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Plenary lectures

MODERN METHODS OF ONCOLOGICAL DIAGNOSTICS - WATER IN THE CELLULAR ENVIRONMENT OF HUMAN BREAST TISSUE

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Despite a very large number of publications, the role of water in the cellular environment of the biological tissue has not been clarified. Characterizing the nano–bio interface is a key challenge in understanding interactions of water in the tissue. Although we often assume that steady state behavior of the bulk water can be translated to the crowded biological environment, this approach must be considerably revised when considering the nano–bio interface. According to our knowledge there are only a few experimental papers monitoring directly interactions, and accumulation of water in the restricted environments of the biological tissue upon realistic crowding conditions. The presentation has focused on deriving a molecular-level picture of the nano-bio interface or, more generally, of water molecules positioned next to any hydrophobic and hydrophilic surfaces of the normal and cancerous tissue. IR and Raman spectra of the ν_s(OH) stretching modes of water at the biological interfaces of the human breast and neck tissues have been recorded and analysed. The results reveal some dramatic changes with water content in the tissue and are of potential relevance both to fundamental problems of interfacial water modelling and to molecular diagnostics of cancer as a 'hydration fingerprint'. We have found that the interfacial water interacting via H-bond with other water molecules and biomolecules at the biological surface as well as free OH vibration of the dangling water are sensitive indicator of pathology discriminating between the normal and cancerous tissue as well as type of cancer.

REFERENCES

[1] Abramczyk, H., Brożek-Pluska, B. (2013). *Chem Rev* DOI: 10.1021/cr300147r.

[2] Abramczyk, H., Brozek-Pluska, B., Tondusson, M., Freysz, E. (2013). *J Phys Chem C **117*** (10), 4999-5013.

[3] Abramczyk, H., Brożek-Płuska, B., Surmacki, J., Jabłońska-Gajewicz, J., Kordek, R. (2012). *Prog Biophys Mol Biol*. ***108***, 74-81.

[4] Brozek-Pluska, B., Jarota, A., Jablonska-Gajewicz, J., Kordek, R., Czajkowski, W., Abramczyk, H. (2012). *TCRT* **4**, 317-331.

[5] Brozek-Pluska, B., Musial, J., Kordek, R., Bailo, E., Dieing, T., Abramczyk, H. (2012). *Analyst* **137** (16), 3773-3780.

[6] Brozek-Pluska, B. , Jablonska-Gajewicz, J., Kordek, R., Abramczyk, H. (2011). *J Med Chem.* **54**, 3386-3392.

MONTE-CARLO SIMULATIONS AND THEIR APPLICATIONS IN SELECTED SYSTEMS OF BIOPHYSICAL INTEREST

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Monte-Carlo simulation is a powerful tool used among others to model complex molecular species and intermolecular processes like excitation energy transfer , charge transfer or aggregation present in biological systems. As typical examples can serve the description of energy transport and trapping in photosynthetic systems or in concentrated solutions of some flavins. However, recently there has been also an increasing demand to generate and/or describe donor-acceptor systems in which so called enhanced energy transfer takes place. This is connected with tries to utilize (but also to understand) energy transfer as a spectroscopic ruler at distances longer than 10nm, an upper limit for Foerster resonance energy transfer. Within this respect two examples will be discussed. First one originating from the interaction between fluorophores and the surface plasmons and the second one originating from selective multiple labelling of proteins by closely located excitation acceptors (antenna effect). Monte-Carlo results will be compared with experimental data. Some details on experimental setups will be also provided.

TRANSCELLULAR AND PARACELLULAR ROUTES OF ION TRANSPORT ACROSS HUMAN BRONCHIAL EPITHELIUM

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Epithelial cells are bound together by tight junctions and form a barrier impermeable to water and ions. The epithelial cell monolayer covers a surface of lungs, pancreas and sweat gland ducts. Both faces of the cell monolayer – apical and basolateral one have different composition of molecules which transport ions and water across the membrane. The defect in ion transport caused by a single gene mutation in leads to cystic fibrosis - the most common fatal human genetic disorder. The defected water transport causes production of too dense and viscous secretion which blocks pancreas ducts and leads to opportunistic inflammations in lungs. There are three hypotheses explaining the mechanism of cystic fibrosis: insufficient water secretion, excessive water absorption and limited secretion of bicarbonate ions.

The ion transport across epithelial cell layer is measured either as the total current flowing through the cell monolayer or by means of radioactive tracers. The obvious weakness of present day studies is lack of possibility to measure simultaneously five ions (Na^+, K^+, H^+, Cl^-, HCO_3^-) involved in ion and osmotic water transport.

In our laboratory we succeed in constructing potentiometric device which allow for simultaneous real time measurement of all ions flowing across the epithelial cell monolayer. In the experiments made on human bronchial epithelial cell line we showed that sodium ions flow from basolateral to apical face via paracellular way (in between cells) while chloride ions uses transcellular route (i.e. via cells.)

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BIOLOGICAL AND MEDICAL APPLICATION OF PHOTOTHERMAL METHODS

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The results obtained with the use of photothermal methods give information on radiative and non-radiative processes of deactivation of dye excited states and a possibility of a photochemical reaction initiation with involvement of their triplet states, on the quantum yield of triplet states and singlet oxygen generation as well as relaxation times of radiationless processes.

Interdisciplinary studies on selected merocyanine dyes [1,2], metaloporphyrins and their derivatives [3,4], dye-semiconductor complexes [5] and dye-protein complexes [6,7] were performed in organic solvents, liquid and solid polymer matrices and in biological systems.

Analysis of results permitted recommendation of dyes for biomedical applications [1-3, 5], as calorimetric references [4] or a probe of the toxic effect of heavy metals (with the help of cyanobacteria as bioindicators) [6] and sensitive indicators of photochemical stability of vegetable oils [7].

REFERENCES

[1] Staśkowiak E., Dudkowiak A., Hanyż I., Wiktorowicz K., Frąckowiak D. (2004). *J Photochem Photobiol A: Chem*. **163,** 127-134.

[2] Staśkowiak E., Dudkowiak A., Wiktorowicz K., Cofta J., Frąckowiak D. (2005). *J Photochem Photobiol A: Chem*. **169**,159-168.

[3] Dudkowiak A., Teślak E., Habdas J. (2006). *J Mol Struct*. **792/793** 93-98.

[4] Plich M., Dudkowiak A., Jurzyk B., Łukasiewicz J., Susz A., Stochel G., Fiedor L., (2013). *Biochim Biophys Acta* **1827**,30-37.

[5] Olejarz B., Bursa B., Szyperska I., Ion R. M., Dudkowiak A. (2010). *Int J Thermophys*. **31,**163-171.

[6] Dudkowiak A., Olejarz B., Banaszek J., Łukasiewicz J., Sikora J., Wiktorowicz K. (2011). *Int J Thermophys.* **32,** 762-773.

[7] Hanyż I., Pieńkowska H., Dudkowiak A., Frąckowiak, D. (2006). *Dyes and Pigments* **70,** 177-184.

MECHANISM OF CHARGE RECOMBINATION IN PHOTOSYNTHETIC REACTION CENTERS FROM PURPLE BACTERIA. THE ROLE OF THE PROTEIN DYNAMICS

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Electron transfer inside a protein is a common phenomenon. Photosynthetic reaction centers are very convenient model systems for studying such a transfer, because the electron carriers inside these proteins are pigments – molecules absorbing in the visible. Absorbing properties of these pigments depend on their redox state. Therefore the electron transport may be easily studied by transient absorption.

Recently, influence of the protein dynamics on the intraprotein electron transfer is in focus of research

activity of a few groups. Results of the studies of back electron transfer from reduced bacteriopheophytin, H_A^-, to oxidized primary donor, chlorophyll dimer, P^+, in reaction centers from purple bacteria *Rhodobacter sphaeroides* will be presented. Using transient absorption technique it was possible to separate "pure" electron transfer reaction from conformational dynamics of the protein, the latter being characterized by two lifetimes: about 0.6 and about 10 ps. Surprisingly, the protein dynamics only weakly decelerates with decreasing temperature from 296 to 77 K.

APPLICATION OF ADVANCED LIGHT MICROSCOPY TECHNIQUES TO STUDY CELLULAR ATP RELEASE

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Mechano-transduction at the cellular and tissue levels often involves the release of signaling molecules. Among them, purines appear to be the most primitive and widespread chemical messengers of local, autocrine and paracrine signaling and intercellular communication in most, if not all, organ and cell systems. Extracellular nucleotides, via interactions with purinergic receptors, regulate a variety of physiological and pathophysiological processes in all organs and tissues. Despite fundamental importance of purinergic signaling, the release mechanisms are not fully understood. Here, I will describe application of total internal reflection fluorescence (TIRF) microscopy and luminescence imaging to study ATP release from lung alveolar A549 cells. With TIRF microscopy we have demonstrated that exocytosis of ATP-laded vesicles contributes to mechano-sensitive ATP release from A549 cells. To investigate stretch-induced ATP release in real-time we used luciferase-luciferin bioluminescence imaging system equipped with electron multiplying EMCCD camera and an image intensifier. It allows low light luminescence detection and imaging of ATP release with 100 ms temporal resolution, ~10 nM sensitivity and dynamic range up to 100 µM. The luminescence imaging was coupled with simultaneous infrared DIC imaging to monitor cells under study. Compared to traditional ATP measurements with luminometry, our imaging system allowed us to: (1) identify single ATP-releasing cells and to monitor their shape and distension during stretch stimulation; (2) to determine absolute ATP concentration and its 2D distribution at the release sites; (3) analyze kinetics of ATP release from single cells. In particular, we found that single stretch of ≥10% induces ATP release sufficient to activate purinergic receptors on neighboring cells up to 150 µm away from the source cell and that stretch-induced ATP release is significantly enhanced in scratch-wound areas.

BIOLOGICAL MEMBRANES: FROM PASSIVE BARRIER TO ACTIVE STRUCTURE – EXAMPLE OF BACTERIAL MEMBRANES

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Our contemporary knowledge concerning the structure and function of biological membranes was founded 40 years ago by publication of the membrane fluid mosaic model. Since that time our view on membranes has strongly evolved. The fluid mosaic model was supplemented by many new aspects or traits, which were necessary to explain the subsequently discovered new membrane properties and/or functions. The most important such supplements are: asymmetry of the individual membrane leaflets and spatial heterogeneity of the membrane composition (presence of domains in the membrane plain). An example of asymmetry can be found in bacteria – it is the structure of the outer membrane of Gram-negative bacteria. Another important feature of the structure of membranes of bacteria like *Escherichia coli* or *Bacillus subtilis* is presence of domains containing negative electric charge (phosphatidylglycerol or cardiolipin). These domains are not static, their localization depends on the phase of bacterial cell cycle. Presence of such lipid domains together with changes of the local curvature of cell membrane plays important role in the control of the cell division and/or sporulation processes. This is because these structures are responsible for binding of proteins participating in division/sporulation processes. In *B. subtilis* appearance of the negative membrane curvature is recognized by DivIVA protein, which binds to membrane in such site and recruits proteins of the Min system. On the other hand the role of lipid domains is confirmed also by the fact that when their formation is corrupted then the division process is blocked. The temporal, periodic changes involve also the lipid composition of membranes, what accommodates the membrane properties to the certain needs of bacteria. For example the drastic changes of membrane lipid composition accompany the sporulation. It was suggested that appearing in this time strong asymmetry of the individual leaflet composition is responsible for the formation of prespore membrane.

The examples given above prove that biological membranes, including bacterial ones, are not only a passive barrier separating cell interior and its surrounding. They play many active roles in numerous cellular processes.

THE RED BLOOD CELL AS A PRACTICAL MODEL OF THE LIVING CELL

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Protection of living organisms against harmful physicochemical factors will be possible if research is continued on the effects of the factors on organisms, and on the molecular mechanisms that lead to such effects. Factors harmful to biological systems include, among others, toxic substances that pollute natural environment and UV radiation that causes oxidation of proteins and lipids. The primary and often the only place of the interaction of biologically active substances and physical factors with organisms is the biological membrane. Therefore the knowledge of that interaction on the molecular and cell level is important. Relevant information can be obtained from biophysical studies applying various physical methods to erythrocytes and extracted erythrocyte membranes (ghosts).

Erythrocytes in many studies are treated as a cell model, and their membrane as an example and model of the biological membrane. Red blood cells, like other morphological elements of blood, can fairly easily and without damage be extracted from the plasma. In addition, they are stable and can be kept long, including possible exchange of the natural medium for an isotonic solution.

Cells or erythrocyte membranes are subjected to modification for a certain time by the studied substances, which are added to the isotonic cell suspension or e.g. exposed to UV radiation. The effect of physicochemical factors on erythrocytes and properties of their membranes can be determined on the basis of e.g. cell shapes, fluidity of membranes, packing order of the polar heads of membrane lipids, and cell hydration, using fluorimetric, microscopic and spectrophotometric methods, including FTIR.

BIOPHYSICAL ASPECTS OF GROWTH RATE ANISOTROPY IN THE *Arabidopsis thaliana* ROOT APEX

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The root apex, like other plant organs, grows symplastically, i.e. in a continuous way. In angiosperms, its growth is determined by the zone of a low mitotic activity known as the quiescent centre (QC). All tissues of the root apex are derived from initial cells surrounding this zone. In the case of *Arabidopsis* these initials are known [1]. A diversity of cell lineages originating from them suggests an interesting variation of growth rates within the apex. However, little is known about this variation, especially in the most apical region including the root cap. The modelling that leads to determination of growth rates in the A. root with a typical shape and cell pattern is presented. At first, the displacement velocity field for the axial section is specified using two types of empirical data, on the velocity profile along the root axis above QC [2], and dimensions of cell packet that develop in the root cap [3]. The velocities vary in length and direction [5]. Next, the linear growth rates are calculated for different points and directions in 3D. As the linear growth rate (R_l) is a tensor quantity [4], the R_l variation is interpreted with respect to principal growth directions. The results indicate a significant R_l anisotropy. The directional preferences depend on position within the apex. In the root proper the rate in the periclinal direction predominates everywhere, while in the root cap the predominating direction varies with distance from the QC. The rhizodermis is distinguished from the neighboring tissues by relatively high contribution of R_l in the anticlinal direction. The degree of growth anisotropy calculated for cell walls may be as high as 25. The changes in the R_l distribution are discussed.

REFERENCES

[1] Dolan et al. (1993). *Development* **119**,71-84.
[2] Van der Weele C. M. et al. (2003). *Plant Physiol.* **132**, 1138-1148.
[3] Campilho A. et al. (2006). *Plant J.* **48**, 619-627.
[4] Hejnowicz Z., Romberger J. A. (1984), *J Theor Bot.* **110**, 93-114.
[5] Nakielski J., Lipowczan L. (2012). *Planta* **236,** 1547-1557.

CRYSTAL OR MEMBRANE DOMAIN? CHARACTERISATION OF ORDER CHOLESTEROL AGGREGATES IN BILAYERS OVERSATURATED WITH CHOLESTEROL – RESULTS OF COMPUTER SIMULATIONS

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Experimental studies, using SL-EPR methods, on phosphatidylcholine-cholesterol (PC-Chol) bilayers demonstrated that for Chol:PC > 1, *cholesterol bilayer domains* (CBDs) do form [1]. Spatial organization, dynamics, and water accessibility to Chol molecules in the domain are different from those in Chol crystals. To characterize in details the dynamics and

spatial organization of Chol molecules in CBD, molecular dynamics (MD) simulations were carried out on: (1) hydrated pure Chol bilayer that models CBD; (2) hydrated PC-Chol bilayer with 50 mol% Chol (PC-Chol50) that models the bulk membrane; (3) Chol crystal. The Chol and PC-Chol50 bilayers were hydrated with 30 water molecules per Chol.

The results of MD simulations demonstrate that static and dynamic properties of the Chol molecules in the Chol bilayer are qualitatively the same as those in the PC-Chol50 bilayer but the dynamics is slower. Moreover, on average, each Chol molecule makes 2.3 hydrogen bonds with water in the Chol bilayer as compared to 1.7 in the PC-Chol50 bilayer [2]. These properties of Chol in the Chol bilayer are at variance with those in the Chol monohydrate [3] and anhydrous [4] crystals, where the Chol molecules are immobile and hydrated by one water molecule or dehydrated.

The results of MD simulations are in close agreement with the SL-EPR results but disagree with those of X-ray diffraction [5]. The latter show that CBD have the same spatial organization and dynamics as Chol crystals. Most likely, Chol crystals that are detected by X-ray diffraction form outside the lipid bilayer and cannot be identified with pure Chol bilayer domains within the lipid bilayer.

REFERENCES

[1] Raguz M., et al. (2011). *Biochim Biophys Acta* **1808**: 1072-1080.
[2] Plesnar E., et al. (2013). *J Phys Chem* B **117**, 8758-8769.
[3] Craven B., (1979). *Acta Cryst* B **35**, 1123-1128.
[4] Shieh H.-S., et al. (1981). *Acta Cryst* B **37**, 1538-1543.
[5] Wachtel E. J., et al. (1991). *Biochim Biophys Acta* **1066**, 63-69.

MOLECULAR MECHANISM OF THE XANTHOPHYLL CYCLE – A COMPARISON OF MODEL SYSTEMS AND NATIVE THYLAKOID MEMBRANES

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Xanthophyll cycle is an important mechanism, involving cyclic transition between three xantho-phylls: violaxanthin, antheraxanthin and zeaxan-thin, localized in chloroplasts and optimizing light harvesting in all higher plants and many groups of algae. In low light or darkness, thanks to the activity of zeaxanthin epoxidase, chloro-plasts convert zeaxanthin via intermediary pro-duct, antheraxanthin to violaxanthin which serves mostly as an antenna, while in strong light an enzyme violaxanthin de-epoxidase is activated and reverses the reaction, which results in zea-xanthin accumulation exerting photoprotective function. Activity of violaxanthin de-epoxidase requires, among others, presence of lipids. Our investigations of model systems revealed that only non-bilayer lipids, in particular those forming inverted hexagonal phases (MGDG, PE) are effective in activating the enzyme. These lipids play several functions. The HII structures formed by them are the docking places for the VDE, they solubilize aggerated violaxanthin better than lamellar lipids and they ensure proper orientation of violaxanthin molecules in the HII phases so they become accessible to de-epoxi-dizing enzyme. In contrary to the lipid model systems where all violaxanthin is dissolved in lipid phase, in thylakoid membranes most of the violaxanthin is associated with LHCII and bound to protein. To be de-epoxidised violaxanthin must be liberated from its binding sites, thus, the mechanism of deepoxidation in in vivo system is more complicated. Similarities and differences in molecular mechanism of violaxanthin de-epoxi-dation in model systems and in native thylakoid membranes will be discussed.

MITOCHONDRIAL POTASSIUM CHANNELS

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Mitochondria are the organelles providing energy to the cell so that it is able to perform basic life functions. They are surrounded by double lipid membrane. The outer membrane of the mitochondria is permeable for the majority of substances, while the transport through the inner one is strictly regulated.

Essential for correct functioning of mitochondria are the ionic channels localised in the inner membrane. They maintain ionic homeostasis in the mitochondria, they are responsible for changes in their volume and take part in generation of pH gradient between the mitochondrial matrix and the cell cytoplasm. So far, the following channels have been identified in the inner mitochondrial membrane: the anion selective channel, magnesium ion selective channel and calcium selective channel (uniporter). Moreover, a group of potassium channels has been identified in the inner membrane, including a channel regulated by Kv1.3 voltage (mitoKv1.3), a channel regulated by ATP (mitoK_{ATP}), a potassium channel of high conductivity regulated by calcium ions (mitoBK_{Ca}) and quite recently, a channel of TASK-3 type. Transportation of potassium inside the mitochondrial matrix changes the volume of mitochondria and the membrane potential, thus influencing the rate of breathing. Although the detail structure and functioning of mitochondrial potassium channels are not fully known yet, the discovery of the proteins involved has brought new information on the regulation of work of mitochondria and the

entire cell. According to some hypotheses these proteins are associated with cell death. The cytoprotective effects with participation of these channels have initiated intense investigation aimed at modulation of these proteins upon cell stress. The investigation is expected to bring new therapies limiting cell death in such conditions and cardiac ischemia or neurodegenerative diseases.

MODIFIED POLY(PROPYLENE IMINE) DENDRIMERS AS CARRIERS OF ANTICANCER DRUGS

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Physiological nucleoside analogs (NAs) are commonly used in anticancer therapy. Most of them have the same way of metabolism and similar mechanism of action. Nucleoside analogs are administered as prodrugs and activated by phosphorylation to 5'-triphosphate form inside the cell. They compete with physiological nucleotides and interfere with nucleic acids during their synthesis. Furthermore, nucleoside analogs may directly cause apoptosis.

Curently used anticancer drugs cause toxicity against normal tissues and leads to numerous side effects. Moreover, nucleoside analogs are not always efficient enough due to several resistance mechanisms such as fast metabolism, low solubility, unfavorable biodistribution, or low specificity of interaction with the cancer cells. Therefore, new drug carrier systems are in search of improving the efficiency and specificity of anticancer drugs.

Maltose modified poly(propylene imine) dendrimers (PPI-m) are promising anticancer drug delivery systems. Dendrimers are characterized by unique structure. Thanks to presence of numerous amino groups PPI dendrimers are highly reactive and well soluble. Thus, this structure are able to create stable complexes with triphosphates of nucleoside analogs. The use of dendrimers as drug devices may improve anticancer therapy by delivering activated nucleosides directly to cancer cells. This strategy may help to overcome the metabolic limitation of chemotherapeutics involving inefficient drug distribution or enzymatic degradation of drugs. Moreover, obtained results have shown that the cytotoxicity of complexed nucleoside analogs is enhanced in comparison to free drugs for leukemic cells.

SPECTROSCOPIC PROPERTIES OF FLUORESCENTLY LABELLED mRNA cap ANALOGUES

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Eukaryotic organisms are characterized by a specific modification at the 5' terminus of mRNA called cap. Due to its unusual chemical structure, cap molecule has unique physicochemical properties and plays a pivotal role in biological processes such as translation, splicing, intracellular transport of RNA. Synthetic analogues of the 5' terminus of mRNA are an important tool in the study of cellular processes involving mRNA. Cap analogues although successful applications in scientific research have limited possibility of use in the study of biological systems.

Fluorescent labelled synthetic analogues potentially allow to obtain more information about investigated biological systems, in which the cap is engaged. Fluorophores used in this type of research should be characterized by high quantum yields, good photostability, long lifetimes in the excited state, absorption spectra out of protein spectral range and, most importantly, their size and chemical properties cannot influence the biological function of cap molecules.

Spectroscopic properties of three dinucleotide fluorescent cap analogues labeled at the ribose of the 7-methylguanosine moiety with either anthraniloyl (Ant) or N-methylanthraniloyl (Mant): Ant-m^7GpppG, Mant-m^7GpppG and 3'Ant-$m_2{}^{7,2'\text{-}O}$GpppG will be presented. Studied synthetic cap analogues were effectively attached to the short RNA transcripts by T7 RNA polymerase and the process of translation of mRNA proceeded with a good efficiency in experiments *in vitro* [1]. During the lecture conclusions about cap analogues conformation and intramolecular interactions will be also presented.

REFERENCES

[1] Ziemniak M., Szabelski M., Łukaszewicz M., Nowicka A., Darzynkiewicz E., Rhoads R. E., Wieczorek Z., Jemielity J. (2013). *RSC Advances* **3(43)**, 20943-20958.

APPLICATION OF THE LASER INTERFEROMETRY IN STUDIES OF BIOPHYSICAL MODEL SYSTEMS

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The laser interferometry is an optical research method that uses interference of two laser beams, one of which is a reference beam and a second one is an information beam. It allows a comprehensive study of diffusion (measurement of the amount and flow of the transported substance, determination of the space-time concentration distribution, measurement of diffusion coefficient of the substance) as well as visualization of the diffusion layers formation and study of their evolution.

The method of laser interferometry was used to test various theoretical models of diffusion, in studies of the substance transport through the membranes, hydrodynamic instability studies, to characterize membrane transport parameters under concentration polarization conditions as well as to study of the influence of gravitational field on the substance transport.

Laser interferometry is now increasingly used in biophysics, biology and medicine.

Modifications of the measurement system, the use of gel systems and immobilisation of molecules in gels allow the interferometric analysis of anomalous diffusion (subdiffusion), the test release and interact-tttion of macromolecules with biologically active substances (antibiotics, lipopolysaccharides, saponins). This method was used recently successfully to the analysis of diffusion of antibiotics (ciprofloxacin or ampicillin) into the water phase from mixtures of neutral or negatively charged liposomes, and antibiotic–liposome interactions.

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