemical synthesis of nucleosides and nucleoside analogs, including those possessing antiviral, antitumor or antibacterial activities. Currently, many such analogs have found applications in the treatment of viral infections such as the herpes simplex virus or the hepatitis B / C virus, or in the teratment of lymphomas [1].

Among the most frequently used biocatalysts are catabolic enzymes such as adenosine deaminase (ADA) [2] and protozoan purine 2′-deoxyribosyltransferases (LmPDT) [3]. In the present poster, we summarize our investigastion on activities of these two enzymes against variuos nucleoside anlogs of potential pharmaceutical applications. Spectroscopic and chromatographic (HPLC) methods were utilized in kinetic measurements.

Adenosine deaminase catalyzes the irreversible deamination of adenosine and deoxyadenosine [2]. It is also active towards many adenosine derivatives, including 6-chloro- and 6-methoxypurine ribosides [2]. Also 2-aminoadenosine and 2-amino-6-chloropurine riboside are good substrates. We have also examined some 8-azapurines, in particular ribosides of a strongly fluorescent 2,6-diamino-8-azapurine (DaaPu) [4]. We found that 9-β-D-riboside of DaaPu is a moderately good substrate for ADA. The etheno- derivative of adenosine – 1,N^6-etheno-2-aza-adenosine turned out to be a non-typical adenosine deaminase inhibitor.

Purine 2′-deoxyribosyltransferase catalyzes the transfer of deoxyriboside from 2′-deoxyribosylnucleoside to a purine free base [3]. We have examined substrate properties of ther fluorescent etheno-derivatives of purine bases as substrates of 2'-deoxyribosyltransferase isolated from *Leshmania Mexicana* [5]. Preliminary studies using HPLC separation of substrates and products suggested that 1,N^6-etheno-adenosine may be a moderatly good substrate for this enzyme, but this finding must be confirmed. No efficient transfer of deoxiribose moiety to other fluorescent etheno-purines was recorded.

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MULTIPLE ENVIRONMENTAL STRESSORS EFFECT ON OXIDATIVE STRESS IN *SPODOPTERA EXIGUAE* LARVAE FROM CONTROL AND CADMIUM STRAIN

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Individuals from populations from differently contaminated area may differ in coping with prooxidant stressors. Examinations, provided for years at Silesian University, on control and cadmium (selected through many generations) strains of *S. exigua* revealed differences in sensitivity to metal and other stressors [1]. In the case of severe pest *S. exigua*; the question how different strains larvae cope with multiple stresses (lower temperature, zinc, spinosad) is important.

This study compared spectrophotometric and spectrofluorimetric assays of: H_2O_2 concentration and TAC (according to [3], [4], [5], [6], respectively) in the 5^{th} stage larvae. The larvae reared on semisynthetic diet, with or without Cd supplementation (44mg/kg dry weight of diet) in 25°C, at 16L:8D and treated during 3 days with 20°C, spinosad (2mg/kg of dry weight of diet) or Zn (200mg/kg of dry weight of diet).

Both methods (spectrophotometric and spectrofluorimetric) revealed similar tendencies, especially lowering of TAC in control strain larvae exposed to Cd and rearing in 20°C, enhancement of TAC after spinosad exposure in Cd strain animals, and after Zn and multiple stressors exposure in both strains individuals. H_2O_2 concentration was enhanced in Cd strain animals reared in 20°C. Lower amounts of H_2O_2 was measured in control animals exposed to Cd and Zn or to all stressors. Lowering TAC was accompanied with decrease of H_2O_2 concentration – in 20°C and cadmium exposure, but only in the case of control animals.

Summarising, main differences between from both strains animals was observed under cadmium and spinosad action.

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THE ROLE OF THE MITOCHONDRIAL BK_{CA} CHANNEL IN DAMAGE OF THE BRONCHIAL EPITHELIAL CELLS CAUSED BY PARTICULATE MATTER

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The main place of deposition of particulate matter (PM) is the epithelium of the respiratory tract. In contact with cells, PM causes an alteration in reactive oxygen species (ROS) levels, leading to inflammatory responses. Moreover PM can damage the mitochondria of epithelial cells, thus inducing cell death. Recently, it has been shown that potassium channels (mitoK) located in the inner mitochondrial membrane are involved in the cytoprotection [1,2,3]. What is more, activation of mitoK channels influences the synthesis of ROS, which may be a key mechanism of cytoprotection. Therefore, it appears that the protection of epithelial cells from PM-induced damage may be related to the activation of potassium channels present in the inner mitochondrial membrane.

To verify the role of mitochondrial large-conductance Ca^{2+}-regulated potassium (mitoBK_{Ca}) channel in cytoprotection in response to stress induced by particulate matter, we performed a series of experiments using patch clamp and oxygen consumption measurement. In the human bronchiolar lung epithelial cell damage model (16HBE14o

SCC150), particulate matter < 4 μm in diameter (SRM-PM4.0) were used (PM4.0).

Earlier, it has been reported that mitoBK$_{Ca}$ channel is present in the inner mitochondrial membrane of epithelial cells [4]. In the current work, using the patch-clamp technique, we have shown that 10 μM quercetin activates the mitoBK$_{Ca}$ channel present in the inner mitochondrial membrane of HBE cells and 300 nM penitrem A abolishes this effect. Additionally, we have shown that 50 μg/ml PM4.0 regulates mitoBK$_{Ca}$ channel activity.

In order to examine the potential role of the quercetin and penitrem A on epithelial cells, measurements of cellular respiration and membrane potential were performed with the use of O2k-technology for high-resolution respirometry in mitochondrial and cell research. We have shown that quercetin at a concentration of 10 μM increased the respiratory rate and depolarizing the mitochondrial membrane. These effects were dependent on the presence of 300 nM penitrem A – an inhibitor of mitoBK$_{Ca}$ channel.

To support our data, we used an analog of quercetin - isorhamnetin, a substance which has one hydroxyl group changed to a methoxyl group. Isorhamnetin has no effect on the mitoBK$_{Ca}$ channel activity, oxygen consumption rate and mitochondrial membrane potential after its application.

A better understanding of the relationship between mitochondrial metabolism and cell physiology could aid the search for effective cytoprotection strategies. Perhaps, by using naturally-derived mitochondrial BK$_{Ca}$ channel activators, we will learn to support and induce these mechanisms to counteract the consequences of PM-induced damage.

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INSIGHTS INTO ACID-BASE PROPERTIES OF THIAMINE AND IT'S PHOSPHATE DERIVATIVES BY SPECTROSCOPIC AND *AB INITIO* STUDIES

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Thiamine (Th) is a water soluble B1 vitamin essential to metabolic processes occurring in living organisms. The molecule consists of the substituted pyrimidine ring connected *via* the methylene bridge to substituted thiazole ring with hydroxyethyl group attached. In nature, there exist phosphorylated derivatives of the vitamin B1 containing one, two or three phosphate groups instead of the hydroxyl group (ThMP, ThDP and ThTP, respectively). The biologically active form is thiamine diphosphate serving as a cofactor for a group of enzymes involved in carbohydrate metabolism. In acidic conditions, thiamine has two positive charges – one on the nitrogen of pyrimidine ring and one on thiazole ring. The pyrimidine moiety undergoes deprotonation upon increasing pH to become singly charged cation at physiological conditions. In alkaline solution, thiazole ring is hydrolised, which leads to the ring opening and formation of a negatively charged thiol [1]. Both the ionic equilibria and the tautomeric states coupled with the protonation shift have the biological importance [2].

The changes in the protonation state are reflected in both the near UV absorption and emission spectra. The equilibrium acidic dissociation constants determined by spectroscopic methods demonstrated that the pK_a value of the pyrimidine ring increases with the increasing number of phosphate groups attached to the distant ethyl chain of the thiazole moiety. This effect is similar to the pK_a changes of N(1)-H of the 7-methylguanosine moiety of the chemical mRNA 5’ cap analogues, where the heterocyclic m^7G base ring that contains the dissociating proton is also separated from the 5’ phosphate groups by the ribose ring [3]. Moreover and contrary to the cap analogues, the thiamine pK_a values in the electronic excited state are significantly higher than those in the ground state. Such a phenomenon was previously found for heterocyclic photobases [4].

To elucidate these relationships for Th and it's phosphate derivatives, *ab initio* computational studies have been performed by using the method based on Møller-Plesset (MP2) perturbation theory [5] for determination of the ground electronic state forms, and the ADC(2) method [6-8] for modelling of the excited state energy landscape. Excited-state calculations might be also helpful in understanding the fluorescence decay mechanism in Th.

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FLUORESCENT SILVER NANOCLUSTERS ON DNA TEMPLATE WITH TWO DOMAINS

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Silver nanoclusters (AgNCs) have attracted special attention due to their facile synthesis, tunable fluorescence emission, and high photostability. [1-3] To prevent aggregation of silver nanoclusters and their oxidation is required a stabilizing scaffold (matrix). [4] Due to the strong interactions of silver cations with bases and DNA phosphate groups, it is possible to design and manufacture DNA-based silver nanoclusters (DNA-Ag NCs). In particular, Ag^+ ions show a strong binding affinity to cytosine bases (C), forming the C-Ag^+-C complex. [5]

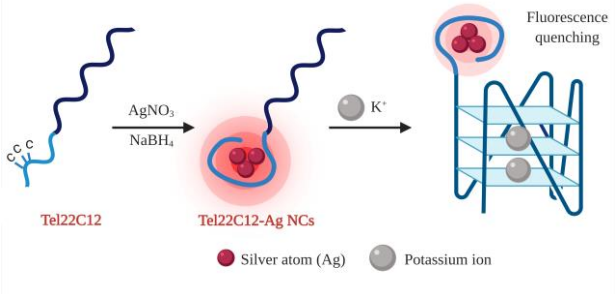

Figure 1. The demonstrative illustration shows the working idea of Tel22C12-AgNCs as potassium sensor [6].

In our previous work, we obtained highly fluorescent AgNCs on DNA template consisting of cytosine-rich (C12) domain integrated with a G-quadruplex DNA. The main idea of such constructed DNA template is that C-rich domain is mainly responsible for nanoclusters formation and serves as the fluorescent tag, whereas G-rich DNA able to forms G-quadruplex serves as a receptor layout for potassium ions. [6] Our studies indicated that the competitive formation of G-quadruplex structure as a result of the binding K+ or Na+ ions has a significant impact on the emission properties of silver nanoclusters and such probe is able to monitor small changes in K+ concentration in the extracellular conditions [6].

The goal of the presented study was to compare properties of DNA-AgNCs synthesized on two oligonucleotides: Tel22C12 and C12Tel22 (Table 1). The studies systems differ in attachment of C12 sequence: Tel22C12 has C-rich domain at 5' end, whereas C12Tel22 at 3' end. We used UV, fluorescence and CD spectroscopy techniques to monitor silver nanocluster formation and to perform spectral characterization of obtained Tel22C12-AgNCs and C12Tel22-AgNCs.

Table 1. The DNA oligonucleotide sequences used in this research.

Name	Oligonucleotide Sequence
Tel22C12	5'-AGG GTT AGG GTT AGG GTT AGG G CC CCC CCC CCC C-3'
C12Tel22	5'-CCC CCC CCC CCC AGG GTT AGG GTT AGG GTT AGG G-3'

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CARBON NANOTUBES AS A PLATFORM FOR OTHER NANOMATERIALS CONTROLLED JUNCTION

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Nanomaterials are a huge and divergent group, which is defined basically by their size, smaller than 100 nm in at least one of their dimensions. The nanomaterials' properties differ from the characteristics of bulk materials. Some nanomaterials are fluorescent as well as redox-active (as semiconductor quantum dots, QDs)[1, 2], some are absorption enhancers due to plasmonic effects (metallic nanoparticles) and there are carbon nanomaterials, which are none of the above. Precisely, carbon nanomaterials might quench fluorescence, act as a final acceptor for electrons as well as just be a neutral platform for the precise and controlled junction of other nanostructures.

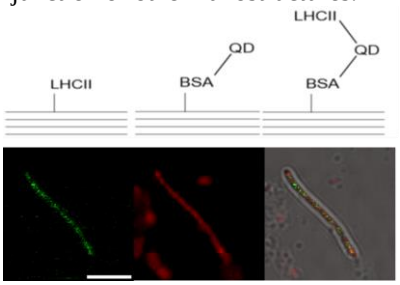

Fig. 1. Scheme of some possible junction versions (upper panel) and (lower panel) an example of a confocal microscope image of a nanotube decorated with QD (green emission, 520-587 nm) and LHCII (red emission, 625-715 nm). Excitation was set at 471 nm. Last image is the overlay of green and red channel with transmission. Scale bar - 5 μm.

Here we explored the last option, decorating carbon nanotubes with cadmium telluride QDs and three types of photosynthetic antennae: light-harvesting complex II (LHCII), phycobilisomes (PBS) and Zn-mesoporhyrine complex with de novo designed alpha-helix bundle, HP49. We optimized the junction to minimize energy stilling by carbon nanotubes, and we observe energy transfer between active components of the nanohybrid. Such constructs can be further improved by other elements, such as enzymes, to serve as biosensors or light-controlled triggers of cell metabolism.

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EFFECTS OF THE AIR PARTICULATE MATTER ON ELECTRICAL PROPERTIES OF THE BIOLOGICAL MEMBRANES

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Urbanized areas, due to the presence of many different emitters of pollutants, are characterized by a deteriorated quality of the atmospheric air, which has an impact on the health of people living there. Out of many types of pollutants, suspended substances in the form of particulate matter (PM) play an important role. In the cells of living organisms, an important role is played by the cell membrane, which protects the inside of the cells against the entry of undesirable substances, and is also a place of interaction with the external environment. Therefore, it is so important to understand how PM interacts with cell membranes.

To verify the influence of PM on electrical properties of biological membranes, we performed a series of experiments using electrophysiological technique like black lipid membrane (BLM) [1]. L-α-Phosphatidylcholine from soybean (azolectin) was used for creating model biological membranes. Additionally, the National Institute of Standards and Technology (USA) samples of particulate matter <4 μm and <10 μm

in diameter (SRM-PM4.0 and SRM-PM10) were used.
In our work, BLM method is based on creating an artificial lipid bilayer on a hole with a diameter of 250 μm separating two compartments filled with solutions, 50 mM KCl on the cis side and 150 mM KCl on the trans side. Then the ionic current flow between two solutions separated by a azolectin membrane are recorded. Parallel, it is also possible to measure changes in the electric capacitance of the membrane.
Proteins such as channels can be incorporated into bilayers, making this technique suitable for mimicking cell membranes [2,3]. In the research work, azolectin membranes were used with and without an artificial channel protein – gramicidin A (5 ng/ml) [4] and particulate matter of two sizes, <4 and <10 micrometers in diameter. Interesting results have been obtained showing that PM in range from 10 to 150 μg/ml reduces the basal ionic current, affecting the lipids that make up the membrane, and that they decrease the channel activity of gramicidin A. The particulates did not change the electric capacity to any significant extent. Additionally, in the study, the epithelial cell culture (HBE) was used to illustrate the toxicity of the PM. We have shown that the PM adversely affects cells, causing morphological changes and increased mortality.
Understanding interaction between PM and biological membranes could aid in the search for effective cytoprotection strategies. Perhaps, by using artificial system, we will learn to support consequences of PM-induced damage.

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THE ROLE OF CFTR IN THE IMPAIRMENT OF HUMAN BRONCHIAL EPITHELIAL CELLS INDUCED BY PARTICULATE MATTER

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Cystic fibrosis (CF) is a genetic disease which affects at least 100 000 people worldwide. The disease is caused by various mutations in gene encoding the CFTR (cystic fibrosis transmembrane conductance regulator) leading to defective protein production, defects in CFTR processing, impaired channel regulation, reduced channel conductance, reduced number of channels or channel stability. The CFTR mutations result in malfunction of many secretory tissues and organs such as airway epithelia, sweet glands, the pancreas and the gastrointestinal tract. However, the CFTR channels are also found in non-epithelial cells such as blood, heart and the brain. The disease leads to difficulty in breathing, frequent lung infections, poor growth and infertility in most males, among others [1].
It was observed that CF patients are at a special risk from air pollution. The environmental exposure of particulate matter (PM) was associated with increased risk of pulmonary exacerbations and a decline in lung function [2]. However, the mechanism by which CF patients are more susceptible to the toxic effects of PM, remains still unclear. Here we investigated the impairment of CF cell function induced by PM administration.
In our study, two different cell lines were investigated. The control cell line was HBE (human bronchial epithelial cell line) with functional CFTR channel. The model for CF was CFBE cell line (human cystic fibrosis bronchial epithelial cell line) with ΔF508 CFTR mutation, which is the most frequent (~66%) type of mutation in CF patients. PM used in the experiments was standard reference material <4um diameter, obtained from NIST. To assess the toxicity of PM, MTT cell viability assay was conducted, with the use of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. Alongside, the cell viability assay using trypan blue staining was performed. To elucidate the potential mechanism for the toxic effects of PM, the reactive oxygen species (ROS) level was determined using fluorescent probe- DCFDA (2',7– 'dichlorofluorescin diacetate).
The results show that there is a significant difference in cell viability of HBE and CFBE cells, upon treatment with different concentrations of PM. It was also discovered that basal ROS level of untreated with PM cells was higher in CF cell line compared to control HBE cells. Additionally, PM induced higher ROS production in CF cells.

In conclusion, we confirmed higher susceptibility of CF cells to PM induced toxicity on cellular level. The effect may be correlated with ROS overproduction. We are committed to elucidate the mechanism further and take into consideration other cell signaling pathways.

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SH-SY5Y CELL LINE AS A MODEL FOR MICROFLUIDIC STUDIES OF NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases (NDDs) describe a group of progressive central nervous system disorders associated with dysfunction and gradual loss of neurons [1]. Alzheimer's disease (AD) and Parkinson's disease (PD) are the most common representatives affecting approximately 55 million people worldwide. Both disorders are characterised by poor early detection and the lack of an effective treatment to reverse or even stop their progression.

Although NDDs are typically associated with the accumulation of abnormal proteins (beta-amyloid and tau for AD and alpha-synuclein for PD), mitochondrial dysfunction is also one of the pathophysiological features contributing to the course of disease [1, 2, 3, 4].

Here we present the SH-SY5Y human neuroblastoma cell line as a model system to mimic NDDs. This cell line exhibits several features that make it a good cellular model for such research [5]:

1. It is of human origin,
2. It can be differentiated to receive neuronal-like cells of a well-defined (e.g. dopaminergic) phenotype by using selected differentiating agents, such as retinoic acid (RA), 12-O-tetradecanoylphorbol-13-acetate (TPA), or cholesterol,
3. It can be used to study PD and AD by using different toxins: 6-OHDA or MPP+ to induce parkinsonian phenotype and streptozotocin for Alzheimer's phenotype.

We monitor the growth of SH-SY5Y cells in different conditions such as medium composition, the concentration of cells during seeding, cell culture substrate material (polystyrene, glass, polycarbonate), or the surface area available for the cells. In addition, we cultivated SH-SY5Y cells on standard 60 mm Petri dishes in a typical cell incubator or on self-projected polycarbonate micro-chambers. SH-SY5Y cells were also differentiated with RA to receive cells with mature neuronal characteristics. Exemplary phase-contrast microscopy images of undifferentiated and RA-differentiated SH-SY5Y cells are shown in Fig. 1.

All the tests were performed to optimize cell culture conditions for the real-time microscopic observation of cells' growth in a microfluidic system and subsequent studies of mitochondria dynamics in NDDs.

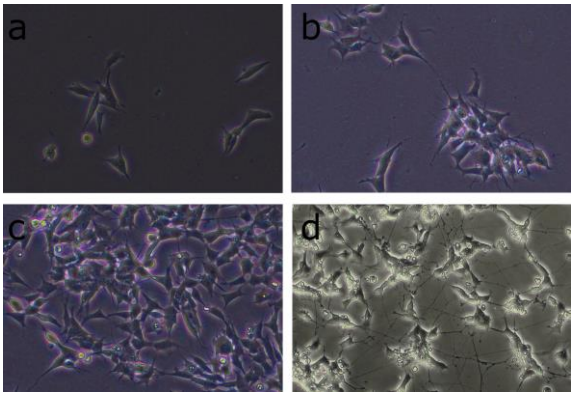

Fig.1. Phase contrast microscopy images of undifferentiated SH-SY5Y cells after 1 (a), 3 (b) and 7 (c) days of culture growth. Cells were seeded with concentration $5x10^4$ cells/ml and cultivated on standard 60 mm PS culture plates. RA-differentiated SH-SY5Y cells after 8 days of RA-treatment (d).

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NANOSTRUCTURE OF THE HYPERTONIC SOLUTION IS A KEY PARAMETER IN OSMOTIC SHOCK-MEDIATED INTRACELLULAR DELIVERY

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The majority of cell biology studies involve delivering various cargos types inside cells. Thus, an efficient method for crossing the cell membrane barrier is required. One of such techniques is the application of osmotic shock. Its principle is based on water flow from a lower osmotic pressure (hypotonic) solution to the one of higher osmotic pressure (hypertonic) through a cell membrane. The hypertonic medium consists of an osmotic pressure-building polymer. The matter in osmotic shock-mediated cellular delivery is the composition of the hypertonic solution.

We checked how the size and the concentration of osmotic polymer affect the effectiveness of intracellular delivery. The effectiveness of each tested polymeric solution was validated using fluorescence correlation spectroscopy FCS to precisely identify the fluorescent cargo inside cells based on the diffusion time. FCS measurements were supported with confocal imaging.

Some of the tested polymeric variants were effective, and some were ineffective. We investigated the nanogeometry of hypertonic solutions to find a key parameter for the success of cellular delivery. The entanglement boundary concentration was calculated for all tested variants using the following equation [1]:

$$R_H = R_g \left(\frac{\frac{4}{3}\pi C R_g{}^3 N_A}{M_w} \right)^{-\beta} \quad (1)$$

Where R_H is the hydrodynamic radius of the polymer, R_g stands for the gyration radius of the polymer, c is for boundary entanglement concentration, β corresponds to Flory's exponent.

A determinant for the osmotic shock-mediated delivery turned out to be the use of polymer (PEG, dextran, or Ficoll) in the entanglement regime (Fig.1).

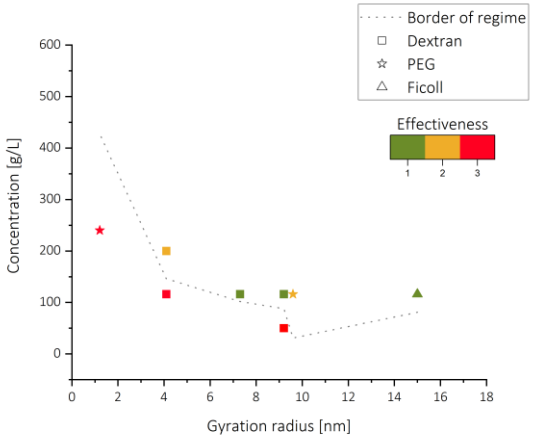

Fig.1. Dependence of polymer concentration on its gyration radius. The use of the polymer in the entangled regime was critical for the efficiency of the cellular delivery process [2].

In summary, we found that the effectiveness of the osmotic shock-mediated cellular delivery strongly depends on the size and concertation of the polymer that builds osmotic pressure. We also showed that high osmotic pressure, built up by the addition of sucrose, is an insufficient parameter. Based on our studies, we proposed the mechanism of pinocytic vesicle swelling in a hypotonic environment [2].

As a result of our research, we have developed a loading reagent called Cell-IN (https://cell-in.eu).

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SPECTROSCOPIC EVIDENCE FOR FORMATION OF COMPLEXES SYNAPIC ACID METHYL ESTERS WITH SERUM ALBUMIN

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The Methyl ester of sinapic acid (MESA) is a molecule with confirmed antioxidant properties and therefore it is important to establish whether it may be transported across a human and an animal organism. For this reason, we investigated MESA interactions with serum albumins: human (HSA), bovine (BSA), rabbit (RSA), and sheep (SSA). Using absorption and fluorescence experiments performed in pH range from 5.9 to 10.7, it was found that MESA formed complexes with every albumin in every checked pH. It was manifested by the appearing new absorption and fluorescence complex bands. Fluorescence intensities were much greater up to 20 times and lifetimes up to 340 times compared to unbound MESA. Results suggested that MESA preferred the hydrophobic binding sites in albumin. The quenching experiments at pH 7.4 showed that the stoichiometry for every albumin was 1 : 1; the binding constant was the highest for HSA, which reached 52 000 M^{-1}. All these results confirmed that MESA can be transported by albumins.

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CALCIUM-MEDIATED AGGREGATION OF INTRINSICALLY DISORDERED POLYANIONIC PROTEINS INVOLVED IN EARLY STAGES OF CORAL BIOMINERALIZATION

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Global warming can affect a wide variety of ecosystems, including marine life. To be able to predict the forthcoming consequences of climate changes, one has to fully understand the molecular basis underlying vital processes governing the development and survival of marine organisms [1]. One of such processes is the formation of coral skeleton.

It was postulated that coral acid-rich proteins (CARPs) secreted to the extracellular space play a significant role in biomineralization. So far, four CARPs have been cloned and partially characterized [2]. Studies show that CARPs change the morphology of calcium carbonate crystals and induce crystallization of aragonite under conditions which do not enable spontaneous precipitation [2].

However, still open questions are (1) what is the molecular mechanism of CARP-dependent microcrystal growth regulation and (2) what phases are formed prior to protein-mediated calcium carbonate crystals [3].

We have cloned for the first time two novel CARPs: secreted acidic protein 1A (SAP 1A) and aspartic and glutamic acid-rich protein (AGARP) of *Acropora millepora* coral species [4]. By means of fluorescence-based techniques like *e.g.* fluorescence correlation spectroscopy, we aim to elucidate what happens at the very beginning of the crystal formation, *i.e.* at the crystal nucleation step.

We observed the process of polyanionic polymers-Ca^{2+} cations aggregation *via* the changes in the autocorrelation curves over time in the range of minutes. Incubation of calcium with fluorescently labeled CARPs has led to the formation of fluorescent microcrystals, which suggests that the proteins not only shape the morphology of crystals but are also present within them.

Our results show that the CARPs-Ca^{2+} aggregates may constitute one of the first steps leading to coral biomineralization.

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THE IMPORTANCE OF LONG-TERM MEASUREMENTS IN GFP FOLDING STUDIES

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Studies on protein folding are of great significance, especially in the context of understanding processes lying under diseases related to protein misfolding and aggregation like type II diabetes or Alzheimer's disease.[1] Protein folding usually takes milliseconds to seconds for small single-domain proteins. Exceptions are molecules, which folding includes proline isomerization or other specific issues resulting in slowdown of folding.[2] One of the slow-folding species is green fluorescent protein containing 10 proline residues, which only one is in *cis* configuration. [3]
Green fluorescent protein (GFP) is a β-barell protein bearing unique chromophore and with its colorful mutants became extensively used biological marker. [4] Because of wide range of application it is important to understand GFP folding and tendency to aggregation.
We performed pH-jump experiments of EGFP (F64L/S65T-GFP) folding and aggregation using stopped-flow technique for short-term (1 000 s) and spectrofluorometer for long-term (24 h) measurements. We compared two processes: refolding and *de novo* folding for protein with and without chromophore, respectively.
Both processes have multi-stage character with the rate of folding and aggregation depending on the presence of chromophore. Thus, it is important to consider these properties of GFP in experiments using it as a marker.

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TYROSINE-MODIFIED POLYETHYLENEIMINES AS PROMISSING siRNA CARRIERS IN LUNG CANCER TERAPY

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Introduction: In recent years, therapies based on RNA interference were extensively studied. RNAi system allow to target any gene of interest what is particularly

important for undrugable diseases. The main concern regarding siRNA therapies is finding a proper delivery vehicle for nucleic acids. Polyethyleneimines (PEI) are a class of synthetic, cationic nanovectors which have found an application in nucleic acid (especially plasmid) delivery. Introducing of tyrosine modification improved their properties for siRNA delivery. The aim of the study was to evaluate the toxicity and efficacy of PEI:siRNA complexes in lung cancer model.
Methodology: The toxicity and gene knockdown efficacy were studied on lung adenocarcinoma cell line (A 549). Cytotoxicity (MTT assay), evaluation of ROS level with H2DCFDA, changes in mitochondrial membrane potential (MMP) using JC - 1 dye, apoptosis/necrosis induction (flow cytometry, caspase induction) were checked in order to evaluate toxic effect of nanoparticles. Gene knockdown efficacy was checked in different models, including normal non-stressed cells and stressing conditions (elevated level of ROS and acidification) and confirmed using qPCR.
Results and conclusion: Complexes of siRNA and tyrosine modified PEIs revealed some cytotoxicity (> 30%), when linear PEIs were used in the complex. Branched PEIs in the complex with siRNA caused very slight decrease in cell viability (<10%). Complexes slightly induced the formation of reactive oxygen species (< 10%) in A 549 cell line and the decrease (ca. 15%) of MMP after 24 hours incubation. Annexin V/ propidium iodide staining revealed that the level of apoptotic/necrotic cells was very low (< than 5%). Caspase 3/7 induction assay confirmed that the level of caspase activity in proliferating cells was comparable to the control, untreated cells. Gene knockdown efficacy was high (> 70%) in non-stressed cells and cells stressed with hydrogen peroxide. Knockdown efficacy in acidic conditions was lower, around 50%. All given results indicate, that tyrosine modified polymers may serve as non-toxic siRNA nanocarriers with a very good knockdown efficacy, even in stressed conditions.

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EXPLORING THE PARAMETER SPACE, CONDITIONS FOR TURING PATTERNS

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How did the leopard get its spots? An innocently simple question about pattern formation which lies at the heart of the field of developmental biology. The main goal of this field is to understand how a living organism develops from a single mother cell. What are the mechanisms that determine the fate of each cell, as well as, how they manage to self-organize to an incredible level of precision despite the noisy environment [1]. In 1952 the famous mathematician Alan Turing [2] was the first one who addressed the question of pattern formation by means of differential equations. He began from the assumption that, during the embryonic stage, the genes of daughter cells are activated by the concentration of certain chemicals he called morphogens. Then, he derived a set of equations governing the dynamics of two morphogens. He was also the first to propose that diffusion may drive a system out of stability into forming distinct periodic patterns. Before him, diffusion was seen as a stabilizing process that forced the system into stabilizing [3]. Now a days his equations are recognized as particular cases of a more general family known as reaction-diffusion equations

$$\frac{\partial}{\partial t} c = f(c) + D \nabla^2 c \qquad (1)$$

where c is the vector of morphogen concentration, f represents the reaction kinetics, D is a diagonal matrix of diffusion constants, and ∇^2 is the Laplacian (usually taken over one, two or three dimensions). It is usually assumed that there are no other morphogens responsible for the pattern formation. This naturally implies the zero flux boundary conditions

$$(n \cdot \nabla) c = 0 \quad \text{for all} \quad r \in \partial B \qquad (2)$$

with ∂B a closed boundary of the reaction-diffusion domain B, n is the normal vector normal to ∂B [4].
Amazingly the reaction-diffusion equations accurately predict the formation of digits, the spots on leopards, stripes on tigers and zebra fishes, spacing of teeth in alligators, among many others. Despite their success and the seven decades of intensive research, the conditions on which such patterns appear are only completely understood in the case of two morphogens. Very recently

a classification in the case of three morphogens was found in terms of topology of biological circuits [5]. In our poster we would like to present the results of current work based on linear algebra and properties of polynomials. We show how to obtain the necessary and sufficient conditions to obtain diffusion driven (Turing) instabilities. We also show, how the constraints have a recursive structure as we increase the number of chemical species. This means that the conditions that lead to Turing patterns with *n* morphogens still hold for *n + 1* morphogens. The difference is that for *n + 1* cases, there are additional regions of instability and with their respective constraints.

Our methods work for any number of morphogens. Nevertheless, as expected, the conditions for instability get progressively more complicated so we limit ourselves up to five morphogen species. We then apply our conditions for a few models of interest to verify their validity and find their full Turing space (regions leading to Turing patterns).

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1,8-DIAZAFLUOREN-9-ONE – A NEW CHALLENGER IN OPTICAL AND NON-FARADIC IMPEDIMETRIC SENSING

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We introduce new spectroscopic and non-faradic impedimetric properties of 1,8-diazafluoren-9-one (DFO) in various environments with an emphasis on rigid matrices. With its selective reactivity towards α- amino acids, 1,8-diazafluoren-9-one (DFO) is commonly used for friction ridge analysis. However, its application potential is much more extensive. The characterization of its unique spectroscopic properties has been the focus of our research group since 2019 [1]. As part of the present work, we identify its significant applicability during optical alpha sensing of amino acids – figure 1. It seems crucial to consider its additional forms - aggregates in the excited state - during optical analyses [2,3,4,5].

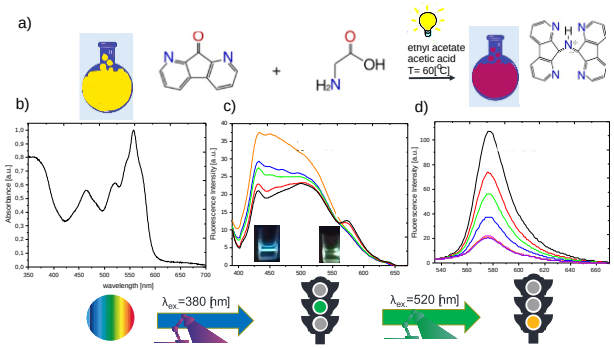

Fig.1. The reaction of DFO with glycine-amino acid (a) and the absorption spectra (b), fluorescence spectra, λex. = 380 nm (c), and fluorescence spectra, λex. = 520 nm (d) of the reaction mixture: DFO with glycine (the molar ratio: 2:1) in ethanol [4]

Improved impedimetric, non-faradaic label-free sensors for the detection of α-amino acids are another important application of the DFO molecule due to its reactivity towards α-amino acids. An electrochemical sensor based on boron-doped carbon nanowalls, porous with excellent

electron transfer, has been proposed- figure 2.

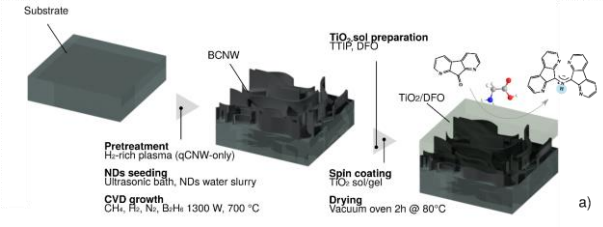

Fig.2. (a) Schematic of the electrode realization.

These new research reports represent a breakthrough in both biomedical and forensic applications.
A fundamentally new approach in the field of modern biocompatible materials and non-toxic environments satisfies the requirements of the current world.

ACKNOWLEDGMENTS

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PEPTIDE HELICITY AND NET CHARGE AS DETERMINANTS OF ANTIBACTERIAL ACTIVITY

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The growing number of drug-resistant bacteria forces the search for alternatives to antibiotic therapies. One possibility could be to use amphipathic cell-penetrating peptides that acquire active, mostly helical, structure near lipid membranes. [1] We assume that peptide helicity contributes to destabilisation of the cell membrane essential for bacterial survival.
Our goal was to examine how the net charge and propensity to form a helix affect the activity of such peptides. Based on literature [2] we designed positively-charged, 11 amino-acid long peptides (Table 1) that could form about three helix turns. Peptides were either purchased or synthesised in-house using solid-phase peptide synthesis with Fmoc strategy. Then, we studied their secondary structure via circular dichroism (CD) spectroscopy in sodium dodecyl sulphate (SDS) and dodecylphosphocholine (DPC) which resemble the prokaryotic and eukaryotic membranes, respectively. [3]
The CD spectra shown for one of the peptides - KALAKLLKKWL-NH_2 (KAL) - confirm adopting a helix near the membrane mimics (Figure 1). Next, the minimum inhibitory (MIC) and bactericidal concentrations (MBC) for Gram-negative *Escherichia coli* K-12 and Gram-positive *Staphylococcus aureus* ATCC 29213 were determined (Table 1).

Table 1. Summary of MIC and MCB assays. q-net charge.

		E. coli		*S. aureus*	
Sequence	q [e]	MIC [μM]	MBC [μM]	MIC [μM]	MBC [μM]
KKLLKKWLKAA-NH_2	6	> 64	> 64	> 64	> 64
KLAKLAKKLAK-NH_2	6	> 64	> 64	> 64	> 64
KALAKLLKKWL-NH_2	5	32	32	> 64	> 64
KALKKLLKAWL-NH_2	5	8	8	> 64	> 64
KALKKLLAKWL-NH_2	5	16	32	> 64	> 64

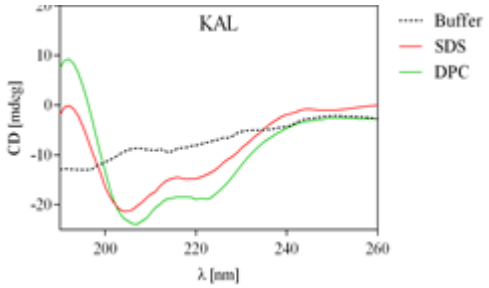

Fig.1. Simulation of KAL peptide active conformation.

The peptides showed activity only against Gram-negative *E. coli* and showed to be ineffective against Gram-positive *S. aureus*. This is probably due to the dense peptidoglycan network in Gram-positive cell wall. The next step will be to stabilise the peptide helical structure with hydrocarbon staples to enhance the antibacterial effect as in the case of anoplin [3]

ACKNOWLEDGMENTS

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INVESTIGATION OF SOME KINETIC PROPERTIES OF NUDT12 TOWARDS DINUCLEOTIDE SUBSTRATES

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Nudt12 was initially identified as NADH diphosphatase, with moderate activity towards substrates including ApppA dinucleotide, FAD or ADP-ribose [1]. Nudt12 possess also ability to hydrolyze m^{7}GpppG and GpppG dinucleotides [2] and structures present on 5' RNA end: the standard mRNA m^{7}GpppN cap, and so called "metabolite" cap structures as NAD or dpCoA [3, 4].
Here, we report the preliminary results of kinetic properties of Nudt12 towards a set of dinucleotide compounds – analogs of standard mRNA cap structure (differing in methylation status of initial guanosine and the type/methylation of the adjacent nucleotide). Nudt12 showed a moderate enzymatic activity towards a majority of tested compounds. However, among those containing adenosine as a second nucleotide were compounds hydrolyzed to the same extent as NADH (e.g. GpppA). Enzyme kinetic analysis showed that they also follow the Michaelis-Menten model, and calculated K_m constant values are within range from around 1 μM - 10μM (e.g. K_m = 1,3 μM ± 0,3 for GpppA) and are close to K_m value for NADH (3,0 μM ± 1,0). Subsequent binding experiments, using differential scannig fluorimetry (DSF) method, and catalytically inactive form of Nudt12, showed that the lower K_m values correlated with the higher thermal protein stabilization (and higher calculated melting temperature T_m values).
Interestingly, compounds containing guanosine as an adjacent nucleotide showed also high thermal stabilization of Nudt12 in DSF experiment, despite they are poor substrates in enzymatic experiments. The first preliminary enzyme kinetic experiments with diguanosine triphosphate suggest Nudt12 undergo here the substrate inhibition – one of the most common deviation from Michaelis-Menten kinetics, that could play role in regulation of enzyme activity [5].

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ROLE OF THE POTASSIUM CHANNEL IN MOLECULAR MECHANISMS OF DNA DAMAGE RESPONSE TO PARTICULATE MATTER EXPOSURE

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Particulate matter (PM) is a well-known air pollutant and its adverse effects on human health are well established. However, the knowledge about the mechanism by which PM exerts its various adverse effects is still incomplete, and detailed *in vitro* studies are highly needed[1]. Understanding molecular mechanisms of DNA damage response (DDR) to genotoxic environmental agents including PM will provide insights for developing novel treatment strategies for neurodegenerative diseases and lung cancer. The potential mechanisms of the PM on these diseases' progression are connected with oxidative stress, mitochondrial dysfunction, and inflammatory response at the cellular level, whereas at the genomic level with genotoxicity[2]. As eukaryotic cells comprise two genomes, nuclear and mitochondrial (mtDNA), it was proposed that oxidative stress in mitochondria will enforce oxidative stress on the nucleus suggesting mitochondrial-nuclear cross-talk. According to recent studies, PM leads to DNA damage *via* base changes, mutations, or DNA double-strand breaks (DSBs)[3]. However, the current knowledge about molecular mechanisms of DNA damage response and repair to PM exposure is limited.

In our studies, we focused on the role of the large-conductance Ca^{2+}-regulated potassium channel (BK_{Ca}) and its potential role in DDR[4]. As a cellular model, we chose human bronchial epithelial cells (16HBE14o-) and generated a 16HBE14o- cell line with BK_{Ca} α subunit knockout using CRISPR/Cas9. Our preliminary studies revealed that exposure to PM<4 μm induced G2/M cell cycle arrest in 16HBE14o- cells depleted for BK_{Ca} channel α subunit. PM exposure also induced DNA damage identified with antibodies to H2AX, which is rapidly phosphorylated at DSBs in this cell line suggesting the role of this ion channel in DDR in a highly interconnected fashion.

Our results suggest that the BK_{Ca} channel can be an important element in molecular mechanisms of DNA damage response and repair after damages caused by urban particle matter. Perhaps our research will contribute to the description of the new protective mechanisms.

ACKNOWLEDGMENTS

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PROTECTIVE PROPERTIES OF YARROW (*Achillea millefolium* L.) AGAINST RED BLOOD CELLS

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Yarrow (*Achillea millefolium* L.) is a plant of the Asteraceae family, which is commonly found in Europe and Asia in temperate regions. It has a high content of polyphenolic compounds. Thanks to its properties it is used as an anti-inflammatory, anti-ulcer and anticancer agent [1,2]. The objective of this study was to test the protective properties of yarrow aqueous extract against biological membranes extracted from erythrocytes.

Yarrow extract was obtained from the Department of Fermentation and Cereals Technology, Wroclaw University of Environmental and Life Sciences.

a detailed quantitative and qualitative analysis of extract was conducted, using the chromatographic (UPLC-DAD, UPLC-ESI-MS) and spectrophotometric (Folin-Ciocalteu) methods. The biological activity of the extract was investigated in relation to erythrocytes and isolated membranes of erythrocytes by using spectrophotometric and fluorimetric methods. Spectrophotometric method was used to determine the effect of the extract on the degree of haemolysis of erythrocytes. The antioxidant activity of yarrow extract towards erythrocyte membranes was determined with fluorimetric methods using AAPH compound as oxidizing agent. The effect of the extracts on the ordering and fluidity of the erythrocyte membrane was tested by fluorimetric method using DPH and Laurdan probes.

The results of hemolytic research showed that yarrow extract does not induce hemolysis, which means there is no destructive action on the erythrocyte membrane. Based on the kinetics of the oxidation process, the concentration which reduces free radicals by 50% was determined. Therd study confirmed high antioxidant activity of the polyphenols contained in extract, compared to that of Trolox®. Fluorescence anisotropy (A) studies of DPH probe showed no significant changes in membrane fluidity in the hydrophobic region under the influence of the tested extract. The changes occurring under the influence of the extract in the hydrophilic part of the membranes were determined by the generalized polarization (GP) value of the Laurdan probe. The GP value was shown to decrease with increasing concentrations of the extract in the membrane.

The study showed that yarrow is a rich source of polyphenolic substances. The polyphenolic compounds contained in the extract reduce the concentration of free radicals, acting as a protective barrier. The high antioxidant activity of yarrow makes it a valuable source of compounds, which can be widely used in the prevention and treatment of many diseases resulting from disorders of oxidative processes in the body.

ACKNOWLEDGMENTS

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CRYSTALLOGRAPHIC STRUCTURES OF *E. COLI* PURINE NUCLEOSIDE PHOSPHORYLASE AND ITS MUTANTS WITH ETHENO-2-AMINOPURINES

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Purine nucleoside phosphorylase (PNP) is one of the enzymes of the purine salvage pathway. It catalyses the reversible phosphorolytic cleavage of the glycosidic bond of purine nucleosides. In many microorganisms the purine salvage pathway is the only source of building blocks for DNA and RNA synthesis, thus PNPs from such pathogenic organisms are potential targets for antimicrobial drugs. Moreover, hexameric bacterial PNPs utilises a wide spectrum of purine bases and nucleosides as substrates, hence are used in enzymatic synthesis of the purine nucleosides, some of them, pharmacologically important, for which chemical synthesis is not easy or tedious [1].

Unexpectedly, some PNPs are active also towards tricyclic, highly fluorescent, ethenoderivatives of purines and purine ribosides, among others towards 1,N^2-etheno-2-aminopurine (1,N^2-ε2AP) and N^2,3-etheno-2-aminopurine (N^2,3-ε2AP). Both of them are substrates for the reversed (synthetic) reaction catalysed by PNP from *E. coli*, but in both cases ribosylation site is rather N^2 than the canonical for PNP N^9 position of the purine base (Figure 1). The ribosylated products, are substrates for the phosphorolytic reaction [2].

To investigate structural basis of these unusual for PNP substrate activity of ε2AP, as well as reasons for the non-canonical rybosylation sites, X-ray diffraction studies were undertaken. We have obtained crystals of the ternary complexes of *E. coli* PNP with both ε2AP isomers and phosphate ion, for three enzyme variants: wild type (WT), D204N mutant mimicking mammalian PNP active site, and D204A/R217A mutant that has significantly modified kinetic properties.

As expected for the *E. coli* PNP structure containing phosphate [1], in most of the complexes examined the active sites of the hexamer are found in the open and

closed conformations. N^2,3-ε2AP is bound in active sites of all investigated protein variants, but its position is different in open and closed active sites. This explains why not only the canonical N^9 riboside is a product of the synthetic reaction. Surprisingly, there is only little electron density in the active sties of the complexes obtained with 1,N^2-ε2AP, suggesting that this ligand is bound only in the small fraction of the active site pockets.

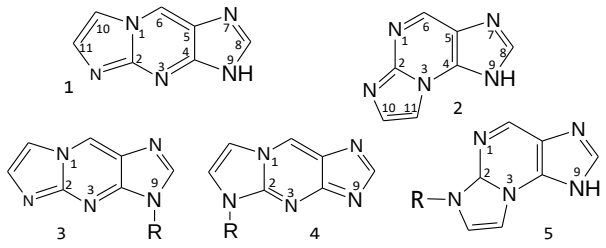

Fig.1. Structures of the nucleobase and nucleoside analogues investigated in this work: 1,N^2-etheno-2-aminopurine (1), N^2,3-etheno-2-aminopurine (2), and the ribosides of the ε2AP isomers (3-5, R = β-D-ribofuranosyl).

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CHARACTERISATION OF THE BINARY MIXED LANGMUIR MONOLAYER COMPOSED OF DPPC AND NEW SYNTHESIZED ALPHA-TOCOPHEROL DERIVATIVE

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Lipid monolayers serve as a simple model of biological membranes and are used to evaluate membrane behavior at a molecular level with inserted ligands as for example drugs, cholesterol or tocopherols (Toc) [1,2,3]. Relatively simple measurements of compression isotherms give insight into conformational order and intermolecular interactions of molecules at monolayer interface. Using this method we were investigated properties of binary mixture of DPPC and carbo analog of Toc devoid of oxygen atom at 1 position in chromanol ring and replaced by methylene group, named 1-carba-alpha-tocopherol (CT).

During experiments the pressure–mean area per molecule (π-A) isotherms of tocopherols and DPPC mixed monolayers were recorded. The obtained from π-A isotherms values of compressibility (C_S^{-1}) [4] and excess area per molecule (ΔA_{exc}) [5] were plotted as function of molar composition of monolayer. To determine possible type of interactions and monolayers stability the thermodynamic properties of mixed monolayers were calculated including the Gibbs energy of excess (ΔG_{exc}) and total energy of mixing (ΔG_{mix}) [6].

The π-A isotherms of Toc and CT shows that pure Toc and its derivatives when spread onto water subphase form a compressible monolayers which exhibit different isotherm shapes. A CT isotherm has collapse pressure (π_c) at 27 mN/m which occurs at 0.39 nm^2 with further plateau. The higher values – compared to Toc - of onset area (A_1) – a value at which the surface pressure is detected, π_c and shape of CT isotherm indicates that molecule during its interaction with subphase has tendency to stay in less ordered gas phase than Toc.

For Toc a C_S^{-1} plot shows that monolayer exists in liquid-expanded (LE) fluid state in full range of surface pressures. CT at low pressure exist in LE state and easily is forming mixed LE and liquid–condensed (LC) phase. The minimum of C_S^{-1} isotherm at about 8 mN/m indicates its phase transition from LE to LC state thus leading to formation of much more ordered structure than Toc.

Increasing presence of CT in DPPC monolayer

is lowering maximum of C_S^{-1} however, opposite to Toc, above 20 mN/m of surface pressure at all concentrations the mixed monolayers remain in LC phase. The surface pressure corresponding to minimum of C_S^{-1} isotherm increases with a CT concentration up to 30 mol% and indicating the formation of equilibrated LE/LC mixed phase as observed in corresponding π-A isotherms.

It shows that for Toc and CT at pressures bellow 10 mN/m a negative sign of ΔA_{exc} and ΔG_{exc} is observed indicating presence of attractive interactions. For CT from value of 10 mN/m in the whole range of concentrations the positive values of ΔA_{exc} and ΔG_{exc} are observed indicating repulsive interactions between components what can lead to phase separation or partial mixing of the components in mixed monolayers. Simultaneously, the increasing surface pressures leads to increasing of positive energy with formation of two maxima at molar fractions of 0,1 and 0,5. The presence of maxima separated by a minimum suggests formation regions enriched in one of components giving rise to partial miscibility.

For CT the negative values of ΔG_{mix}, similar like for Toc, indicate on monolayer stability however for CT its decreases fast with increasing surface pressure.

The results show that in the case of CT, replacement of O1 in the chromanol ring by methylene group leads to a change in the electron distribution in the chromanol ring and its different behavior in mixed DPPC monolayer compared to parent compound.

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CHARYBDOTOXIN REDUCES THE MIGRATION OF GLIOBLASTOMA U87MG CELLS

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Glioblastoma is characterized by intensive proliferation and migration of cells, with the two phases separated from each other [1]. Multiple ion channels are involved in the proliferation and migration of glioblastoma cells, including large-conductance calcium-activated potassium channels (BK) [2]. The aim of the study was to investigate the effect of charybdotoxin (ChTX), a BK channel blocker, on the migration of cells of the U87MG cancer line. To determine the number of migrating cells, Petrie dishes with silicon inserts were used. Cells moved to the gap created by the removal of the insert. In the initial 8 hours after the insert removal, the number of cells in the gap was significantly lower, when charybdotoxin was present in bath solution (Fig.1).

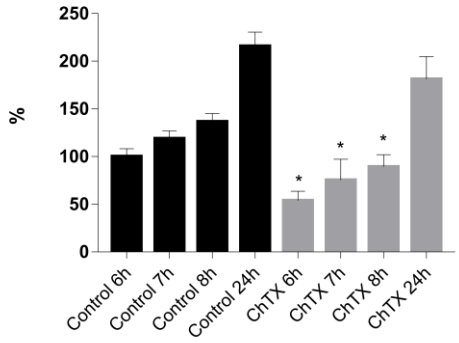

Fig.1. Charybdotoxin reduces the number of migrating cells. The columns represent the percentage of migrated cells as compared to the control. 100% represents the average number of migrating cells in the control 6 hours after the removal of the silicone insert. n=6. * p<0.05 One-way ANOVA.

U87MG cells are characterized by high expression of BK channels in the plasma membrane. The activity of BK channels in the presence of charybdotoxin was tested using the patch clamp, single channel technique. High activity of BK channels in the presence of charybdotoxin was observed in non-migrating cells (50% of n=21 patches). In contrast, the majority of migrating cells (83% of n=18 patches) did not show BK channel activity under the same conditions (Fig.2). The activity of BK channels in the presence of charybdotoxin may result from the expression of β4 subunit in BK channels in proliferating cells, which significantly reduces the sensitivity of BK channels to charybdotoxin and iberiotoxin [3]. Changing the cell status from proliferating to migrating may be associated with dissociation of the β4 subunits from the BK channels.

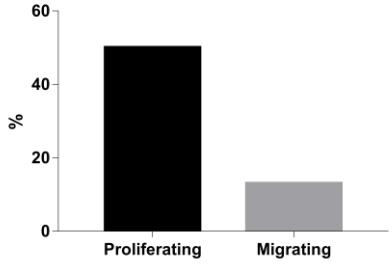

Fig.2. The BK channel in migrating cells is sensitive to ChTX. The columns represent the percentage of patches where channel activity in the presence of ChTX was observed.

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CHLOROPHYLL FLUORESCENCE RELAXATION ANALYSIS FOR THE MONITORING OF PHOTOSYNTHETIC CAPACITY OF MICROALGAE EXPOSED TO ANTIFOULING COATINGS

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Chlorophyll fluorescence kinetics provides detailed information about the electron transfer reactions in photosystem II (PSII) [1, 2]. In this research the flash-induced chlorophyll fluorescence relaxation was used to study the PSII efficiency of the sequential electron transfer steps in green microalga *Chlamydomonas reinhardtii* exposed to antifouling coatings. The response of algae to antifouling paints was monitored over a short-term period of 24 hours. Fluorescence multiscaling analysis showed that photosynthetic activity of algae is mainly dependent on the concentrations of copper and zinc contained in the coatings. The fluorescence kinetics courses (Fig. 1) were fitted using a function described by the equation (1),

$$F(t) = y_0 + \sum_{i=1}^{n} A_i \cdot e^{(-t/t_i)} \quad (1)$$

where *F(t)* is a fluorescence value at time *t*, A_i- is the amplitude of the fluorescence relaxation phase, t_i- is a characteristic lifetime and y_0 - is the stable minimal fluorescence at the end of the decay. The time dependent analysis of lifetime (t_i) and amplitude (A_i) values of the fluorescence kinetic components revealed a high variability under exposure of algae to antifouling paints. A significant decline in the maximum quantum yield of primary photochemistry (F_V/F_M) was observed within the first 1 hour of exposure to coatings (Fig.2). Interestingly, after this time, the recovery of F_V/F_M was noticeable. This research shows that the fluorescence relaxation kinetics is a sensitive indicator of altered intersystem electron transfer processes in PSII caused by biocides, and, as a non-destructive method is feasible in antifouling tests. The study indicate that algal cultures

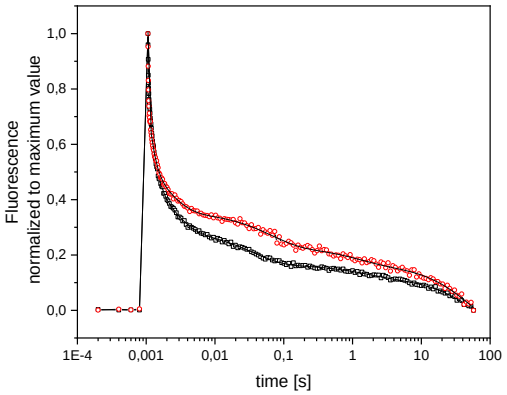

Fig.1. The flash-induced chlorophyll *a* fluorescence relaxation in *Chlamydomonas r.* cells exposed to antifouling paints (red, circle symbols) for 24 hours. The black trace represents the control (black, square symbols).

may adapt to the environmental toxic Cu^{2+} and Zn^{2+} released from antifouling paints.

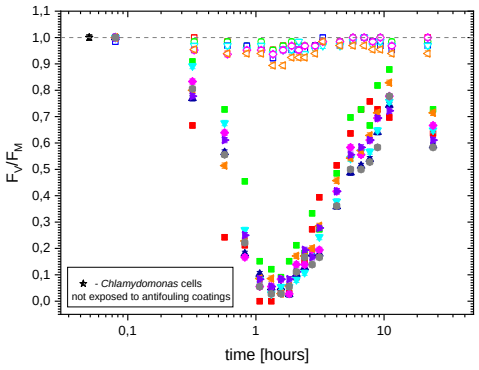

Fig.2. Maximal photosystem II efficiency (F_V/F_M) in algal cultures exposed to different antifouling coatings. F_V/F_M was normalized to the maximum value. Open symbols represent the coatings of low toxicity, full symbols refer to highly toxic ones.

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METHODS OF EXPOSING LIVING ORGANISMS TO ELECTROMAGNETIC FIELDS IN LABORATORY CONDITIONS

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Exposure to radio-frequency electromagnetic fields (EMF) has increased significantly in recent decades. There are numerous research about the influence of EMF on living organisms, however the results of these experiments are still not unambiguous, primarily because of non-homogenous and imprecise methods of exposing living organisms to EMF in laboratory environment. [1,2] Therefore this is necessary to find a method which enables comparison between results derived from different laboratories.

There are many methods of generating electromagnetic fields in laboratory conditions, including Helmholtz coil (frequently custom-made) [2,3], magnetotherapy applicator [4], cylindrical exposure unit [5]. All of these methods are incomparable. Moreover, it is hard to determine real parameters of electromagnetic fields. There is also the problem, where a model organism should be located and what intended parameters are in reality. As a matter of fact, in most publications this trouble is not raised, even though this is a key issue in this type of research.

In the presented studies, we are proposing a different manner of generating electromagnetic fields in laboratory conditions, which is appropriate for exposing living organisms to this factor. This way includes using Function Generator, which is attached to Faraday cage, constructed in such a manner that can allow only specific EMF (in presented experiment there are fields of the background - occurring in urban environment). In this construction there are specified conditions, which are measured consequently through the meters of induction and frequency. Also oscilloscope is connected to the function generator and it measures all of the parameters.

Thanks to precise and proper way of generating electromagnetic fields in laboratory conditions, results of future research will be comparable and the outcome will be no longer in doubt, which is much-needed for public opinion information and real science.

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PHYTIC ACID ENHANCES ELECTRICAL EXCITABILITY IN MACROPHYTE *NITELLOPSIS OBTUSA*

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In response to local external stimuli, plant cells generate electrical signals which propagate through the plant body and induce a systemic response by altering various physiological functions. Despite the relatively well-understood principles of the transmembrane ion current dynamics during electrical excitation, the molecular basis of action potential (AP) generation in plants remains obscure [1]. It is known that the initial membrane depolarization is caused by Ca^{2+} influx into the cytoplasm, but its regulation is still veiled. Based on a paradigm borrowed from animal physiology, inositol 1,4,5-trisphosphate (IP_3) has been supposed as a Ca^{2+} channel activator [2]. However, plants do not possess

animal-like IP_3 receptors. The attention has been shifted to inositol hexakisphosphate (IP_6), also known as phytic acid, as a possible second messenger capable of action potential initiation [3].

In the present study, electrophysiological investigations were carried out using freshwater macroalgae *Nitellopsis obtusa* model system. Two-electrode current clamp technique was employed to register electrically-elicited APs. Cells were externally exposed to various concentrations of phytic acid.

After 30 min exposure phytic acid enhanced *Nitellopsis obtusa* excitability by hyperpolarizing AP excitation threshold. The AP peak membrane potential also was decreased, thus, the AP amplitude remained unchanged, indicating that the magnitudes of Ca^{2+} and Cl^- fluxes during excitation remained unaffected.

Our research showed that phytic acid is a modulator of plant electrical signaling. These results call for more in-depth investigations of the molecular identity of ion channels and second messenger cascades involved in plant electrogenesis and analysis of the role of phytic acid in these processes.

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EFFECT OF BROMINATED FLAME RETARDANTS ON THE LEVEL OF LIPID PEROXIDATION AND PROTEIN OXIDATION IN HUMAN RED BLOOD CELLS

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Brominated flame retardants (BFRs) are synthetic compounds whose task is to reduce the flammability of polymeric materials, which are commonly used in industry including electronics, construction, transport, as well as to produce everyday objects. The widespread use of these compounds is associated with human exposure to BFRs [1]. The most used BRF is tetrabromobisphenol A (TBBPA), whose annual production of TBBPA exceeds 220,000 tonnes [2].

The aim of this study is to evaluate the extent of lipid peroxidation and protein in human red blood cells – erythrocytes exposed to TBBPA and TBBPS - tetrabromobisphenol S. TBBPS is substitute of TBBPA, which has recently been introduced to the market. TBBPS has less harmful effects on human blood cells but is yet insufficiently researched to determine whether it is safe to use and whether it will allow the complete abandonment of TBBPA use. Erythrocytes were isolated from erythrocyte-leukocyte platelet buffy coats purchased from the Regional Centre of Blood Donation and Blood Treatment, Lodz, Poland. Next, erythrocytes suspensions in PBS with 5% hematocrit were treated with TBBPA and TBBPS and stimulated for 24 hours in 37°C. The concentration range for TBBPA was 10; 12,5; 15; 20, 30 µg/ml and TBBPS was 10; 20; 30; 50; 100 µg/ml. Different concentrations of individual compounds were chosen due to their different hemolytic potential.

The degree of lipid peroxidation was determined by cytometric analysis using the BODIPY™ 581/591 fluorescent label, where oxidation of the polyunsaturated butadienyl part of the dye shifts the fluorescence emission peak from 590 nm to 510 nm. The level of RBC protein oxidation was determined using Protein Carbonyl Fluorometric Assay Kit (Cayman Chemical's). Protein carbonylation, i.e., post-translational modification that produces protein-carbonyl adducts, occurs during oxidative stress. The test uses the rhodamine B hydrazide reaction to measure the content of protein carbonyls in biological material. Formation of fluorescent carbonyl-RBH protein hydrazone is analyzed at excitation wavelength 560 nm and emission wavelength 585-595 nm.

The obtained results indicated that TBBPS in the tested concentration range increased neither the degree of lipid peroxidation nor protein oxidation. Similar results were observed for the concentration range tested for TBBPA. To sum up, TBBPA and TBBPS do not significantly influence the level of lipid peroxidation and protein oxidation in the concentrations studied.

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ANTIOXIDANT EFFICIENCY ASSESSMENT OF SELECTED PLANT EXTRACTS BY UV-VIS AND FLUORESCENCE SPECTROSCOPY

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For centuries plants were sources of medicines that were used to cure daisies in humans and animals. Especially phenols, polyphenols, and flavonoids constitute major groups of compounds having natural antioxidant properties and are present in plants and plant extracts. Phenolic compounds such as quercetin, rutin, catechin, genistein, caffeic acid, chlorogenic acid, and gallic acid are among the most popular. Recently much attention has been paid to the influence of natural compounds present in everyday 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 their antioxidant performance.

One of the sources of the possible antioxidants is the fruits of the European bird cherry (*Prunus padus*) which is a European and Asian native tree of a Rosaceae family. The bark, leaves, and fruits have been known in the field of folk medicine, considering their antibacterial, diuretic, antirheumatic, styptic, and other performance. 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.

This contribution is one of the first to assess the antioxidant potential of bird cherry fruit of water, methanol, ethanol, and acetone extracts and their antioxidant efficiency against oxidation of PC liposomes using spectroscopic methods. The extracts were prepared with the use of each above-mentioned eluents and dissolved in water prior to measurements.

Basic characteristics confirming the presence of antioxidants in the extracts were performed with the use of HPLC, GC-MS, and UV-Vis spectroscopy accompanied by ATR-FTIR measurements.

The total luminescence spectra with maxima at 314-318 nm, 325-355 nm, and 428-435 nm were ascribed to the presence of phenolic acids and tocopherols. The antioxidant properties of extracts and their inhibition properties against lipids peroxidation in PC liposomes were determined by fluorogenic probes DCF-H and C11-BODIPY581/591. The measured antioxidant properties against generated free radicals in aqueous and lipogenic phases revealed differences between extracts depending on their physicochemical properties with the greatest potential for acetone extract and sirup. Moreover, studies with membrane model systems using PC liposomes in the liquid-crystalline state have shown that investigated extracts are able to delay oxidation processes not only in homogenous but also in a heterogeneous environment including model biological membranes. Reported high antioxidant properties of raw sirup, squeezed directly from bird cherry fruits may also arise from the synergistic effect which has been observed between compounds detected in other systems. Finally, the extracts and sirup could be considered as food supplements of naturally occurring antioxidants with big application potential in the food industry.

THE APPLICATION OF PROTIDE TECHNOLOGY IN SYNTHESIS AND BIOLOGICAL EVALUATION OF 5' CAP ANALOGS

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Interest in the study of nucleic acids and their components have grown over the previous years. Among them there are analogs of 5'-end of mRNA, the cap. Its base structure is composed of a 7-methylguanosine bound to the first transcribed nucleotide via an unusual 5',5'-triphosphate bridge. The cap has numerous important functions in cellular processes, however, its acting in initiation of translation draws a lot of attention as promising target to fight cancers.[1] During this process, cap interacts with eukaryotic initiation factor 4E.[2] It is known, that elevated level of this protein leads to oncogenesis.[3] Therefore, synthesis of cap analogs that are able to effectively bind eIF4E is especially important.

Nonetheless, application of 5' cap analogs as inhibitors is limited by their poor translocation properties. One of the most efficient solution, called "ProTide" approach, is transforming nucleotides into aryl phosphoramidate prodrugs (Fig. 1).[4]

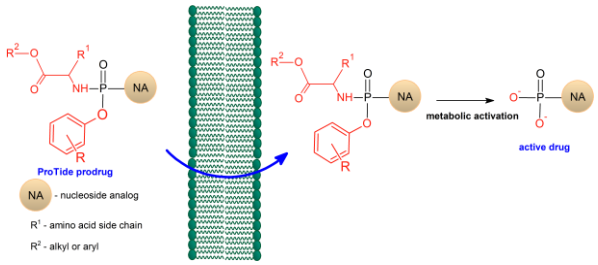

Fig.1. Mechanism of action of ProTide nucleoside analogs.

It has been previously shown, that additional substituents in the N2 position of guanosine enhance inhibitory properties of 5' cap derivatives.[5] For that reasons, herein we report the ProTide technology approach employed to N^2-modified mRNA cap analogs (Fig. 2).

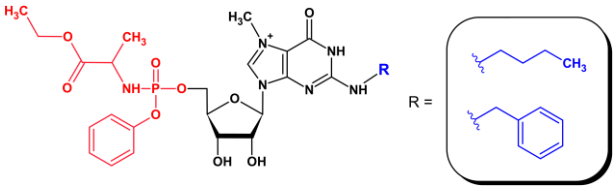

Fig.2. Obtained ProTide 5'cap analogs with additional substituents at N2 position.

Presented newly synthesized compounds were tested using different biophysical and biological methods. Cap analogs in prodrug form do not show inhibitory properties, thus, their susceptibility to metabolic activation was determined. Eventually, their ability to inhibit translation after their turnover into active derivatives was also examined.

ACKNOWLEDGMENTS

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ENERGY AND ELECTRON TRANSFER BETWEEN QUANTUM DOTS AND CYTOCHROME *C* QUANTIFIED BY TRANSIENT ABSORPTION SPECTROSCOPY

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Fluorescent semiconductor nanocrystals, commonly known as quantum dots (QDs), constitute the group of nanoparticles offering one of the greatest and most versatile potential in bionanotechnology. In crowded biological environment (e.g. cell interior, extracellular matrix, artificial bionanohybrid assemblies) QD, serving as multi-electron and photoenergy supplier, may participate in different electron transfer (ET) and charge transfer (CT) pathways simultaneously [1]. In this work, the system composed of colloidal cadmium telluride (CdTe) QDs and cytochrome *c* protein (Cyt *c*) was used to evaluate the contribution of both processes in Cyt *c*-induced ET/CT from QDs.

We applied the transient absorption spectroscopy to study the early photodynamics in the QD-Cyt c system. We used two types of CdTe QDs with different emission maximum (QD570 and QD650) and recorded the absorption transients in the excited QD+Cyt *c* mixtures. We observed efficient quenching of QDs emission by Cyt *c* and estimated the contribution of electron transfer and other mechanisms to this process. In QD and Cyt *c* mixtures, 25% of excited QD electrons quickly (~30 ps) relaxes to the ground state and 75% is quenched by Cyt *c*. The primary quenching mechanism is energy transfer but electron transfer and photoreduction of Cyt *c* makes the significant contribution (~8%). The lifetime of reduced Cyt *c* is ~1 ms and the fraction with unmeasurable decay time is observed. We speculate that back electron transfer from reduced Cyt *c* occurs and the fraction of Cyt *c* is stably reduced.

On the basis of our results we postulate that QDs are electron donors in photoreduction of Cyt *c*. The process of electron transfer has a significant contribution in the

quenching of colloidal CdTe QDs by Cyt *c*.

ACKNOWLEDGMENTS

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STRUCTURAL CHANGES IN BOVINE SERUM ALBUMIN INDUCED BY SODIUM DODECYL SULFATE

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Investigation of effects of surfactants on proteins has many practical applications. An important aspect of these interactions is the kinetics of structural changes in proteins induced by surfactants such as sodium dodecyl sulfate (SDS). These changes are achieved in millimolar concentrations, not molar concentrations as for traditional denaturants. Of particular interest is opportunity to follow simultaneous temporal changes in the secondary and tertiary structural changes. Kunio Takeda and coworkers were the first to start protein kinetic research in the 1980s[1], including bovine serum albumin (BSA) by stopped-flow measurements using the detections of circular dichroism and absorbance[2]. In more recent times, such research was undertaken by Daniel Otzen and coworkers[3,4].

Stationary circular dichroism experiments performed at FUV and NUV showed that changes in spectra are already observed for micromolar concentrations of SDS. After analyzing the FUV spectra with the BestSel program, we can see that in the case of SDS concentration below and above CMC, the population of helixes, antiparallel beta sheets and turns decreases.

Table 1. The percentage of secondary structure of BSA obtained from the analysis of circular dichroism spectra with the BestSel.

BSA [μM]	SDS [mM]	Helix	Anti-parallel	Turn	Others
10	0	65.3	10.6	11.1	13
10	4	49.1	7.3	9.1	34.5
10	40	48.1	9.9	10.4	31.5

Kinetic measurements of circular dichroism indicate that changes in the secondary structure of the protein are at least two-step (the higher the concentration of SDS, the more distinguishable are the individual steps) and occur in a very short time of ~ 50 ms. The ionic strength of the solution above 50mM causes that the individual steps in the reaction progress curves are no longer visible. In the tertiary structure, the structural changes probably occur in the instruments dead time of 1 - 2 ms.

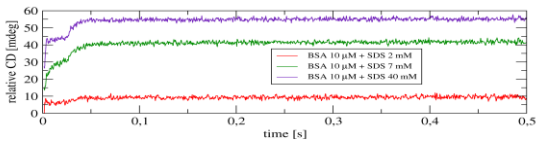

Fig.1. Relative progress curves BSA-SDS, excitation 220 nm.

The fluorescence spectra show that SDS quenches the protein fluorescence already in micromolar concentrations, moreover it shifts the fluorescence maximum towards shorter wavelengths, the same is for absorption maximum.

The results of analytical ultracentrifugation may indicate that SDS coats the protein molecules and lengthening them. Based on the distribution of the sedimentation coefficient, we can conclude that in a solution containing no BSA, SDS micelles are formed at its concentration equal to 5 mM, while in the presence of protein at a concentration of 10 mM.

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DRUG RELEASE KINETICS AND TRANSPORT MECHANISMS FROM VARIOUS POLYMERIC TRANSDERMAL PATCHES

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Mathematical models are an important tool to predict the release behavior of the drug from a matrix in the function of time. They allow to determine some physical parameters e.g. drug diffusion coefficient, release constant, release exponent. The modeling of drug release from delivery systems allows understanding and elucidation of the transport mechanisms. Most of the existing mathematical release kinetic models is based on the diffusion equation.

The aim of the study was to determine, using appropriate models, the mechanism of transport of indomethacin (IND) from adhesive polymeric matrices. The in vitro release experiments were carried out for transdermal patches containing IND incorporated (5% w/w) in two types of polymer matrices: silicone (SSA) and acrylic (DT2). The effect of additional liquid excipients, namely silicone oil (SO), isopropyl myristate (MIP) and propylene glycol (PG) was investigated. The following kinetic parameters were considered: release constants, release exponents and diffusion coefficients.

Comparative analysis of the release of IND from silicone and acrylic patches with excipients was conducted using *in vitro* dissolution model (USP apparatus 5, paddle over disk 75 rpm at 37°C, medium: phosphate buffer pH 7.4). Concentration of IND in the acceptor medium was assayed with HPLC/UV. Modelling of the obtained release curves was performed with OriginPro 2021.

A semi-empirical power law equation (Korsmeyer-Peppas model)

$$Q = k_{KP} t^n \quad (1)$$

where Q is cumulative amount of the drug released at time, k_{KP} is release constant and n – release exponent, was used to describe drug release processes [1, 2]. The release curves of IND for both polymeric matrices are presented in Fig. 1 and Fig. 2, respectively.

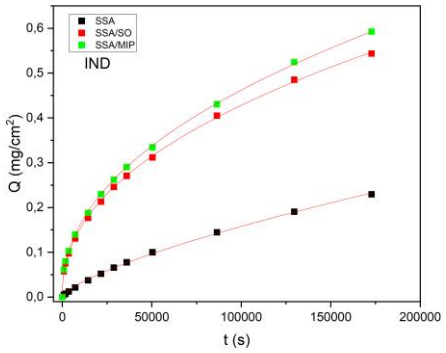

Fig.1. Release curves of indomethacin for silicone matrices.

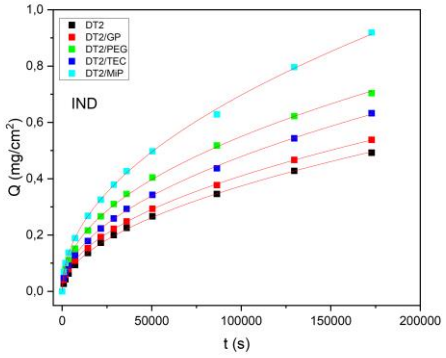

Fig.2. Release curves of indomethacin for acrylic matrices.

Table 1. Release parameters obtained from the power law equation (Korsmeyer-Peppas model)

IND	**Korsmeyer-Peppas Model**			**Release mechanism**
	$k_{KP} \cdot 10^{-5}$ [mg /cm^2 s^n]	n	R^2	
SSA	4,5270± 0,4713	0,7084± 0,0088	0,99921	anomalous transport
SSA/SO	250,0000± 10,4606	0,4466± 0,0037	0,99961	quasi-diffusion
SSA/MIP	247,0000± 9,9411	0,4543± 0,0035	0,99965	quasi-diffusion
DT2	99,4221± 5,6308	0,5151± 0,0049	0,99945	anomalous transport
DT2/GP	136,0000± 3,8174	0,4957± 0,0024	0,99986	diffusion
DT2/PEG	243,0000± 12,1739	0,4709± 0,0044	0,99951	quasi-diffusion
DT2/TEC	154,0000± 6,4945	0,4988± 0,0037	0,99969	diffusion
DT2/MIP	236,0000± 13,1455	0,4940± 0,0048	0,99940	diffusion

The analysis of the obtained kinetic parameters for SSA and DT2 matrices with excipients follows quasi-diffusion ($n < 0.5$) or diffusion processes and anomalous diffusion for pure systems (Table 1). Modification of the matrices with liquid components affects the drug transport process, which becomes more effective and this effect is more visible for silicone patches.

ACKNOWLEDGMENTS

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IS THIOFLAVIN T AN APPROPRIATE MARKER TO MONITOR GFP AMYLOID FORMATION?

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Thioflavin T (ThT) is the widely used and convenient tool for identifying amyloids both in vivo and in vitro because of large enhancement of its fluorescence emission upon binding to β-sheet fibrils [1].
The green fluorescent protein, GFP, characterized by mainly β-sheet structure is a popular fluorescent marker, commonly used to observe localization, identification, migration of cell components and also to monitor the folding or aggregation of other proteins [2].

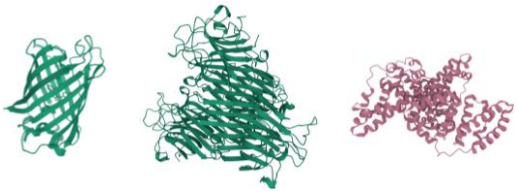

Fig.1. Spatial structure of EGFP [3] (left) [3], concanavalin A [4] (middle) and albumin [5] (right).

To check whether the aggregation of GFP can be tested with thioflavin T and whether the presence of GFP does not interfere with the measurements of other protein amyloidosis using thioflavin T, fluorescence measurements of the interaction of ThT with the natively β-sheet - concanavalin A, EGFP, and α-helix protein - bovine albumin were performed. It was found that thioflavin T binds to rich in β-sheet structures, regardless of they are native or aggregated. This should be considered when using this marker in the study of amyloidosis of natively rich β -sheet proteins.

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ESTABLISHMENT OF A COLONY FORMATION ASSAY TO DETERMINE THE DOSE-RESPONSE TO A GENOTOXIC AGENT – FINE PARTICULATE MATTER (PM_4)

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The colony formation assay (clonogenic assay) is a well-known gold standard method, developed in the mid-50s, as a basic cell survival assay to study the cytotoxic effect of radiation and cytotoxic agents. The clonogenic assay has been used by numerous researchers to measure the self-renewing capacity of various mammalian cell model systems *in vitro*. This method is based on the ability of a single cell to grow into a colony that consists of at least 50 cells. The assay tests every cell in the population for its ability to undergo 'unlimited' division. As clonogenic assay is a widely used method of choice to determine cell reproductive death after treatment with ionizing radiation, in this study we utilized and optimized the method to determine the dose-response to a genotoxic agent – fine particulate matter (PM_4) in different cell lines. We used particulate matter from the National Institute of Standards and Technology (NIST): Fine Atmospheric Particulate Matter (Mean Particle Diameter < 4 μm) (SRM 2786). The Standard Reference Material (SRM) is intended for use in evaluating repeatable experiments as it was described as a suitable surrogate sample for the study of authentic street particles [1]. We optimized the colony-forming assay in different adherent human cell lines for our toxicological studies using protocols described previously by Wassermann et al. (1990)[2], Danielsen et al. (2008)[1], Franken et al. (2006)[3], and Brix et al. (2021) [4].

Our clonogenic survival experiment included three distinct components: 1) pre-treatment and post-treatment strategy of the cell monolayer in tissue culture flasks with particulate matter PM_4 at various concentrations and time points, 2) preparation of single-cell suspensions and plating an appropriate number of cells in 6-well plates, and 3) fixing, staining and counting colonies following a relevant incubation period. Here we demonstrate the general procedure established and optimized in our laboratory for performing the clonogenic assay using adherent cell lines. Also, we aimed to describe the calculation of the plating efficiency and survival fractions after exposure of cells to PM_4. Finally, we determined the cell survival curve which describes the relationship between the dose of a genotoxic agent and the number of surviving cells.

The clonogenic assay should become a standard tool to evaluate cellular reproductivity in response to cytotoxic or genotoxic effects of particulate matter. It estimates the capability of cells to maintain their reproductive integrity over an extended period of time. This is a significant feature as it shows phenotypic effects that require time and quite a few cell divisions to develop, therefore this array has advantages over other short-term colorimetric cytotoxicity assays based on MTT, MTS, and XTT.

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INVESTIGATION INTO THE MECHANISM OF SYNERGISTIC ANTIMICROBIAL ACTION OF POLYENE-BASED ANTIBIOTICS AND THE SELECTED 1,3,4-THIADIAZOLE DERIVATIVES

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Thiadiazole derivatives are widely reported to possess

broad array of biological activities such as the antitumor, antibacterial, or antifungal [1-2]. Also, recent reports point at the potential ability of those compounds to enhance the activities of commercially available antibiotics such as amphotericin B (AmB) or Kanamycin [3-5]. Such synergistic effects were investigated in detail by our group, and particularly for mixtures of 4-(5-methyl-1,3,4-thiadiazole-2-yl) benzene-1,3-diol (C1) and AmB [4-5]. These studies did not involve the mechanistic studies and hence our current work aims at more detailed examination of the mode of action of the synergistic system mentioned.

Based on the electronic absorption and stationary fluorescence data a disaggregation of AmB micelles caused by the interaction of AmB with C1 molecules was suggested. This effect is evidenced by a notable positioning change of the AmB absorption maximum in PBS, wherein the band characteristic of the AmB aggregate shifted from 345 nm to approximately 335 nm and the shift was attributed to the addition of the C1 aliquot. These data are consistent with the fluorescence spectroscopic results, which revealed a disappearance of the AmB aggregate emission band upon the addition of C1. Also, the TCSPC data obtained are in-line with the steady state fluorescence and electronic absorption results, and suggested that the active form of AnB-C1 tandem is characteristic of a particular fluorescence lifetime. Furthermore, the additional stationary and time resolved fluorescence anisotropy studies evidenced the aggregation-dependence of the synergistic action of C1-AmB system. The experimental data were additionally supported with quantum chemistry calculations.

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FUNDING

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RNA OLIGONUCLEOTIDE BEACON BIOSENSING SYSTEM FOR NEURODEGENERATIVE BIOMARKERS DETECTION

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Neurodegenerative diseases (ND) related to ageing, like Parkinson's disease (PD) and Alzheimer's disease (AD), are a challenge for modern medicine due to the not clear genesis and hampered diagnosis [1]. Detection of specific microRNAs (miRNAs), which are deregulated in ND, may help understand the molecular mechanisms behind those diseases and contribute to developing novel treatments [2].

Due to the mitochondrial dysfunction, a hallmark of the ND [3], we selected mitochondria-related microRNAs and evaluated their expression level. For this purpose, we designed an optical biosensing system for detecting miRNA in neuronal cells in vitro – a molecular beacon based on oligonucleotide hairpin with the fluorescent dyes and quenchers, complementary to the sequence of the selected miRNAs (Fig.1). Delivery of probes to the cells SH-SY5Y in vitro was performed in three ways. We used commercially available lipidic transfection reagents: Lipofectamine™ 2000 (Invitrogen) and Cell-In™ (Institute of Physical Chemistry PAS), as well as exosomes isolated from the SH-SY5Y cell line.

Fig.1. Example structure and sequence of the molecular beacon with fluorophore HEX and quencher TAMR.

Designed probes have allowed detecting and imagining the expression level of three tested miRNAs implicated in neurodegenerative diseases (miR-16-5p, miR-7, miR-34a) [4,5,6]. To validate obtained results, we performed RT-qPCR using TaqMan™ MicroRNA Assays (Applied Biosystems). After completion of the validation of the detection method, we treated cells with three compounds responsible for controlling the fusion/fission in mitochondria, i.e. mitochondrial division inhibitor (mdivi-1), dynamin Drp1 inhibitor drug (dynasore) and rapamycin. Next, we measured the changes in the expression of selected miRNAs. Our observation of changes in the level of two miRNAs (miR-16-5p and miR-7-5p) indicates that a designed optical biosensing system allows us to detect specific miRNAs in vitro. Moreover, we observed that the exosome delivery system appeared to be more efficient than commercially available transfection reagents.

ACKNOWLEDGMENTS

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ORNITHINE BASED SIDEROPHORES AS PEPTIDE NUCLEIC ACID CARRIERS TO *E. COLI* CELLS

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Antibiotic resistance is a threat to public health due to the increasing number of resistance mechanisms and rapid spread of resistance genes within the bacterial population. Gene targeting could be a promising approach for discovering new effective antimicrobials. To achieve this, peptide nucleic acid (PNA), a nucleic acid mimic, can be used for silencing specific genes to regulate gene expression in bacteria [1]. Unfortunately, the drawback of PNA includes its poor membrane permeability.

Iron is crucial to microbial growth and is obtained by bacteria using siderophores (iron chelators) [2]. Secreted siderophores capture ferric iron and are retrieved by bacterial transport system inside the cell [3]. Thus we consider siderophores as potential carriers of PNA. Our hypothesis is that conjugation of a PNA oligonucleotide to ornithine based synthetic siderophore will result in PNA uptake into Gram-negative bacteria.

We use ornithine derivative (N-Δ-hydroxy-N-Δ-acetyl-ornithine) as a building block for siderophore, because it provides hydroxamate groups capable of binding ferric iron using oxygens in an octahedral geometry (Figure 1). PNA oligomers were synthesized and conjugated with linear and cyclic siderophores via the copper-calyzed azide-alkyne cycloaddition (CuAAC). Purity and identity analysis of the synthesized compounds were performed by RP-HPLC and mass spectrometry. Functionality of the synthetic siderophores in PNA transport was tested on *E. coli* wild type and *Δfur* strains carrying plasmids expressing red fluorescent protein (RFP). The RFP-silencing assay was performed, and the percentage of bacterial cells with silenced RFP-fluorescence was measured using flow cytometry. Iron coordination properties of the

synthesized siderophores were also determined using circular dichroism spectroscopy.

Fig. 1. Structure of the designed linear (1) and cyclic (2) ornithine based siderophores with azide linkers.

ACKNOWLEDGMENTS

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THE EFFECT OF INGESTED Cd AND Cu ON THE STRUCTURAL PROPERTIES OF HUNTING WEBS PRODUCED BY *STEATODA GROSSA* (THERIDIIDAE) SPIDERS

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The study aimed to assess whether copper and cadmium administered via ingestion to *Steatoda grossa* spiders (Theridiidae) affects the selected structural properties of the produced hunting webs. The study was carried out on webs produced by adult females (F) and males (M) of spiders, which originated from a multi-generation experimental laboratory colony. *S. grossa* spiders build small, tangled, three-dimensional cobweb snares (Fig. 1).

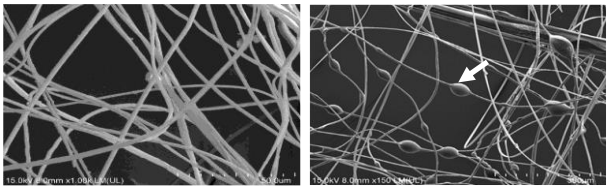

Fig.1. SEM images of spider web pattern examined under a Hitachi SU 8010 FESEM. The web consisting of an irregular tangle of dry silken fibers and threads with glue regions (arrow).

Three experimental groups, the control (CT), copper (Cu-int) and cadmium (Cd-int) group, were distinguished. The CT was fed *Drosophila hydei* flies grown on standard, uncontaminated medium, while Cu-int and Cd-int groups were fed prey grown on a medium supplemented with $CuSO_4$ (0.234 mM) and $CdCl_2$ (0.248 mM), for three month. Assessment of structural changes in hunting webs produced by *S. grossa* spiders was based on measurements of thread diameter, as the predators are known to actively control this parameter depending on environmental conditions [1, 2, 3]. Air-dried webs of adult *S. grossa* F and M were analysed under a scanning electron microscope (SEM; Hitachi SU8010 field emission scanning electron microscope, FESEM) (Fig. 2).
Eenergy dispersive X-ray microanalysis (EDX) was applied to identify the elemental composition of silk fibers from CT and Cd or Cu-treated samples.

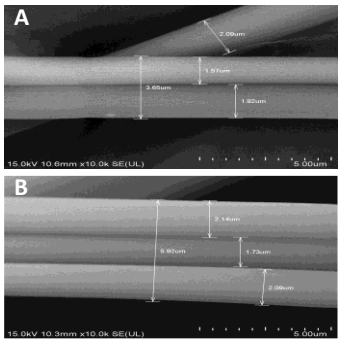

Fig.2. Example SEM images of web threads. A: single-, double- and B: multi-stranded threads

F and M showed different reactions to Cd and Cu supplied through food. Diameter of single strands found as single-stranded threads or in double- and multi-stranded threads produced by the M was on average 2-fold lower than in F from CT and Cd- or Cu-int groups. Cd-int F spun webs with smaller single-strand diameters than the control individuals. Moreover, in webs produced by F from the Cu-int group, multi-stranded threads were by 37% thinner than in webs of control F. EDX microanalysis enabled identification of elements such as C, N, O, Na, K, but not Cu and Cd in fiber-web samples from M and F *S. grossa*.

We conclude that changes in the structural properties of silk fibers spun by *S. grossa* F exposed to Cd or Cu contaminated food may be result energy allocation to energetically costly detoxifying mechanisms triggered as a defense reaction of the organism to the applied metals.

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BIOPHYSICAL STUDIES OF THE INTERACTION OF OPTICALLY ACTIVE IODOLACTONES WITH DNA AND MEMBRANES OF CANCER CELLS AND THEIR POTENTIAL ANTITUMOR ACTIVITY

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Lactones are bioactive compounds that possess various interesting biological properties. Those that have an aromatic ring show high antitumor activity. In our previous research, we synthesized a series of β-aryl-δ-iodo-γ-lactones differing in substituents at the benzene ring which exhibited cytotoxic activity against the selected cancer cell lines: Jurkat (human T-cell leukaemia) and D-17 (canine osteosarcoma), GL-1 (B-cell leukaemia) and CLBL-1 (B-cell lymphoma cel line) [1].

A number of studies have demonstrated that the membranes of cancer-altered cells are more fluid than those of healthy cells. Moreover, the higher fluidity of these membranes is closely related to their invasive potential, proliferation and metastases ability [2]. Therefore, the mechanism of this anticancer effect can be explained by a determination of interactions between the compound with anticancer potential and the membrane of cancer cell and its effect on the biophysical parameters of membranes.

Thus, the aim of this work was to determine the effect of selected two enantiomeric iodolactones with benzelodioxol ring on physicochemical properties (like fluidity and polarity of the membrane) of cancer cells: Jurkat and GL-1 using steady-state and time resolved fluorescence spectroscopy [3]. Furthermore, measurements of the interaction of plasmid DNA with the tested lactones were performed using time correlated single photon counting mode applied into fluorescence correlation spectroscopy (TCSPC-FCS) (PicoQuant, Olympus). Additionally, the aim of this study was also to determine the potential antitumor activity of the mentioned compounds against a panel of canine lymphoma/leukemia cell lines: GL-1, CLBL-1; CLB70, CNK89, CL-1. In this study, the antiproliferative activity of the compounds was determined using MTT assay and the proapoptotic activity was evaluated by annexin V staining.

The interactions of iodolactones with membranes of cancer cells (Jurkat and GL-1) were determined using two fluorescence probes: Laurdan and DPH. The results of these studies showed that the compounds cause an increase of an order in the hydrophilic-hydrophobic region of the membrane of both cell types and a slight decrease in fluidity in the hydrophobic region relative to the GL-1 line. Fluorescence lifetime of Laurdan, labeling Jurkat cancer cells membrane does not change in the presence of both tested compounds. The results of the DNA interaction studies indicate the lack of interaction of the of both iodolactones with tested plasmid. Results of antitumor activity showed that the most sensitive lines to the tested compounds were: CLBL-1 and CLB70 - in these lines the compounds had antiproliferative and proapoptotic effects. Enantiomer of iodolactones with 4S,5R,6S configuration showed more potent anticancer activity compared with its 4R,5S,6R antipode.

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PYRIDOXAL 5'-PHOSPHATE AND ITS PRECURSOR, PYRIDOXAL HYDROCHLORIDE ARE ACTIVE AGAINST *H. PYLORI*

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Helicobacter pylori is responsible for several serious diseases: chronic active gastritis, peptic ulceration, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma. Unfortunately, standard therapies used to combat this pathogen fail in more than 20% of cases, hence there is a need for new drugs based on new molecular mechanisms [1].

Dong and Fromm [2] have shown that pyridoxal 5'-phosphate (PLP) leads to almost complete inactivation of adenylosuccinate synthetase (AdSS) from *E. coli* by formation of a Schiff base with a lysine residue. Therefore we decided to characterize interactions of PLP with AdSS from *H. pylori* 26695 strain, and the influence of PLP and its metabolic precursors on the replication of *H. pylori* in order to check if these compounds could be used for eradication of this bacterium.

Even at saturation of all enzyme substrates PLP at the concentration higher than 100 μM leads to 86% inhibition of the AdSS activity, while at concentration 13.3 μM it causes 50% inhibition. When PLP is allowed to interact with the enzyme without the GTP present, after about 4 hours at 0.03 μM it causes 50% inactivation, while 8.1 μM PLP leads to enzyme activity drop by about 97% already after 10 minutes.

Effects of PLP and its metabolic precursors, pyridoxal hydrochloride (PI-h), pyridoxine, pyridoxine hydrochloride, and pyridoxamine dihydrochloride, in the concentrations up to 5 mM, on the replication of the three *H. pylori* strains, 26695, N6 and P12 were studied as described previously [3]. Inhibition was observed only for PLP and PI-h. These compounds slow down the growth of all tested *H. pylori* strains in a similar manner (Figure 1). Determined minimal inhibitory concentrations (MICs) are 618 μg/ml (2.5 mM) and 509 μg/ml (2.5 mM), respectively.

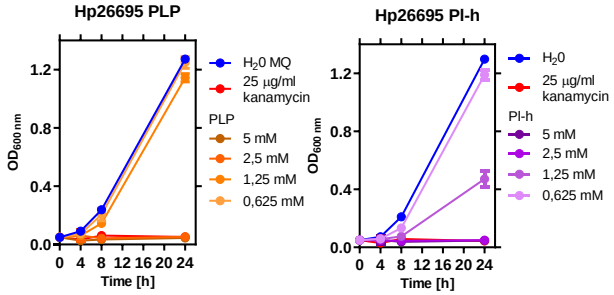

Fig.1. Growth curves of *H. pylori* 26695 in the presence of various concentrations of PLP (left panel) and PI-h (right panel).

Although these MICs are rather high, the results obtained show that PLP and PI-h are able to stop the proliferation of *H. pylori*. Since all known treatments to combat this pathogen consist of at least two drugs, the next step will be to determine joined effect of PLP and PI-h with these medicines to check if PLP and PI-h can replace one of them in the combined therapies. We also plan to obtain the X-ray structure of PLP with AdSS from *H. pylori* in order to characterize in detail the enzyme-ligand interactions and design PLP analogues with an optimized structure, causing more potent inhibition, which should result in lowering the MIC.

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IMPROVEMENT PHOTOSTABILITY OF FLUOROPHORE AND ENHANCING THE FLUORESCENCE SIGNAL ON THE MATRIX WITH SILVER NANOPARTICLES

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The aim of the work is to develop a platform for high-sensitivity fluorescence detection for biological objects on a biosensor. Our study used the phenomenon of fluorescence enhancement on the surface of metals (MEF - Metal enhanced fluorescence), which are responsible for: local field enhancement near the surface of the metal (LSPR - Localized Surface Plasmon Resonance), plasmon coupling and the effect of radiative decay engineering (RDE). [1] Localized surface plasmon resonance (LSPR) is related to the interaction of a specific wavelength of light with oscillating electrons on the surface of metal nanoparticles. The consequence of these phenomena may be shorter life times, increased quantum efficiency, and a reduced background signal level. [2] The condition for the enhancement is a very close distance between the fluorophore and the surface of metal nanoparticles (5-90nm) and the specific size of the nanoparticles, with diameters much smaller than the wavelength of the excitation light. [3] The phenomenon of metal enhanced fluorescence can be modeled by changing the size, shape, homogeneity of metal nanoparticles, as well as the distance between the nanoparticles or the method of applying them to the matrix surface. [4] The ability to scatter and absorb light by the silver nanoparticles enables the possibility to control size of the synthesized particles, by detecting the position of the absorption band assigned to the surface plasmon phenomenon, in the range of 400 to 530nm. The larger the size of the silver nanoparticles, the absorption band is shifted towards longer wavelengths.

The tested matrix consisted of properly prepared cover slips on which silver nanoparticles were deposited by a chemical synthesis method. The silver colloid islands formed on the glass surface were monitored by measuring the absorption spectra. The biosensors developed in this study must be characterized by high photostability and high enhancement of the fluorescence signal. Therefore, for testing matrices coated with silver nanoparticles, we used a photodegradable and photoreactive compound, with low quantum efficiency. The matrix we are looking for should ensure an increase in photostability and limit or fully inhibit photoreactions due to the shortening of the life time in the excited state and increase the intensity of emission several times. Our criteria are met by the hypericin that we used for this study. Hypericin is a compound naturally occurring in plants (St. John's wort), it is used as a marker in diagnostics and photodynamic therapy [5]. This compound is characterized by low quantum efficiency of fluorescence and is easily photodegradable under the influence of light. In the excited state hypericin can undergo various photochemical reactions. The study results showed the enhancement of the hypericin fluorescence signal on the surface of the matrix with silver nanoislands and a significant improvement in its photostability.

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INTERACTIONS OF PLATINUM NANOPARTICLES AND DAUNOMYCIN – BINDING, RELEASE, AND BIOLOGICAL ACTIVITY

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Nanomedicine is an interdisciplinary field attracting extensive and constantly rising attention of the scientific community. Nanoparticles, including metallic nanoparticles, are proposed to serve more and more roles in diagnostics and therapy of patients, including, but not limited to, imaging, acting as delivery vessels or therapeutics themselves [1]. Platinum nanoparticles (PtNPs), noble metal-based nanoparticles, possess features that make them extraordinary. Namely, their large surface to mass ratio coupled with high reactivity make them excellent vessels for drug delivery. What is more they are known to convert radio waves to heat,

characteristic that may be exploited in the tumor imaging [2].

Daunomycin (DAU), on the other hand, is an anthracycline drug commonly used in treatment of acute leukemias, but also other types of cancer, including breast, lung and ovarian cancers. DAU physico-chemical and biological properties are well established making the drug a perfect candidate for the analysis of potential interactions with PtNPs [1].

We used spectrofluorimetry as well as dynamic light scattering to assess interactions between chosen PtNPs (namely PtNPs with diameter of 50 and 70 nm) and DAU. The first method exploits DAU fluorescence properties and quenching induced by interactions with other molecules. The latter measures hydrodynamic diameter and allows comparison of this parameter between PtNPs alone and PtNPs-DAU mixture. Subsequently, we analyzed release of DAU from these complexes employing dialysis in three different pHs, namely natural pH of 7.4, slightly acidic pH 6.4 of cancer microenvironment and pH 5.4 of lysosomes. In order to better assess the release patters we decided to preincubate PtNPs with DAU for 24 hours. Finally, we evaluated the biological effects of DAU complexation with PtNPs using Ames test, also including the 24 h preincubation.

Observed quenching of DAU fluorescence upon titration with PtNPs as well as changes of hydrodynamic diameter of PtNPs induced by DAU indicate direct interactions and aggregation of these chemicals. Dialysis experiments revealed that DAU release from PtNPs-DAU aggregates is both pH and PtNPs size dependent. PtNPs-DAU mutagenic activity evaluated with Ames test led to conclusion that PtNPs influence DAU mutagenic activity. Observed effect is most pronounced after 24 h incubation and in the highest PtNPs concentrations.

Presented results imply aggregation of DAU with PtNPs that influences DAU biological activity. What is more, stability of the obtained aggregates appears as pH and PtNPs size dependent. These findings indicate the need to investigate interactions of different sizes of PtNPs with anticancer drugs, as such research provide knowledge on nanoformulations and may lay foundation for the new chemotherapy methods.

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INFLUENCE OF DENDRIMERS AND THEIR COMPLEXES WITH siRNA ON CELLS

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Neurodegenerative illnesses have become a global epidemic with no possibility of prevention or even permanent cure [1]. Many brain-associated diseases remain undertreated because many therapeutic molecules cannot cross the endothelial and blood-brain barriers [2].

Dendrimers are promising alternative to conventional way of delivering drugs to the brain. Since dendrimers are so highly versatile transporters, they offer a lot of scope for designing a structure carrying a particular biologically active compound. Combining dendrimers with drugs or medical nucleic acids can improve treatment outcomes by increasing the solubility of the therapeutics, modifying their pharmacokinetics, and improving bioavailability [1,2,3].

The aim of this study was to test the cytotoxic and haemolytic properties of dendrimers that allowed to select plausible nanocarrier candidate for further investigation. The selected dendrimer was then combined with siRNA directed against genes responsible for the development of Alzheimer's disease. Then the haemotoxic and cytotoxic properties of the complex were studied in the same manner. Cytotoxicity studies were performed using the MTT assay against the brain microvascular endothelial cell line HBEC-5i. The haemolytic properties were investigated using human erythrocytes.

It was found that the tested compounds significantly reduce cell viability and have haemolytic properties. Larger damage is due to the haemotoxic effect of the not complexed dendrimer than that of dendriolexes. It was observed that the dendrimer in complex with siRNA in comparison to the non-complexed dendrimer shows higher toxicity towards endothelial cells at the highest tested concentration.

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PHYSICOCHEMICAL PROPERTIES OF DENDRIMERS AND siRNA COMPLEXES

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Dendrimers, due to their unique structural features, can be purposed in many fields of science. The greatest interest of scientists is the application of dendrimers as carriers of therapeutics. One of the most important potential applications of dendrimers is their use in gene therapy as transporters of nucleic acids, such as siRNA. A promising tool for the transport of siRNAs are positively charged dendrimers. These nanoparticles are thoroughly complex with siRNAs through electrostatic interactions. Positively charged complexes have increased enzymatic resistance and enhanced cellular uptake, thereby increasing its transport into cells. Gene transfer offers the potential to provide long-lasting treatments and possibly cures for illnesses that were previously untreatable or had symptom-focused treatments [1,2]. In recent years, scientists have made great strides in better understanding the mechanisms behind the development of Alzheimer's disease, yet no therapies are available to cure Alzheimer's disease, and the available resources can only alleviate symptoms or slow its progression. Alzheimer's disease is considered a polygenic disease; however, the strongest risk factor is the common polymorphism of the three alleles of the apolipoprotein E (APOE) gene. Therefore, one way to effectively slow the disease progression is through cellular mechanisms that enable the selective silencing of specific genes [3].

This study, was performed to check whether analyzed dendrimers bind to siRNA creating complexes. In order to characterize the dendriplexes, we measured their size, zeta potential. Zeta potential and hydrodynamic diameter of the nanoparticles were measured using zetasizer. Dendrimer – siRNA interactions were performed using gel electrophoresis and circular dichroism.
Results obtained in gel electrophoresis, zeta potential, and circular dichroism show that all dendrimers interacted with siRNA creating dendriplexes.

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INTRAMOLECULAR DISULPHIDE BRIDGE IN HUMAN 4E-T AFFECTS ITS BINDING TO eIF4E1a PROTEIN

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The mRNA 5'cap is a key determinant of gene expression in eukaryotic cells, which among others is required for cap dependent translation and protects mRNA from degradation. These properties of cap are mediated by several proteins [1]. One of them is 4E-Transporter (4E-T), a big (above 100 kDa) and mostly unstructured protein, which plays an important role in translational repression, mRNA decay and P-bodies formation [2]. It is also one of several proteins that interact with eukaryotic initiation factor 4E (eIF4E), a cap binding protein, which is the main component of translation initiation machinery. 4E-T has two 4E

binding motifs at its N-terminus: canonical YXXXXLΦ and the second, non-canonical [3].
Studying the interactions between human eIF4E1a factor and the N-terminal fragment of 4E-T having both 4E binding motifs, we have observed that, under reducing conditions, 4E-T binds to eIF4E1a about 250-fold stronger than under non-reducing conditions (Table 1).

Table 1. Parameters for the complexes of human eIF4E1a protein with 4E-T(1-68) variants in the absence or presence of TCEP.

Variants of 4E-T(1-68)	TCEP	K_D [nM] from ITC	ΔT_m from DSF
h4E-T(1-68)wt	–	800 ± 96	9.5 ± 0.5
h4E-T(1-68)wt	+	2.7 ± 0.5	14.5 ± 0.5
h4E-T(1-68)C26A	–	~10	12.5 ± 0.5
h4E-T(1-68)C26A	+	1.5 ± 0.5	15.0 ± 0.5
h4E-T(1-68)C26AC50A	–	3.1 ± 0.2	14.5 ± 0.5

In contrast to 4E-T from other organisms, the N-terminal fragment of human 4E-T possesses two cysteine residues able to form a disulphide bridge, located before and after the canonical 4E-binding motif.

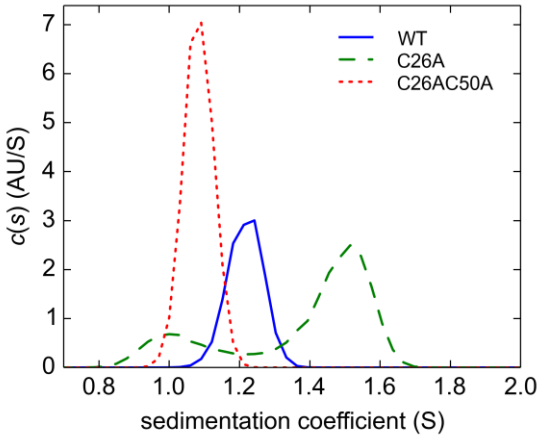

Fig.1. Sedimentation coefficient distribution c(s) of h4ET(1-68) variants under non-reducing conditions.

The analysis of wild-type 4E-T and its single (C26A) and double (C26AC50A) cysteine mutants by sedimentation velocity experiments (Fig. 1) and size-exclusion chromatography has shown that wild-type 4E-T forms an intramolecular disulphide bridge which probably blocks access to the canonical 4E binding motif.

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Historical session

PAMIĘCI TRZECH WIELKICH BIOFIZYKÓW: DAVIDA SHUGARA, STANISŁAWA PRZESTALSKIEGO I LECHA WOJTCZAKA

K. Dołowy

Katedra Fizyki i Biofizyki, Instytut Biologii, SGGW

David Shugar (1915-2015) urodził się w Polsce, ale wkrótce wyemigrował wraz z rodzicami do Kanady. ukończył studia i doktoryzował się z fizyki na Uniwersytecie McGilla w Montrealu. W czasie wojny pracował nad urządzeniami do zwalczania łodzi podwodnych. Podczas ery makkartyzmu aresztowano go i oskarżono o szpiegostwo. Po uniewinnieniu przeniósł się do Instytutu Pasteura w Paryżu, a w roku 1952 został zaproszony przez prof. Leopolda Infelda do objęcia Zakładu Biochemii w Państwowym Zakładzie Higieny w Warszawie oraz pracował w Instytucie Biochemii i Biofizyki PAN. W roku 1965 stworzył Katedrę Biofizyki na Wydziale Fizyki Uniwersytetu Warszawskiego. Zajmował się biofizyką molekularną: kinetyką enzymów i budową kwasów nukleinowych. Był Przewodniczącym Polskiego Towarzystwa Fizyki Medycznej.

Stanisław Przestalski (1927-2017) ukończył studia z fizyki na Uniwersytecie Wrocławskim w 1951 i tam doktoryzował się w 1964 roku. Po studiach został asystentem profesora Jana Nikliborca, który w 1952 zaproponował mu zorganizowanie Katedry Fizyki (przemianowanej później Katedrę Fizyki i Biofizyki) w nowo tworzonej Wyższej Szkole Rolniczej (później przemianowanej kolejno na Akademię Rolniczą i Uniwersytet Przyrodniczy). Był jej kierownikiem przez 45 lat. Sławne były organizowane przez niego w latach 70tych, 80tych i 90tych Szkoły: Biophysics of Membrane Transport, co było głównym tematem jego zainteresowań naukowych. Był Przewodniczącym Polskiego Towarzystwa Biofizycznego.

Lech Wojtczak (1926-2019) ukończył studia z biologii na Uniwersytecie Łódzkim w 1950. Od 1947 pracował w Instytucie Biologii Doświadczalnej im. Marcelego Nenckiego włączonym w 1952 w struktury Polskiej Akademii Nauk. W 1954 otrzymał na Uniwersytecie Łódzkim stopień kandydata nauk. W IBD PAN kierował kolejno pracowniami: Izotopów (1961–1963), Enzymologii (1963–1971), Biochemii Lipidów i Błon Biologicznych (1971–1991), Bioenergetyki, Błon Biologicznych i Regulacji Metabolizmu. W swoich badaniach zajmował się własnościami błony mitochondrialnej i przemianami energetycznymi w komórce zwierzęcej. Był Przewodniczącym Polskiego Towarzystwa Biochemicznego.

POLSKIE TOWARZYSTWO BIOFIZYCZNE: TOWARZYSTWO SPRZYJAJĄCE TWÓRCZOŚCI, PRZYJAZNE I Z KLASĄ – Z PAMIĘTNIKA BIOFIZYKA

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Moją bramą do Polskiego Towarzystwa Biofizycznego, czyli naszego PTBF, był Oddział Lubelski. Funkcję przewodniczącego oddziału sprawował wówczas pan prof. Adam Paszewski, emerytowany już profesor Uniwersytetu Marii Curie-Skłodowskiej, człowiek o śmiałych poglądach, obdarzony niezwykłą charyzmą i poczuciem humoru. My staraliśmy się rezonować z jego osobowością i wyznawanymi wartościami, co sprawiało, iż Towarzystwo postrzegałem jako grono osób nie tylko obdarzonych pasją poznawczą, ale również ludzi z klasą! Stanowiło to swojego rodzaju rekompensatę za aktywność w obszarze nauki postrzeganej przez wielu jako niedookreślony, przez co mniej wartościowy. Wkrótce zorientowałem się, że każdy z oddziałów PTBF w kraju ma podobnych naszemu, swoich mentorów, gwiazdy promieniujące jaskrawym i ciepłym blaskiem na firmamencie polskiej biofizyki. Nieprzypadkowo użyłem określenia „ciepłym", bowiem wielu z nas, młodych adeptów nauki, mogło się przekonać, iż tuzy naszej dyscypliny naukowej, na przekór pierwszej impresji o ich surowości, okazywali się ludźmi nie tylko przystępnymi, ale również życzliwymi. Któż z nas, wygłaszających wówczas swoje pierwsze seminaria, komunikaty konferencyjne czy też pracujących nad rozprawą doktorską nie spotkał się z wyrozumiałością, życzliwością oraz daleko idącym wsparciem merytorycznym starszych wiekiem oraz będących na wyższych szczeblach kariery akademickiej kolegów. Z licznego grona „mistrzów" w obszarze polskiej biofizyki przywołam może jedynie kilka: panią prof. Danutę Frąckowiak z Poznania, pana prof. Stanisława Przestalskiego z Wrocławia czy też panią prof. Wandę Leyko z Łodzi. Uważam siebie za szczęściarza, że mogłem poznać te znaczące osobowości, spotkać się z ich mądrością i zaznać ich legendarnej życzliwości. Z perspektywy lat mogę powiedzieć, że taka postawa okazała się dla młodych swoistym zobowiązaniem, „pozytywnym" wirusem, jako przekazywana na kolejne pokolenia profesorów biofizyki. Myślę, że wiele mądrości jest w stwierdzeniu, iż dobry, życzliwy i pogodny klimat współpracy kształtuje pozytywnie poziom twórczości, a przecież właśnie twórczość nadaje sens naszej aktywności naukowej. Chciałoby

się powiedzieć: szczególnie w obszarze biofizyki, która stanowi najbardziej fascynujący obszar badawczy. Nie zrobię jednak tego zważając, że biofizycy są również ludźmi skromnymi.

KRÓTKA HISTORIA POCZĄTKÓW POLSKIEJ BIOFIZYKI

B. Lesyng[1,2]

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Biofizyka jako dziedzina wiedzy została jak się wydaje po raz pierwszy nazwana przez Davida Burnsa, wykładowcę chemii fizjologicznej na Uniwersytecie w Glasgow, autora książki *An Introduction to Biophysics*, wydanej w roku 1921. Z kolei Max-Planck Institut fuer Biophysik w swojej informacyjnej broszurze podkreśla rolę Borisa Rajewskiego, który w roku 1923 stworzył pojęcie „biophysik" jako dziedziny obejmującej m.in. badania wpływu promieni rentgenowskich na organizmy żywe. Można się domyślać, że motywacją do wprowadzenia takiego określenia były m.in. osiągnięcia i Nagroda Nobla w roku 1903 dla Marii i Pierre Curie za badania nad odkrytym przez Becquerela zjawiskiem promieniotwórczości i po raz drugi w roku 1911 dla Marii Curie za odkrycie polonu i radu, a dalej wykorzystania „promieni X" w medycynie. Z tego też powodu potrzeby medycyny stały się pierwszym motorem rozwoju dziedziny wiedzy, który obecnie nazywamy biofizyką. W Polsce po raz pierwszy intelektualny potencjał tej dziedziny dostrzegł fizyk, prof. Stanisław Kalandyk, który na przełomie 1921/1922 stworzył Katedrę Biofizyki na Wydziale Lekarskim Uniwersytetu Poznańskiego. Prof. Kalandyk własnym nakładem w roku 1934 wydał „Podręcznik fizyki dla medyków i biologów". W tym też okresie Maria Skłodowska-Curie przekazuje 1 gram radu Instytutowi Radowemu (obecnie Narodowy Instytut Onkologii im. Marii Skłodowskiej-Curie – Państwowy Instytut Badawczy w Warszawie), co uruchomiło w Polsce badania i zastosowania, które obecnie nazwalibyśmy biofizyką radiacyjną.

Prawdziwym katalizatorem zastosowania metodologii i formalizmu nauk fizycznych w innych naukach przyrodniczych - w tym w biologii, była wszakże książka *What is Life* wydana w roku 1944 przez światowej klasy fizyka i noblisty, Erwina Schroedingera. Od tego też czasu obserwuje się wzrastające i znaczące zainteresowanie fizyków naukami biologicznymi i biomedycznymi.

Jeżeli chodzi o wspomnianą wyżej Katedrę Biofizyki, to w czasie okupacji została ona zlikwidowana ale otworzona zaraz wojnie i we wrześniu 1945 roku doc. Cezary Pawłowski, który został jej kierownikiem, rozpoczął wykłady dla studentów Wydziału Lekarskiego. Z historią Katedry z okazji jej 100-lecia można dokładniej zapoznać się w materiałach Uniwersytetu Medycznego im. Karola Marcinkowskiego w Poznaniu [1].

Poniższy krótki przegląd obejmuje okres do roku 2000 i też w niepełnym zakresie. Stworzenie pełnego przeglądu musiałoby być znacznie obszerniejsze i jest naturalnie też znacznie trudniejsze. Ta historia może wszakże stanowić punkt wyjścia do takiej przyszłej analizy.

Istotnym elementem rozwoju polskiej biofizyki było utworzenie w roku 1957 Instytutu Biochemii i Biofizyki PAN w Warszawie (patrz: https://ibb.edu.pl/en/institute/history/) oraz w roku 1966 przez prof. Davida Shugara (http://www.biogeo.uw.edu.pl/shugar_en.html) pierwszej w Polsce i jednej z pierwszych w Europie Katedry Biofizyki (później Zakład Biofizyki IFD) na Wydziale Fizyki UW. Historia rozwoju nauk fizycznych na UW, w tym biofizyki na Wydziale Fizyki, została opisana przez prof. A.K. Wróblewskiego w zbiorowym opracowaniu na 200-lecie UW [2], jak również przez dr Macieja Gellera z okazji 75-lecia Wydziału Fizyki UW [3]. Kolejnym zakładem na wydziale fizyki, był Zakład Biofizyki Molekularnej Instytutu Fizyki UAM, zorganizowany w roku 1988 przez profesorów Adama Patkowskiego oraz Andrzeja Dobka, którzy byli jego pierwszymi kierownikami.

Można zadać sobie pytanie - kim właściwie jest biofizyk ? Pozwolę sobie przytoczyć tutaj żartobliwe powiedzenie prof. Shugara, że biofizykiem jest każdy naukowiec zajmujący się biofizyką, który na dodatek ma na to jeszcze dwóch świadków. Analizując zatem wczesne prace polskich biofizyków wyobrażam sobie że teraz ja mogę być jednym takim „świadkiem", a ponieważ do czasu rozpoczęcia konferencji może nie pojawić się „drugi świadek" mogę więc dość swobodnie klasyfikować zarówno prace biofizyczne jak i polskich biofizyków.

Z punktu widzenia aktualnej klasyfikacji w dziedzinie nauk ścisłych i przyrodniczych mamy dwa rodzaje biofizyk, przypisane odpowiednio do nauk fizycznych oraz nauk biologicznych. Osobiście, trochę chętniej czytam prace tej „pierwszej biofizyki".

W latach 1951 – 1972 w szeregu ośrodkach naukowych powstawały zespoły uprawiające badania w następujących obszarach:

- biofizyka (makro)cząsteczek biologicznych,
- bioenergetyka,
- biofizyka komórek, tkanek i narządów ze szczególnym uwzględnieniem biofizyki membran biologicznych,
- biofizyka teoretyczna/matematyczna oraz
- biofizyka radiacyjna.

W szczególności powstawały następujące zespoły: w Poznaniu kierowany przez prof. Danutę Frąckowiak, w Łodzi przez prof. Wandę Leyko, w Krakowie przez prof. Wojciecha Froncisza, we

Wrocławiu przez profesorów Wacława Hendricha i Stanisława Przestalskiego oraz w pewnym zakresie przez profesorów Lucjana Sobczyka i Henryka Chojnackiego, w Toruniu przez prof. Józefa S. Kwiatkowskiego, na Śląsku przez prof. Tadeusza Wilczoka a w Warszawie, poza prof. Shugarem, również przez profesorów Kazimierza L. Wierzchowskiego i Jana W. Doroszewskiego. W nieco późniejszym okresie (1976) prof. Ewa Skrzypczak, we współpracy z prof. Doroszewskim, stworzyła Pracownię Fizyki Biomedycznej (później Zakład Fizyki Biomedycznej IFD) - ponownie na Wydziale Fizyki UW. Dla studentów biofizyki wykładali tacy wybitni profesorowie jak kwantowy chemik Włodzimierz Kołos oraz molekularny biolog i biofizyk prof. Lech K. Wojtczak.
Informacje uzupełniające do tego omówienia zawarte są w „Kalendarium początków rozwoju biofizyki w Polsce", który stanowi załącznik do tego omówienia.

Istotnym etapem rozwoju polskiej biofizyki było powstanie Polskiego Towarzystwa Biofizycznego (PTBF), które w tym roku obchodzi 50-cio lecie swojego istnienia i zorganizowanie przez łódzkie środowisko naukowe pod przewodnictwem prof. Wandy Leyko roku 1973 w Uniejowie I-go Zjazdu PTBF. Kolejne zjazdy były zorganizowane ponownie w Uniejowie (1976), w Oleśnicy k/Wrocławia (1978), w Kozubniku k/Katowic (1979) i w Książu k/Wrocławia (1983). Przerwa, która się pojawiła, wynikała z wprowadzeniu w roku 1981 stanu wojennego w Polsce. Należy również podkreślić, że w roku 1976 pod redakcją Wandy Leyko został wydany pierwszy tom wydawnictwa PTBF, „Zagadnienia Biofizyki Współczesnej". Kolejne tomy były wydawane corocznie do roku 1991. W roku 1992 wydawnictwo zostało zmienione na formę angielskojęzyczną o nazwie *Current Topics in Biophysics*. Pierwszymi edytorami tej wersji byli F. Jaroszyk, P. Jaśkowski, D. Frąckowiak, S. Przestalski, oraz A. Dobek (więcej szczegółów w „Kalendarium").

W międzyczasie odbyło się szereg bardzo wartościowych dla rozwoju biofizyki i zbliżonych nauk interdyscyplinarnych konferencji i szkół. Należy m.in. do nich, począwszy od roku 1972, seria konferencji zorganizowana przez prof. Macieja Wiewiórowskiego i jego współpracowników w Dymaczewie k/Poznania na temat *Synteza, struktura i właściwości transferowych kwasów nukleinowych*. Od roku 1976 (lub trochę wcześniej) konferencje te przybrały międzynarodowy charakter pod nazwą *Synthesis, Structure and Chemistry of Transfer Ribonucleotide Acid and their Components*. W późniejszym okresie, w latach 1985-1993, organizowane były międzynarodowe konferencje przez środowiska poznańskie, krakowskie i wrocławskie o bardziej ogólnym profilu pt. *Spectroscopy, Structures and Functions of Materials and Systems of Fundamental Importance to Biology and Medicine*". Podobna tematyka skoncentrowana na kwasach nukleinowych była też przedmiotem dużej konferencji zorganizowanej z okazji 80-ciolecia prof. Shugara przez IBB PAN oraz Zakład Biofizyki w Warszawie (1995, *Symposium on Structure and Biological Functions of Nucleic Acid Components and their Analogues, and Related Topics*). Z kolei we Wrocławiu profesorowie Przestalski i Wierzchowski począwszy od roku 1974 organizowali zimowe szkoły „*Winter Schools of Biophysics*", które w miarę upływu czasu poświęcane były tematyce błon biologicznych (rok 1976, *Biophysics of Membrane Transport*). Z kolei środowisko warszawskie kładło trochę większy nacisk na strukturę i funkcje białek organizując np. w roku 1993 w Zaborowie k/Warszawy sympozjum zatytułowane *Struktura białek i konformacja peptydów* oraz szereg innych konferencji poświęconych tej tematyce.

Intensywny rozwój strukturalnych i funkcjonalnych badań złożonych układów biomolekularnych z wykorzystaniem m.in. metod molekularnej spektroskopii był motywacją wydania w roku 1989/90 bardzo dobrej 5-cio tomowej monografii zatytułowanej *Biospektroskopia*, pod redakcją dr hab. Jacka Twardowskiego z Uniwersytetu Jagiellońskiego.

Na szczególną uwagę zasługują dwa ciągi międzynarodowych konferencji: *Molecular Aspects of Chemotherapy* oraz *International Conferences on Inhibitors of Protein Kinases* (IPK). Pierwszy ciąg konferencji w odstępach dwuletnich od roku 1986 organizował w okolicach Gdańska prof. Edward Borowski ze współpracownikami i we współpracy z prof. D. Shugarem, drugą serię od 1998 roku w Warszawie, również w odstępach dwuletnich organizował prof. D. Shugar ze współpracownikami. Należy podkreślić, że materiały konferencji były na ogół drukowane w czasopismach o zasięgu międzynarodowym. W przypadku pierwszych konferencji IPK materiały były drukowane w *Pharmacology and Therapeutics*, czasopiśmie którego IF wynosi obecnie powyżej 12. Przegląd badań oraz inicjatyw wykraczających również poza warszawskie środowisko naukowe zostało zreferowane w krótkich przeglądowych pracach profesorów K. L. Wierzchowskiego (IBB PAN) oraz R. Stolarskiego (Katedra Biofizyki -aktualnie Zakład Biofizyki IFD - Wydziału Fizyki UW) z okazji 100-nych urodzin prof. Shugara, [4-5]. Ponadto krótka historia konferencji kinazowych została zreferowana w tym samym tomie przez B. Lesynga [6].

Należy dodatkowo zwrócić uwagę, że dwójka wybitnych badaczy struktur układów biomolekularnych, w tym odkrywców struktury proteazy wirusa HIV-1, dr Maria Miller oraz prof. Alexander Włodawer, pracownicy *National Laboratory for Cancer Research, Frederick, USA*, to absolwenci lat 60/70 specjalizacji biofizyki na Wydziale Fizyki UW, a prof. Władysław Minor, (*Distinguished Professor Molecular Physiology and Biological Physics*) na *University of Virginia* to absolwent specjalizacji fizyki ciała stałego w tym samym okresie, również na Wydziale Fizyki.

Oddzielnym wątkiem jest powstanie i rozwój zespołów teoretycznej biofizyki molekularnej a później również bioinformatyki. W latach 70-tych ośrodki rozwijające oraz stosujące metody chemii kwantowej lub jak kto woli molekularnej mechaniki kwantowej, stanowiły istotną podstawę biofizyki teoretycznej układów biomolekularnych wszelkiego rodzaju. Ośrodkami tymi był przede wszystkim zespół prof. Włodzimierza Kołosa na Wydziale Chemii Uniwersytetu Warszawskiego oraz zespoły prof. Józefa. S Kwiatkowskiego i Wiesława Woźnickiego na Wydziale Matematyki, Fizyki i Chemii Uniwersytetu Mikołaja Kopernika w Toruniu, zespół prof. Henryka Chojnackiego na Wydziale Chemicznym Politechniki Wrocławskiej i częściowo prof. Lucjana Sobczyka na Uniwersytecie Wrocławskim, jak również zespoły prof. Kazimierza Gumińskiego oraz Alojzego Gołębiewskiego na Uniwersytecie Jagiellońskim. W szczególności w latach 1976-1989 ośrodek toruński organizował w Bachotku świetne letnie szkoły zatytułowane *Zaawansowane metody chemii kwantowej* w których uczestniczyli młodzi pracownicy naukowi teoretycznej chemii i fizyki (w tym teoretyczni biofizycy) wielu ośrodków akademickich. Uczestniczką tych szkół jako słuchacz była m.in. dr Angela Merkel, późniejsza kanclerz RFN i jej przyszły mąż dr Joachim Sauer (aktualnie profesor na Uniwersytecie Humboldta w Berlinie).

Korzystając z doświadczeń i oprogramowania zespołów prof. W. Kołosa oraz J. S. Kwiatkowskiego, jak również ze wsparciem prof. D. Shugara, zaawansowane jak na tamte czasy metody mechaniki kwantowej zostały szybko zaimplementowane i dalej rozwijane w Katedrze Biofizyki (Później Zakład Biofizyki IFD) przez doktorów Macieja Gellera, Bogdana Lesynga i Andrzeja Pohorille oraz ich współpracowników. Zespół ten przez szereg lat posiadał najbardziej zaawansowaną w Polsce lokalną infrastrukturę informatyczną służącą badaniom teoretycznym. Zarówno ta infrastruktura jak i doświadczenia w dziedzinie informatyki stosowanej oraz współpraca Bogdana Lesynga w latach 90-tych z Markiem Niezgódką z Wydziału Matematyki, Informatyki i Mechaniki Uniwersytetu stanowiły podstawę utworzenia pierwszego w Polsce centrum superkomputerowego - Interdyscyplinarnego Centrum Modelowania Matematycznego i Komputerowego UW (ICM UW). Przegląd teoretycznych modeli układów biomolekularnych i stosowanych technik symulacyjnych lat 80-tych i 90-tych zawarty jest w pracach [7-8]. Z tego środowiska wywodzą się teoretyczni biofizycy oraz bioinformatycy pracujący w doskonałych ośrodkach naukowych na świecie jak: Andrzej Pohorille (*NASA Ames Research Research Center* oraz *University of California San Francisco*), Krzysztof Fidelis (*Lawrence Livermore National Laboratory*, później *University of California at Davies*), Adam Godzik (*The Scripps Research Institute*, później *University of California Riverside*), Krzysztof Kuczera (*Harvard University*, później *The University of Kansas at Lawrence*) czy Tomasz Wesołowski (*University of Southern California* w Los Angeles, później *Université de Genève*).

Środowisko teoretycznej biofizyki wsparło zarówno intelektualnie jak i osobowo środowisko polskich bioinformatyków. Historia rozwoju polskiej bioinformatyki opisana została w przeglądowej pracy [9] przez profesorów Janusza Bujnickiego (Międzynarodowy Instytut Molekularnej i Komórkowej Biologii w Warszawie) oraz Jerzego Tiuryna (Wydział Matematyki, Informatyki i Mechaniki UW).

Na podkreślenie zasługuje też fakt, że pierwszy Festiwal Nauki w Polsce został w roku 1997 zorganizowany z dominującym udziałem biofizyków środowiska warszawskiego. Pomysłodawcą był prof. D. Shugar, który rok wcześniej w czasie swojego pobytu w Edynburgu zapoznał się z tamtym festiwalem. Pierwsza wstępna decyzja została podjęta w gronie trzech osób, D. Shugara, B. Lesynga oraz matematyka M. Niezgódki. W maju lub czerwcu 1996 roku doszło do spotkania tymczasowego komitetu organizacyjnego złożonego z 12-tu przedstawicieli różnych dziedzin wiedzy. Dyrektorem pierwszych festiwali został dr hab. Maciej Geller a przewodniczącą Rady Programowej prof. Magdalena Fikus – również biofizycy. W następnych latach praktycznie wszystkie większe ośrodki naukowe w Polsce organizowały i organizują dalej festiwale nauki.

Ograniczenia na rozmiar tekstu oraz bardzo duży zakres biofizycznych badań realizowanych w okresie późniejszym, nie pozwalają na szczegółową analizę tej tematyki. Pozwolę więc sobie tylko wymienić nazwiska młodzieży lat 70-tych, która doskonale bawiła się na konferencjach i w/w szkołach, którzy niestety są już teraz na emeryturze lub są bliscy emerytury, którzy jednak stworzyli nowe prężne zespoły i/lub kontynuowali tematykę szkół swoich dawnych mistrzów. Są to m.in. tacy profesorowie jak: Jan Antosiewicz, Edward Darżynkiewicz, Agnieszka Bzowska oraz Ryszard Stolarski (Zakład Biofizyki IFD, Wydział Fizyki UW), Wojciech Bal, Jarosław Poznański oraz Piotr Zielenkiewicz (IBB PAN, Warszawa), Katarzyna Cieślak-Blinowska (Zakład Fizyki Medycznej, Wydział Fizyki UW), Andrzej Dobek, oraz Adam Patkowski (Wydział Fizyki, UAM, Poznań), Krzysztof Dołowy (Katedra Fizyki i Biofizyki, Szkoła Główna Gospodarstwa Wiejskiego w Warszawie), Włodzisław Duch oraz Wiesław Nowak (Wydział Fizyki, Astronomii i Informatyki Stosowanej, UMK, Toruń), Wiesław Gruszecki (Katedra Biofizyki IF UMCS w Lublinie), Mariusz Jaskólski (Wydział Chemii, UAM, Poznań), Andrzej Koliński oraz Krzysztof Wożniak (Wydział Chemii, UW), Adam Liwo (Wydział Chemii, Uniwersytet Gdański), Marta Pasenkiewicz-Gierula i Tadeusz Sarna (Wydział Biochemii, Biofizyki i Biotechnologii, UJ), Andrzej Sokalski (Wydział Chemii, Politechnika

Wrocławska), Zbigniew Wieczorek (Katedra Fizyki i Biofizyki, Uniwersytet Warmińsko-Mazurski, Olsztyn), jak również nieżyjący już Marek Cieplak, który stworzył prężne Środowiskowe Laboratorium Fizyki Biologicznej w Instytucie Fizyki PAN, w Warszawie.

Autor zdaje sobie sprawę, że nie wszystkie istotne elementy historii rozwoju polskiej biofizyki zostały w tym krótkim przeglądzie podjęte. Wynika to zarówno z ograniczeń czasowych w jakich ten przegląd miał powstać jak i z braku szczegółowej wiedzy o aktywności naukowej i organizacyjnej innych ośrodków akademickich. Braki te zostaną uzupełnione w procesie tworzenia wspólnego serwisu informacyjnego PTBF.

Na zakończenie należy również zwrócić uwagę, że powyższy krótki przegląd zawiera jedynie marginalne informacje na temat akademickiej, edukacyjnej aktywności środowiska biofizyków. Zawarcie pełniejszej informacji musiałoby znacznie rozszerzyć ten krótki przegląd, a jego przygotowanie również wymaga znacznie więcej czasu. Tematyka ta będzie najprawdopodobniej przedmiotem oddzielnej analizy jednego z naszych biofizyków seniorów.

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