Posters

MOLECULAR DYNAMICS SIMULATIONS OF α-SYNUCLEIN AGGREGATION

M. M. Anila [1], B. Różycki[2], M. Chwastyk[1]

[1]Laboratory of Biological Physics, Institute of Physics, Polish Academy of Sciences, Aleja Lotników 32/46, 02-668 Warsaw, Poland
[2]Division of Theoretical Physics, Institute of Physics, Polish Academy of Sciences, Aleja Lotników 32/46, 02-668 Warsaw, Poland

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.

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

M. Araszkiewicz[1,2], U. Kaźmierczak[3], A. Korgul[1], K. Maliszewska-Olejniczak[4], Z. Szefliński[3], K. Tymińska[2]

[1] Faculty of Physics, University of Warsaw, Pasteura 5, 02-093 Warsaw, Poland
[2] National Centre for Nuclear Research, Andrzeja Sołtana 7, 05-400 Otwock, Świerk, Poland
[3] Heavy Ion Laboratory at the University of Warsaw, Pasteura 5A, 02-093 Warsaw, Poland
[4]Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences – SGGW, Nowoursynowska 166, 02-787 Warsaw, Poland

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.

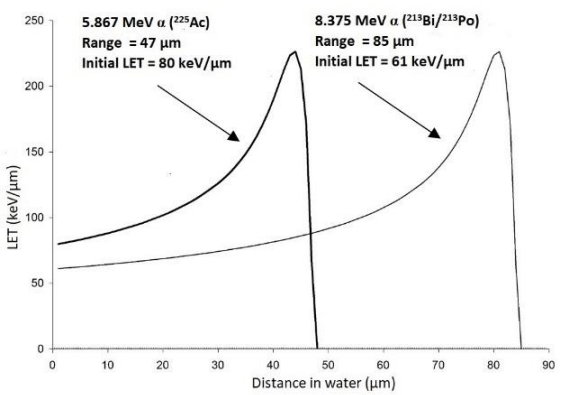

Fig.1. Changes in LET with distance in water (tissue) for 2 α-particles with different initial kinetic energies [3].

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

P. Bełdzińska[1], K. Butowska[1,2], G. Gołuński[1], M. Jamrógiewicz[3], A. Moliński[4,5], J. Piosik[1]

[1]Laboratory of Biophysics, Intercollegiate Faculty of Biotechnology UG&MUG, University of Gdańsk, Gdańsk, Poland
[2]Department of Cellular and Molecular Medicine, School of Medicine, University of California San Diego, La Jolla, San Diego, California, USA
[3]Department of Physical Chemistry, Faculty of Pharmacy, Medical University of Gdańsk, Gdańsk, Poland
[4]Department of Biomedical Physics, Faculty of Physics, Adam Mickiewicz University in Poznań, Poznań, Poland
[5]NanoBioMedical Centre, Adam Mickiewicz University in Poznań, Poznań, Poland
e-mail: patrycja.beldzinska@phdstud.ug.edu.pl

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

A. Budzińska, K. Wójcicki, Ł. Gałgański, W. Jarmuszkiewicz

Laboratory of Mitochondrial Biochemistry, Department of Bioenergetics, Faculty of Biology, Adam Mickiewicz University, Uniwersytetu Poznanskiego 6, 61-614 Poznan, Poland

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

M. Chwastyk, M. Cieplak

Laboratory of Biological Physics, Institute of Physics, Polish Academy of Sciences, Aleja Lotników 32/46, 02-668 Warsaw, Poland

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.

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

H. Cichocka[1], J. Wierzchowski[2], A. Stachelska-Wierzchowska[2], B. Wielgus-Kutrowska[1]

[1] Division of Biophysics, Institute of Experimental Physics, University of Warsaw, Pasteura 5, 02-093 Warsaw, Poland;
[2]Depratment of Biophysics, University of Warmia & Mazury in Olsztyn, 4 Oczapowskiego St., 10-721 Olsztyn, Poland

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

A. Czuba, K. Rozpędek, A. Kafel, A. Pompka

Faculty of Natural Sciences, University of Silesia, Katowice, Poland

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

A. Dąbrowska, A. Łukasiak, M. Zając, P. Bednarczyk

Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences, ul. Nowoursynowska 159, 02-776 Warsaw

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

M. Duszka[1], M. F. Rode[2], A. Niedźwiecka[1]

[1]Laboratory of Biological Physics and [2]Molecular Photophysics Group, Institute of Physics, Polish Academy of Sciences, Aleja Lotnikow 32/46, PL-02668, Warsaw, Poland

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

P. Filipczuk, A. Dembska

Bioanalytical Chemistry Department, Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland

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]

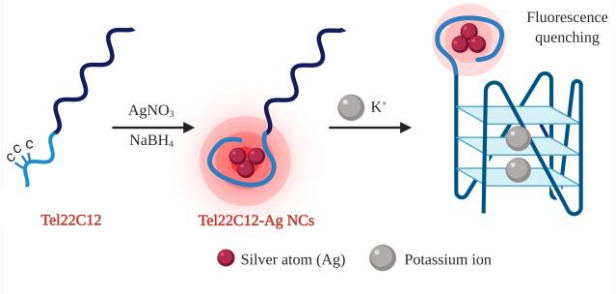

Figure 1. The demonstrative illustration shows the working idea of Tel22C12-AgNCs as potassium sensor [6].

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

J. Grzyb, J. Maciejewski, J. Sławski

Department of Biophysics, Faculty of Biotechnology, University of Wrocław, ul. F. Joliot-|Curier 14a, 50-387 Wrocław, Poland

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.

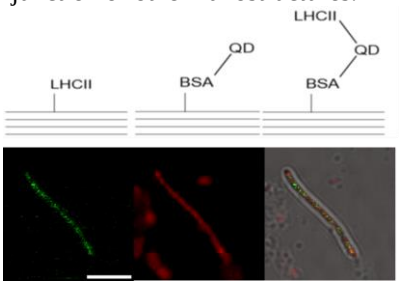

Fig. 1. Scheme of some possible junction versions (upper panel) and (lower panel) an example of a confocal microscope image of a nanotube decorated with QD (green emission, 520-587 nm) and LHCII (red emission, 625-715 nm). Excitation was set at 471 nm. Last image is the overlay of green and red channel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

ACKNOWLEDGMENTS

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

J. Hoser, A. Dąbrowska, P. Bednarczyk

Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences – SGGW

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.

ACKNOWLEDGMENTS

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

S. Jaworowska, P. Bednarczyk, A. Łukasiak

Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences, Warsaw, Poland

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.

ACKNOWLEDGMENTS

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

E. Kalwarczyk, D. Woźnica, S. Jakiela

Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences – SGGW, Warsaw, Poland

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.

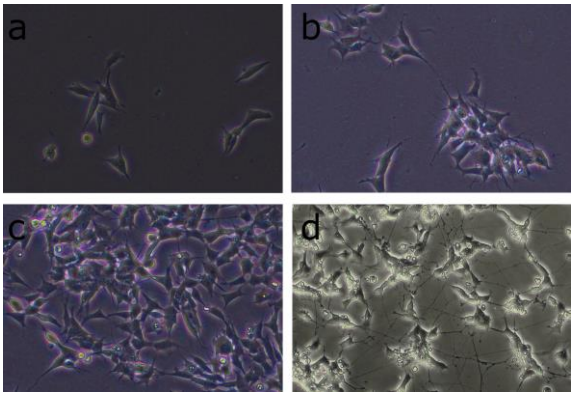

Fig.1. Phase contrast microscopy images of undifferentiated SH-SY5Y cells after 1 (a), 3 (b) and 7 (c) days of culture growth. Cells were seeded with concentration $5x10^4$ cells/ml and cultivated on standard 60 mm PS culture plates. RA-differentiated SH-SY5Y cells after 8 days of RA-treatment (d).

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

A. Karpińska, A. Zgorzelska, K. Kwapiszewska, R. Hołyst

Department of Soft Condensed Matter, Institute of Physical Chemistry PAS, 01-224, Warsaw, Poland

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).

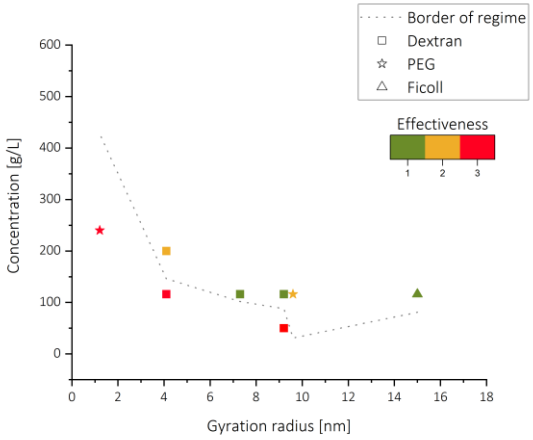

Fig.1. Dependence of polymer concentration on its gyration radius. The use of the polymer in the entangled regime was critical for the efficiency of the cellular delivery process [2].

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

A. Kasparek, B. Smyk

Department of Physics and Biophysics, University of Warmia and Mazury in Olsztyn, Oczapowskiego 4, 10-719 Olsztyn, Poland

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

B.P. Klepka, A. Niedźwiecka

Laboratory of Biological Physics, Institute of Physics, Polish Academy of Sciences, Aleja Lotnikow 32/46, PL-02668, Warsaw, Poland

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

J. Krasowska, B. Wielgus-Kutrowska

Division of Biophysics, Faculty of Physics, Institute of Experimental Physics, University of Warsaw, Pasteur 5, 02-093 Warsaw, Poland

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

M. Kubczak[1], M. Karimov[2], A. Ewe[2], K. Miłowska[1], A. Aigner[2], M. Ionov[1], M. Bryszewska[1]

[1]Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Poland, PomorskaStr. 141/143, 90-236 Lodz. malgorzata.kubczak@biol.uni.lodz.pl, katarzyna.milowska@biol.uni.lodz.pl; maria.bryszewska@biol.uni.lodz.pl, maksim.ionov@biol.uni.lodz.pl
[2]Rudolf-Boehm-Institute for Pharmacology and Toxicology, Clinical Pharmacology, Faculty of Medicine, Leipzig University, Germany, HärtelStr. 16-18, 04107 Leipzig. michael.karimov@medizin.uni-leipzig.de, alexander.ewe@medizin.uni-leipzig.de, achim.aigner@medizin.uni-leipzig.de

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.

ACKNOWLEDGMENTS

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EXPLORING THE PARAMETER SPACE, CONDITIONS FOR TURING PATTERNS

H. Larraguível, M. Zagórski

University of Warsaw and Jagiellonian University

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).

ACKNOWLEDGMENTS

Our work was financed by the Polish National Agency for Academic Exchange (NAWA), Polish Returns "Principles of information decoding in developmental systems".

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

A.Lewkowicz[1], P. Bojarski[1], M. Pierpaoli[2], K. Walczewska-Szewc[3], M. Czarnomska M[4], L. Kułak[5], S. Pogorzelski[1], R. Bogdanowicz[2]

[1]Faculty of Mathematics, Physics, and Informatic, University of Gdańsk; 57 Wita Stwosza Street, 80-308 Gdańsk, Poland; aneta.lewkowicz@ug.edu.pl, piotr.bojarski@ug.edu.pl
[2]Faculty of Electronics, Telecommunications, and Informatics, Gdańsk University of Technology, 11/12 Gabriela Narutowicza Street, 80-233 Gdańsk, Poland;
[3]Institute of Physics, Faculty of Physics, Astronomy, and Informatics, Nicolaus Copernicus University in Toruń, 5 Grudziądzka Street, 87-100 Toruń, Poland;
[4]Faculty of Law and Administration, University of Gdańsk; 6 Jana Bażyńskiego Street, 80-309 Gdańsk, Poland;
[5]Faculty of Applied Physics, Gdańsk University of Technology 11/12 Gabriela Narutowicza Street, 80-233 Gdańsk, Poland;

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].

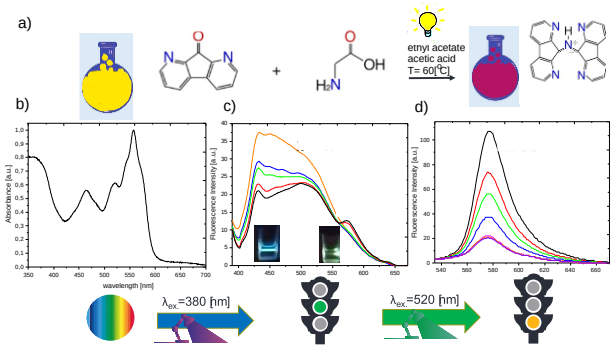

Fig.1. The reaction of DFO with glycine-amino acid (a) and the absorption spectra (b), fluorescence spectra, λex. = 380 nm (c), and fluorescence spectra, λex. = 520 nm (d) of the reaction mixture: DFO with glycine (the molar ratio: 2:1) in ethanol [4]

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.

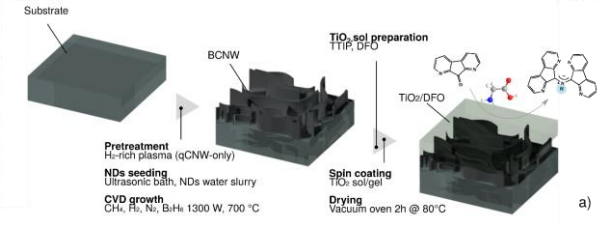

Fig.2. (a) Schematic of the electrode realization.

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

M. Lobka[1,2], J. Macyszyn[2,3], M. Burmistrz[2], J. Trylska[2]

[1] Faculty of Physics, University of Warsaw, Pasteura 5, Warsaw 02-093, Poland,
[2] Centre of New Technologies, University of Warsaw, Banacha 2C, Warsaw 02-097, Poland,
[3] Doctoral School of Exact and Natural Sciences, University of Warsaw, Banacha 2C, Warsaw 02-097, Poland

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

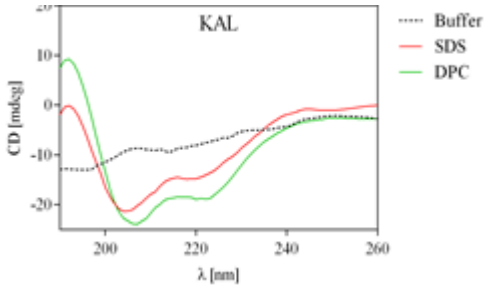

Fig.1. Simulation of KAL peptide active conformation.

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]

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INVESTIGATION OF SOME KINETIC PROPERTIES OF NUDT12 TOWARDS DINUCLEOTIDE SUBSTRATES

M. Łukaszewicz[1], A. Ferenc-Mrozek[1], E. Bojarska[1], J. Stępiński[1], E. Darżynkiewicz[1,2]

[1] Division of Biophysics Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Pasteura 5, 02-093 Warsaw, Poland
[2] Centre of New Technologies, University of Warsaw, Banacha 2c, 02-097 Warsaw, Poland

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

K. Maliszewska-Olejniczak[1], K. Pytlak[2], B. Kulawiak[2], P. Bednarczyk[1]

[1].Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences – SGGW, Warsaw, Poland
[2] Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

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.

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

K. Męczarska[1], D. Bonarska-Kujawa[1], S. Cyboran-Mikołajczyk[1], J. Oszmiański[2]

[1]Department of Physics and Biophysics, Wrocław University of Environmental and Life Sciences, Wrocław, Poland;
[2]Department of Fermentation and Cereals Technology, Wrocław University of Environmental and Life Sciences, Wrocław, Poland

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.

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

M. Narczyk[1], M. Krystian[1], D. Matczak[1], K. Pachocka[1], A. Stachelska-Wierzchowska[2], J. Wierzchowski[2], A. Bzowska[1], B. Wielgus-Kutrowska[1]

[1]Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Pasteura 5, 02-093 Warsaw, Poland
[2]Department of Physics and Biophysics, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland

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.

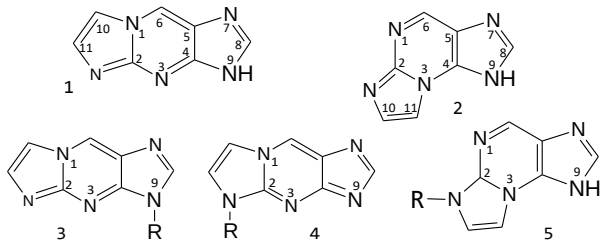

Fig.1. Structures of the nucleobase and nucleoside analogues investigated in this work: 1,N^2-etheno-2-aminopurine (1), N^2,3-etheno-2-aminopurine (2), and the ribosides of the ε2AP isomers (3-5, R = β-D-ribofuranosyl).

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

G. Neunert[1], W. Kamińska[2], R. Hertmanowski[2], P. Siejak[1], A. Baj[3], S. Witkowski[3], K. Polewski[1]

[1]Department of Physics and Biophysics, Faculty of Food Sciences and Nutrition, Poznan University of Life Sciences, Wojska Polskiego 38/42, 60-637 Poznan, Poland
[2]Institute of Materials Research and Quantum Engineering, Faculty of Materials Engineering and Technical Physics, Poznan University of Technology, Piotrowo 3, 60-965 Poznan, Poland
[3]Institute of Chemistry, University of Białystok, Ciolkowskiego 1k, 15-245 Białystok, Poland

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.

ACKNOWLEDGMENTS

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CHARYBDOTOXIN REDUCES THE MIGRATION OF GLIOBLASTOMA U87MG CELLS

E. Nurowska[1], O. Gójski[1], B. Dworakowska[2]

[1] Department of Pharmacodynamics, Centre for Preclinical Research and Technology, Faculty of Pharmacy, Medical University of Warsaw, 02-097 Warsaw, Poland;
[2] Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences, 02-776 Warsaw, Poland

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).

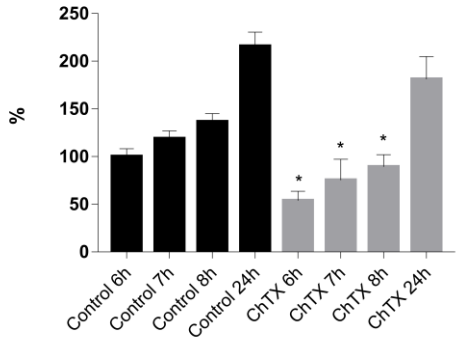

Fig.1. Charybdotoxin reduces the number of migrating cells. The columns represent the percentage of migrated cells as compared to the control. 100% represents the average number of migrating cells in the control 6 hours after the removal of the silicone insert. n=6. * p<0.05 One-way ANOVA.

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.

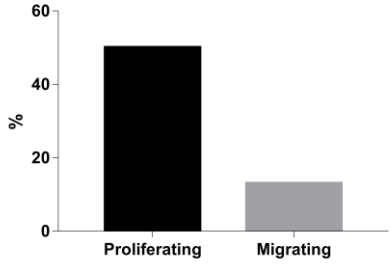

Fig.2. The BK channel in migrating cells is sensitive to ChTX. The columns represent the percentage of patches where channel activity in the presence of ChTX was observed.

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

A. Orzechowska[1], A. Czaderna-Lekka[2], M. Trtílek[3], K. Wątor[4]

[1] Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Al. Mickiewicza 30, 30-059 Kraków, Poland
[2] August Chełkowski Institute of Physics, University of Silesia in Katowice, 75 Pułku Piechoty 1, 41-500 Chorzów, Poland
[3] Photon Systems Instruments, Drásov 470, 664 24 Drásov , Czech Republic
[4] Department of Hydrogeology and Engineering Geology, Faculty of Geology, Geophysics and Environmental Protection, AGH University of Science and Technology, Al. Mickiewicza 30, 30-059 Kraków, Poland

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

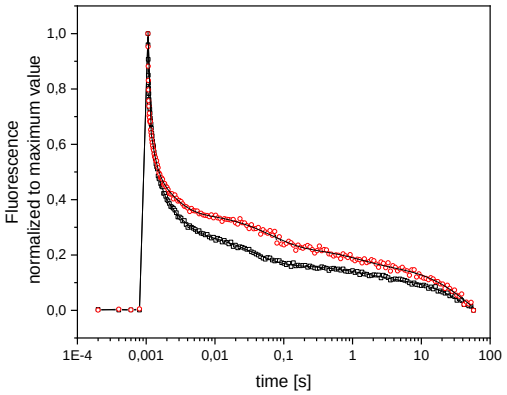

Fig.1. The flash-induced chlorophyll *a* fluorescence relaxation in *Chlamydomonas r.* cells exposed to antifouling paints (red, circle symbols) for 24 hours. The black trace represents the control (black, square symbols).

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

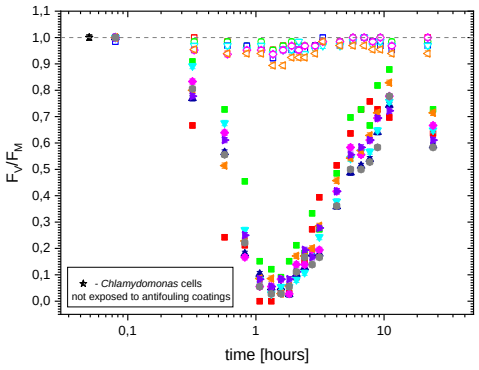

Fig.2. Maximal photosystem II efficiency (F_V/F_M) in algal cultures exposed to different antifouling coatings. F_V/F_M was normalized to the maximum value. Open symbols represent the coatings of low toxicity, full symbols refer to highly toxic ones.

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METHODS OF EXPOSING LIVING ORGANISMS TO ELECTROMAGNETIC FIELDS IN LABORATORY CONDITIONS

W. Porc, K. Rozpędek, A. Babczyńska

Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia, Katowice, Poland

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*

V. Pupkis, I. Lapeikaite, V. Kisnieriene

Department of Neurobiology and Biophysics, Institute of Biosciences, Life Sciences Center, Vilnius University, 7 Sauletekio ave., LT-10257 Vilnius, Lithuania

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

A. Pyrzanowska, B. Bukowska, A. Krokosz

Department of Biophysics of Environmental Pollution, Faculty of Biology and Environmental Protection, University of Lodz, Poland

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

P. Siejak[1], G. Neunert[1], W. Smułek[2], J. Nowak-Karnowska[3], A. Dembska[3], K. Polewski[1]

[1] Department of Physics and Biophysics, Poznan University of Life Sciences, Poznań, Poland
[2] Institute of Chemical Technology Engineering, Poznan University of Technology, Poznań, Poland
[3] Department of Bioanalytical Chemistry, Faculty of Chemistry, Adam Mickiewicz University

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

I. Siekierska[1,2], M. Łukaszewicz[3], R. Worch[4], M. Jankowska-Anyszka[2], K. Piecyk[2]

[1]Centre of New Technologies, University of Warsaw, Poland
[2]Laboratory of Synthesis of Organic Nanomaterials and Biomolecules, Faculty of Chemistry, University of Warsaw, Poland
[3]Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Poland
[4]Laboratory of Cell Biophysics, Nencki Institute of Experimental Biology, Warsaw, Poland

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]

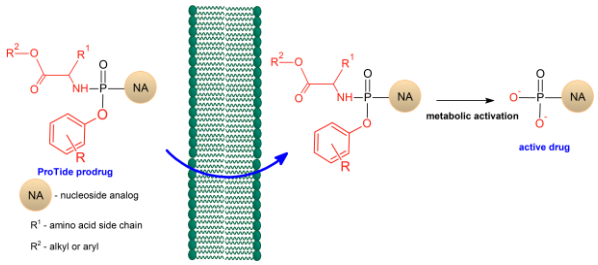

Fig.1. Mechanism of action of ProTide nucleoside analogs.

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).

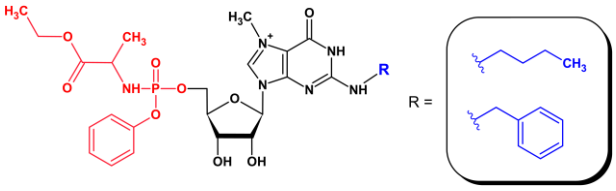

Fig.2. Obtained ProTide 5'cap analogs with additional substituents at N2 position.

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.

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

J. Slawski[1], S. Szewczyk[2], G. Burdziński[2], K. Gibasiewicz[2], J. Grzyb[1]

[1] Department of Biophysics, Faculty of Biotechnology, University of Wrocław, ul. F. Joliot-Curie 14a, 50-383 Wrocław, Poland
[2] Faculty of Physics, Adam Mickiewicz University in Poznań, ul. Uniwersytetu Poznańskiego 2, 61-614 Poznań, Poland
[a] Corresponding author's e-mail: jakub.slawski@uwr.edu.pl

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

K. Stachurska

Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Warsaw, Poland

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.

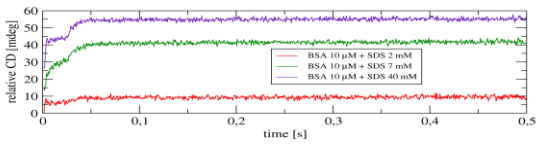

Fig.1. Relative progress curves BSA-SDS, excitation 220 nm.

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

J. Strankowska[1], B. Mikolaszek[2], M. Sznitowska[2]

[1] Institute of Experimental Physics, Faculty of Mathematics, Physics and Informatics, University of Gdansk, Wita Stwosza 57, Gdansk, Poland
[2] Department of Pharmaceutical Technology, Medical University of Gdansk, Hallera Street 107, Gdansk, Poland

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.

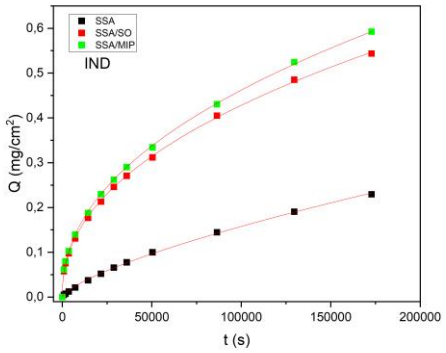

Fig.1. Release curves of indomethacin for silicone matrices.

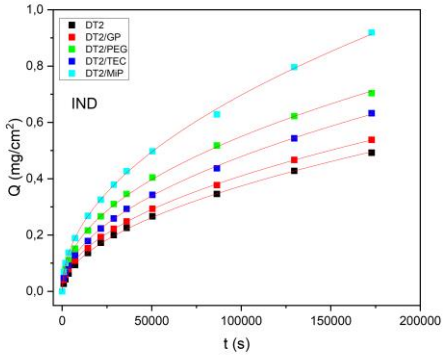

Fig.2. Release curves of indomethacin for acrylic matrices.

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.

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

Z. Sulej, J. Krasowska, B. Wielgus-Kutrowska

Biophysics Division, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Pasteura 5 St., 02-093 Warsaw, Poland

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].

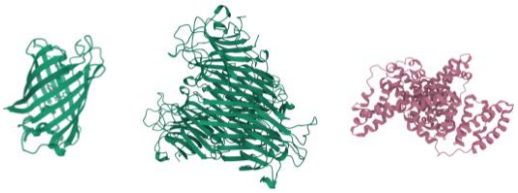

Fig.1. Spatial structure of EGFP [3] (left) [3], concanavalin A [4] (middle) and albumin [5] (right).

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)

W. Szymański, P. Bednarczyk, K. Maliszewska-Olejniczak

Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences – SGGW, Warsaw, Poland

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

L. Ślusarczyk[1], G. Czernel[1], D. Karcz[2], J. Hooper[3], M. Gagoś[4], J. Chavez[5], J. Kimball[5], L. Ceresa[5], Z. Gryczyński[5,6], I. Gryczyński[6], A. Matwijczuk[1*]

[1]Department of Biophysics, Faculty of Environmental Biology, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland.
[2]Department of Chemical Technology and Environmental Analytics (C1), Faculty of Chemical Engineering and Technology, Cracow University of Technology, Warszawska 24, 31-155 Kraków, Poland.
[3]Department of Theoretical Chemistry, Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Krakow, Poland.
[4]Department of Cell Biology, Institute of Biology, Maria Curie-Skłodowska University, 20-033 Lublin, Poland.
[5]Department of Physics and Astronomy, Texas Christian University, Fort Worth, TX, 76129, USA.
[6]Department of Microbiology, Immunology, and Genetics, Center for Fluorescence Technologies and Nanomedicine, University of North Texas Health Science Center, Fort Worth, TX, 76107, USA.
* Correspondence: arkadiusz.matwijczuk@up.lublin.pl

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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RNA OLIGONUCLEOTIDE BEACON BIOSENSING SYSTEM FOR NEURODEGENERATIVE BIOMARKERS DETECTION

W. Z. Świtlik[1], M. Stobiecka[2], S. Jakiela[2]

[1]Department of Biochemistry and Microbiology, Institute of Biology, Warsaw University of Life Sciences-SGGW, Warsaw, Poland
[2]Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences-SGGW, Warsaw, Poland.

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.

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

U. Tsylents[1,2], M. Burmistrz[1], M. Wojciechowska[1], J. Trylska[1]

[1] Centre of New Technologies, University of Warsaw, Warsaw, Poland.
[2] Doctoral School of Exact and Natural Sciences, University of Warsaw, Warsaw, Poland.

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

G. Wilczek, M. Stolarczyk, I. Potocka, K. Surmiak-Stalmach, K. Wiśniewska, K. Rozpędek

Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia, Katowice, Poland

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).

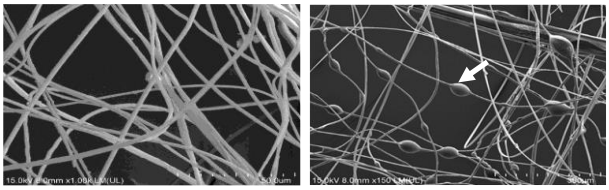

Fig.1. SEM images of spider web pattern examined under a Hitachi SU 8010 FESEM. The web consisting of an irregular tangle of dry silken fibers and threads with glue regions (arrow).

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.

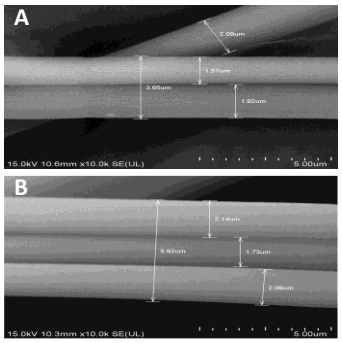

Fig.2. Example SEM images of web threads. A: single-, double- and B: multi-stranded threads

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

A. Włoch[1], T. Kral[1,2], A. Pawlak[3], H. Pruchnik[1], J. Sykora[2], M. Hof[2], W. Gładkowski[4]

[1] Department of Physics and Biophysics, Wrocław University of Environmental and Life Sciences, Wrocław, Poland
[2] Department of Biophysical Chemistry, J. Heyrovsky Institute of Physical Chemistry of the Czech Academy of Sciences, Prague, Czech Republic
[3] Department of Pharmacology and Toxicology, Wrocław University of Environmental and Life Sciences, Wrocław, Poland
[4] Department of Food Chemistry and Biocatalysis, Wrocław University of Environmental and Life Sciences, Wrocław, Poland

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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Teresa Kral and Martin Hof greatly acknowledge the Czech Science Foundation (EXPRO project GX19-26854X).

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

M. I. Wojtyś[1,2], W. Maksymiuk[1], A. Bubić[3], I. Leščić Ašler[3], E. K. Jagusztyn-Krynicka[2], A. Bzowska[1]

[1]Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Pasteura 5, 02-093 Warsaw, Poland
[2]Department of Bacterial Genetics, Institute of Microbiology, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland
[3]Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička cesta 54, 1000 Zagreb, Croatia

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.

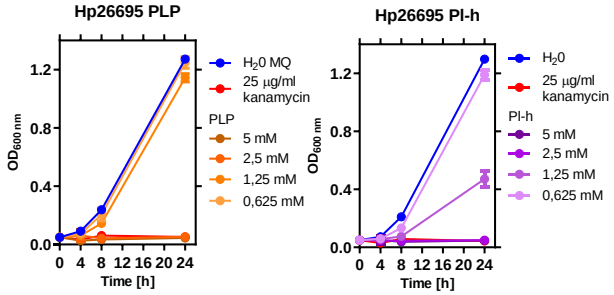

Fig.1. Growth curves of *H. pylori* 26695 in the presence of various concentrations of PLP (left panel) and PI-h (right panel).

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

G. Wołąkiewicz, M. Szabelski, M. Pietrzak

Department of Physics and Biophysics, University of Warmia and Mazury in Olsztyn, Oczapowskiego 4, 10-719 Olsztyn, Poland

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

M. Zakrzewski, P. Bełdzińska, J. Piosik, G. Goluński

grzegorz.golunski@ug.edu.pl

Laboratory of Biophysics, Intercollegiate Faculty of Biotechnology University of Gdansk & Medical University of Gdansk, Gdansk, Poland

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

S. Zawadzki [1,2], K. Miłowska [1]

[1] Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Poland
[2] Bio-Med-Chem Doctoral School of the University of Lodz and Lodz Institutes of the Polish Academy of Sciences, University of Lodz, Poland

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

S. Zawadzki [1,2], E, Okła [1,3], K. Miłowska [1]

[1] Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Poland
[2] Bio-Med-Chem Doctoral School of the University of Lodz and Lodz Institutes of the Polish Academy of Sciences, University of Lodz, Poland
[3] Doctoral School of Exact and Natural Sciences University of Lodz, Poland

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

J. Żuberek, M. Warzecha, M. Dobrowolski, A. Modrak-Wójcik

Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Poland

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.

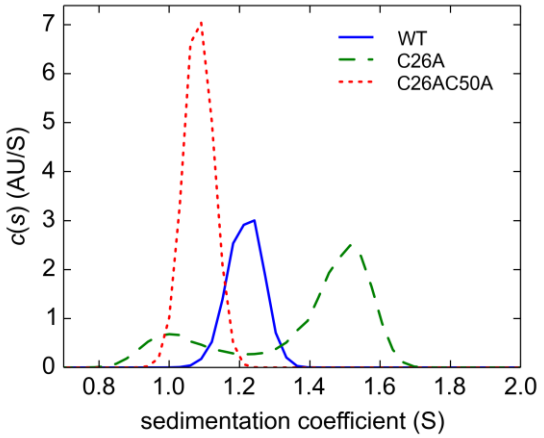

Fig.1. Sedimentation coefficient distribution c(s) of h4ET(1-68) variants under non-reducing conditions.

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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