Current Topics in Biophysics 2018, 41 (suppl A), 7-18 DOI: 10.2478/ctb-2018-0003

Plenary lectures

SINGLE-CELL ANALYSIS OF BACTERIAL DNA REPAIR AND MUTAGENESIS

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The accurate detection and repair of DNA damage is crucial for genome stability in all organisms. Despite extensive characterization of DNA repair pathways using genetics and biochemical assays, it remains unclear how repair proteins perform their function within the cellular environment and how different repair pathways are coordinated in the cell. I will present our developments of single-molecule imaging and microfluidics techniques to investigate the mechanisms of DNA repair and mutagenesis at the single-cell level. A key advantage of these methods is their ability to resolve biological heterogeneity and dynamic behaviour. By avoiding population averaging, we found that mutation rates are dynamically regulated by DNA damage responses. We show that stochastic fluctuations in the expression of DNA repair factors affect cell survival and modulate the rates of mutagenesis in single bacterial cells.

SINGLE-MOLECULE IMAGING OF DNA GYRASE ACTIVITY IN LIVING *ESCHERICHIA COLI*

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DNA gyrase has an essential role in bacterial chromosome maintenance via controlling level of chromosome supercoiling. During transcription and replication, moving polymerases introduce positive supercoiling, which will block the enzymes if not removed. By using live cell imaging, we observed that gyrase forms replication dependent foci. However, Photoactivated-Localization Microscopy revealed that majority of active gyrase molecules were evenly distributed throughout the chromosome with only ~10 molecules associated with replication machinery. This is surprising due to high speed of the replisome (up to 1000bp/s, leading to 100 supercoils/s) and slow catalytic cycle of gyrase (2s). This conundrum was resolved by the observation that gyrase molecules in the vicinity of the replisome remained immobile for >8 seconds, while gyrase far from the replisome for only ~ 2 seconds. Together, we found that gyrase display two modes of action; single catalytic events dominate in maintaining a steady-state supercoiling while ahead of replication gyrase performs multiple rounds of catalysis.

HYBRID PHOTOSYNTHESIS

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No abstract available

EXTREMOPHILIC PHOTOSYSTEM I: IMPROVING NATURE FOR SOLAR ENERGY CONVERSION

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One of the main challenges facing mankind is to advert the anthropogenic climate change associated with uncontrollable release of CO_2 and other greenhouse gases due to burning of fossil fuels. Photosynthesis, where solar energy, water and carbon dioxide are transformed into chemical energy in the form of carbon-based molecules, serves as a blue print for developing sustainable technological solutions based on solar energy conversion [1]. I will present our recent efforts on construction of the biohybrid photoelectrodes and the whole solar cells that employ a highly efficient molecular machine of extremophilic photosystem I (PSI), serving as light harvesting/charge separating component of unprecedented robustness [2]. The focus of the present work in our laboratory is to generate an ordered architecture of PSI complexes through their intermolecular interaction with the natural electron donor, cytochrome c_{553}. Several configurations of the PSI-based devices will be presented together with their photoelectrochemical properties, pointing towards the important role of the conductive cytochrome *c* bioconjugation factor in enhancing the photocurrent output through promoting the specific orientation of PSI with respect to the electrode surface [3,4].

ACKNOWLEDGEMENTS

The research in the laboratory is supported by the grants no. DZP/POLTUR-1/50/2016 funded by the Polish National Centre for Research and Development and OPUS grant no. 756 UMO-2014/15/B/NZ1/00975, funded by the Polish National Science Centre.

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MOLECULAR ANALYSIS OF INTRINSICALLY DISORDERED REGIONS OF NUCLEAR RECEPTOR ULTRASPIRACLE

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Nuclear receptors (NRs) are a group of transcription factors activated by the presence of small lipophilic compounds. Typically, NRs are composed of two highly preserved, globular domains and some disordered regions. One of the globular domains contains zinc-fingers and is responsible for binding to specific DNA sequences. The other, the ligand binding domain (LBD) binds ligands but also various cofactors which play a role in modulation of a NR activity. NRs usually form dimeric complexes with the same or a different NR and the main oligomerization site is just present in the LBD. The biggest differences in the amino acids composition and the length of the polypeptide chains of various NRs but also various isoforms of the same NR are observed in the N-terminal domain (NTD) [1, 2]. Despite, many years of studying of NRs our knowledge regarding the influence of NTD on the overall activity of NRs is limited. In our work we decided to investigate the molecular properties and the potential role of NTD of *Helicoverpa armigera Ultraspiracle* (HaUsp). Prior *in vitro* analyses, *in silico* analyses were performed and obtained results clearly indicate that NTD of HaUsp is highly disordered. For subsequent research we used recombinant full-length HaUsp and HaUsp deprived of the NTD (HaUsp_ΔNTD). The DNA binding activity of both forms was investigated with electrophoretic mobility shift assay (EMSA) using two different specific probes. Obtained results indicated that the NTD has significant influence on the interaction of the protein with DNA. In the presence of NTD different pattern of protein-DNA interaction could be observed. Subsequently, both forms were analyzed with small-angle X-ray scattering (SAXS). Determined structural parameters indicated that the Usp forms oligomers, mainly dimers. However, depending on which form of protein was analyzed, formation of dimers was observed at different concentration. These results suggest that full-length HaUsp forms more stable dimers. Molecular models reconstructed from SAXS data by means of EOM [3] helped us to get closer to the explanation of such phenomena.

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DETERMINATION OF MOLECULAR INTERACTIONS IN SOLUTIONS FROM THE MOTION OF SINGLE MOLECULE

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Molecular interaction in solution takes apart in all types of biochemical reactions and can be quantified by equilibrium and rate constants of the reactions. Due to the presence of molecular interactions, the free motion of each molecule involving in the reaction is affected to the extent depending on these constants. In this presentation, I will introduce you a convenient and reliable method mainly based on one type of single-molecule technique called fluorescence correlation spectroscopy (FCS) for the qualitative and quantitative determination of molecular interactions in solutions from the motion of single molecule. The diffusion coefficients of probes, equilibrium and rate constants for the molecular interaction were determined from the analysis of experimental data using proper theoretical models for FCS. The validity and reliability of our method has been proved by several examples of intermolecular interactions in this thesis, such as protein-surfactant interactions (1)_ENREF_1 and dye-micelle interactions (2, 3). Our work paves a promising application of FCS toward the quantitative determination of molecular interactions in chemical and biological systems.

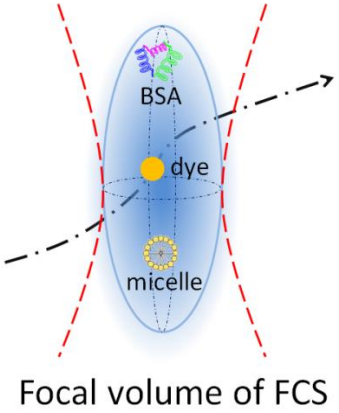

Fig.1. Focal volume of FCS

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FUNCTIONAL AND STRUCTURAL ANALYSES OF LHCII-GRAPHITE ELECTRODE ELECTROCHEMICAL SYSTEM

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The electrochemical properties and changes in the arrangement of LHCII complexes immobilized on screen-printed graphite electrodes (GE) were investigated by broad range of physicochemical methods. We used the cyclic voltammetry (CV) and differential puls voltammetry (DPV) for detail characterization of LHCII-GE multilayer whereas the photocurrent response was analyzed by chronoamperometry. The interaction of LHCII complexes with a graphite layer was analyzed by low temperature fluorescence, scanning electron microscopy (SEM), atomic force microscopy (AFM), and confocal laser scanning microscopy (CLSM). LHCII in watery glutaraldehyde cross-linked matrix is immobilized on GE by physical adsorption and later inserted into graphite pores by a potential treatment. Obtained data showed that LHCII complexes located inside the graphite layer reveal intact structure and generate photocurrent without soluble redox mediators. The intensity of photocurrent is proportional to light intensity and dependent on GE potential. Furthermore, the reduction-oxidation specific peaks for LHCII-GE were noted. Results suggest that effective photocurrent generation depends on specific potential difference between LHCII and GE as well as on LHCII and GE connection area.

The project was supported by the National Science Centre (NCN), Poland, decision number DEC-2013/09/B/NZ1/01111

LHCII ANTENNA COMPLEX PHOSPHORYLATION DURING DARK-CHILLING STRESS IN CHILLING TOLERANT AND CHILLING SENSITIVE PLANT SPECIES

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Low temperatures are one of the abiotic stress factors which affect the growth and productivity of plants, however, depending on their evolutionary background, tolerance of plants to low temperature varies between species and cultivars. Since chloroplast, and thus photosynthesis, are primary target of chilling stress, then regulatory processes of photosynthetic light reactions, LHCII phosphorylation among them, should be involved in chilling response and tolerance. We examined two plant species: chilling sensitive runner bean (*Phaseolus coccineus* L.) and chilling tolerant pea (*Pisum sativum* L.) and analyzed changes in the LHCII phosphorylation level during dark-chilling stress. We found that the dark-chilling treatment of pea leaves induced almost complete dephosphorylation of LHCII proteins, while subsequent photo-activation of chilled leaves caused a return of LHCII phosphorylation to the control level. This behavior of LHCII is consistent with the literature data. On the contrary after dark-chilling of runner bean leaves strong LHCII phosphorylation comparing to the control conditions was observed. Similarly to pea, after photo-activation of bean leaves LHCII phosphorylation came back to the control level. Such different response of LHCII phosphorylation processes to dark-chilling stress suggest that LHCII phosphorylation might be a part of plant adaptation mechanism to grow in low temperatures.

ACKNOWLEDGMENTS

Presented work was financed by the National Science Centre, Poland grant No.: 2016/23/D/NZ3/01276

CHLOROPLAST WAKE UP

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Photosynthesis is a process functioning efficiently at high span of light intensities. Numerous, complex regulatory mechanisms, operating to adjust photosynthetic activity to actual light intensity, include excitation quenching under overexcitation conditions. Such a mechanism can be followed by monitoring of lifetimes of chlorophyll fluorescence in vivo. Surprisingly, very similar type of quenching has been observed in intact chloroplasts under extremely low light intensities [1]. During talk, this mechanism will be presented and discussed.

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ORIENTATION OF CAROTENOIDS IN LIPID MEMBRANES

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Xanthophylls can play different biological roles. Their major properties relay on modification of the physical properties of lipid membranes and protection against oxidative damage. Localization and especially orientation of xanthophyll molecules in lipid membranes is important for understanding of the molecular mechanism responsible for carotenoid functions. On the presentation will be given the new method which enable to determine orientation of a polyene molecule in a single model lipid bilayer membrane [1]. By using two complementary microscopy methods: fluorescence and Raman imaging approaches applied to giant unilamellar vesicles it was possible to asses not only the orientation but also the molecular organization conformation of studied molecules. The average angle of xanthophylls (lutein and zeaxanthin incorporated into DMPC) relative to the membrane normal was determined to be ~40 deg [2]. Such a result corresponds well with the thickness of the DMPC membrane and the distance between the hydroxyl groups of chosen xanthophylls.

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PHOTOSYNTHETIC PROTEINS IN PHOTOVOLTAICS

Krzysztof Gibasiewicz

Photosynthetic reaction centers are natural optoelectronic devices converting the light energy into electrical current with quantum efficiency close to one. They are responsible for nearly all energy driving the life on Earth and accumulated in fossil fuels. During my talk I will present a few recent biohybrid photovoltaic systems containing purple bacteria reaction centers or photosystem I. Hopefully, further progress in this field will bring breakthrough constructions allowing cost-effective conversion of sunlight energy.

MODELLING OF CATHODIC AND ANODIC PHOTOCURRENTS FROM RHODOBACTER SPHAEROIDES REACTION CENTERS IMMOBILIZED ON TITANIUM DIOXIDE

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Among possible solutions for meeting future energy demands, solar cells based on photosynthetic reaction centers (RCs) from the purple bacterium *Rhodobacter sphaeroides* are considered. The proposed construction is similar to that of a dye sensitized solar cell, consisting of a TiO_2 mesoporous layer but with natural pigment-protein complexes used instead of an artificial dye. Genetically engineered RCs are used with an additional

extramembrane peptide tag to facilitate binding to TiO_2. For construction of the photoactive electrode, TiO_2 pastes prepared according to two different preparation procedures and containing two different TiO_2 nanoparticle sizes are used. With TMPD (*N,N,N′,N′*-tetramethyl-*p*-phenylenediamine) as electrolyte, biohybrid working electrodes produce a photocurrent that is the net product of cathodic and anodic photocurrents. For explanation of the observed photocurrents, a mathematical kinetic model is proposed that includes: (1) an anodic current due to injection of electrons from the triplet state of the RC primary donor (P) to the TiO_2 conduction band, (2) a cathodic current due to reduction of P^+ by surface states of TiO_2, and (3) transient cathodic and anodic peaks due to oxidation/reduction of TMPD/$TMPD^+$ on the conductive glass (FTO) substrate. This model explains: (1) the origin of the photocurrent spikes appearing after turning on and off the illuminating light, (2) the reason of appearance of the positive or negative net stable photocurrents depending on experimental conditions, as well as (3) the overall efficiency of the constructed cell. The model may be a used as a guide showing how to improve the photocurrent efficiency of the presented system as well as other biohybrid constructions, after introducing proper adjustments into it.

ACKNOWLEDGEMENTS

DJKS and MRJ acknowledge support from the Biotechnology and Biological Sciences Research Council of the UK (project BB/I022570/1). RB and KG acknowledge support from the National Science Center, Poland (project entitled ”Bio-semiconductor hybrids for photovoltaic cells” no. 2012/07/B/NZ1/02639).

INSIGHTS INTO THE IN VITRO MECHANISMS OF ANTICANCER ACTIVITY OF DIRUTHENIUM-1

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The anticancer activity of diruthenium-1 complex *in vitro* was evaluated against a panel of human cancer cell lines: HepG2 (liver hepatocellular carcinoma), MCF-7 (estrogen responsive breast adenocarcinoma), MDA-MB-231 (triple negative breast adenocarcinoma) and A549 (lung alveolar adenocarcinoma), on the basis of its ability to induce apoptosis, DNA damage and inhibition of cell cycle and proliferation. The effect of the complex on the intracellular redox state was also investigated.

The results indicated selective anticancer activity of diruthenium-1 toward MCF-7 cells at the nanomolar IC_{50} concentration (77 nmol/L) vs. the micromolar concentration IC_{50} (>1 μmol/L) for the rest of the cell lines.

The molecular mechanisms responsible for the anticancer activity of the complex in human breast cancer cells are mainly conditioned by its ability to: *i)* generate ROS burst; *ii)* induce apoptosis and necrosis of cancer cells*; iii)* interact with DNA and cause its extensive damage; *iv)* inhibit the cell cycle by arresting cancer cells at the G2/M check point. These changes are related to the concentration of diruthenium-1, the duration of the cell treatment and the post-treatment time.

EXCITATION DYNAMICS IN PHOTOSYSTEM I IMMOBILIZED ONTO CONDUCTIVE AND NON-CONDUCTIVE SURFACE

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Photosystem I (PSI) is a well-described pigment-protein complex with a quantum efficiency of photon to electron conversion near unity. This promising feature combined with high stability beyond the natural lipid membrane causes this complex to become the object of interest for biophotovoltaic applications. The main evidence of intactness of the structure and function of PSI in many artificial systems was its photoelectrochemical response. However, an additional evidence of stability could be obtained by a detailed study of the first steps of energy and electron transfer processes. Time-resolved fluorescence studies of the excitation dynamics in PSI suspended in solution and PSI deposited onto FTO conductive glass as a substrate showed significant acceleration of the excitation decay in PSI after immobilization [1].

The next step was to focus on the possible origin of such acceleration. We formulated two alternative working hypotheses: 1) the acceleration results from electron injection from PSI to the conducting surface; 2) the acceleration is caused by dehydration and/or crowding of PSI proteins deposited on the glass substrate. To resolve this issue, femtosecond transient absorption experiments were performed, with PSI being prepared in three states: (1) in aqueous solution, (2) deposited and dried on glass surface (either conducting or non-conducting), and (3) deposited on glass (conducting) surface, but being in contact with aqueous solvent. The kinetic traces for all systems with PSI deposited on substrates are almost identical and they decay significantly faster than the kinetic traces of PSI in solution [2]. Therefore, we assume that the accelerated excitation decay in PSI-substrate

systems is caused mostly by dense packing of proteins. Despite spectral and dynamic modification in PSI after immobilization, we conclude that those proteins remains fully functional in terms of energy and electron transfer processes.

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FLUORESCENCE ENHANCEMENT OF PHOTOSYNTHETIC COMPLEXES

Karolina Sulowska

No abstract available

DYNAMICS OF H_2O MOLECULES AS DEDUCED FROM BIREFRINGENCE STUDIES

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As a liquid, water plays a critical role in biological systems. A partial charge separation between oxygen and hydrogen atoms in H_2O molecule creates a net permanent dipole moment. Such molecule can interact favorably with charged species.

Water is an excellent hydrogen bonding solvent. It has a balanced number of hydrogen bond donors and acceptors and it has the smallest moment of inertia among small molecule polar liquids and as such demonstrates the fastest solvent relaxation dynamics. In the liquid phase H_2O molecules form a disordered fluctuating network of intermolecular hydrogen bonds.

Optical Kerr effect and Raman measurements in pure water at 298 K with 38 fs IR light pulses revealed three main components of the induced birefringence decay. The fast one, <50 fs, 500 and 1700 fs, due to the Raman modes, to a structural relaxation of the hydrogen-bonded network and to a molecular and/or cluster Brownian motion of water, respectively.

The motions of biomacromolecules depend on the structure and dynamics of water. These motions take place over a many time-scales: from ns (diffusion of H_2O in the first solvation shell of protein), through ps (amino-acid side motions) to sub-ps (librational and phonon-like motion of H_2O). The motions in a large range of frequencies can be studied by the OKE, the anisotropic Raman scattering and the dielectric techniques including THz-TDS. Using these methods one can observe changes in the spectra of biomacromolecules in water solution in the range 10 GHz – 30 THz.

In the lecture the fundamentals of THz radiation, as well as optical and THz Kerr effect will be reminded. THz–OKE measurements of three water samples of deionized, distilled and buffered (PBS) water will be reported and analyzed [1, 2]. These media were chosen in order to study the effect of ions presence on water behavior in the ultrafast time scale. The water most interesting from the point of view of living cells studies is the one significantly ionized. Therefore, discrimination between ultrafast effects resulting from internal H_2O properties from those resulting from H_2O – ions interactions are very important. These two effects may be connected to difference in the fluctuations of the network of intermolecular hydrogen bonds of water molecules in the presence or absence of ions and cations in solution. These fluctuations are expected to significantly alter water birefringence amplitude and its dynamics.

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EVOLUTION OF *ESCHERICHIA COLI* UNDER MECHANICAL STRESS INDUCED BY NANOPARTICLES

Kinga Matula[1]*, Łukasz Richter[1], Marta Janczuk-Richter[1], Wojciech Nogala[1], Mikołaj Grzeszkowiak[2], Barbara Peplińska[2], Stefan Jurga[2], Elżbieta Wyroba[3], Szymon Suski[3], Henryk Bilski[3], Adrian Silesian[4], Hans A. R. Bluyssen[4], Natalia Derebecka[5], Joanna Wesoły[5], Joanna M. Łoś[6,7], Marcin Łoś[6,7], Przemyslaw Decewicz[8], Lukasz Dziewit[8], Jan Paczesny[1], Robert Hołyst[1]

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Bacterial evolution under antibiotic pressure is currently the topic of great importance, as only recently strains resistant to all known available drugs emerged. Recently Baym *et al.*[1, 2] and Zhang *et al.*[3] demonstrated fast (within a few hours) evolution of *Escherichia coli* under antibiotic selective pressure in a heterogeneous environment. Here we show that instantaneous physical stress in a homogeneous environment also induces fast evolution of *E. coli*. We exposed *E. coli* in a stirred medium to a large number of collisions with sharp zinc oxide nanorods. The pressure exerted on the bacterial cell wall (up to 10 GPa) induced phenotype changes. The bacteria's shape became more spherical, the density of their periplasm increased by 15 % and the average thickness of the cell wall by 30 %.

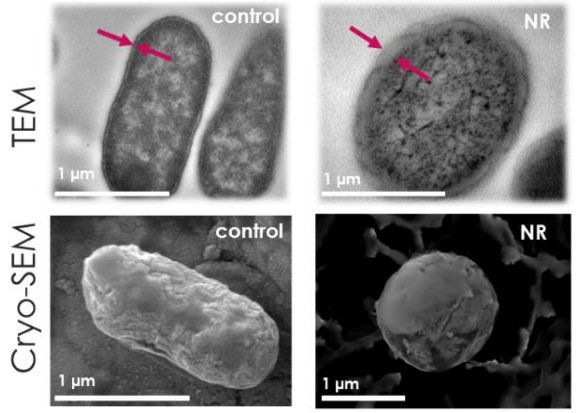

Fig.1. Transmission electron microscopy (TEM) and cryo-scanning electron microscopy (Cryo-SEM) of control *E. coli* (control) and the bacteria after exposure to ZnO nanorods (NR).

Such *E. coli* cells appeared almost as Gram-positive bacteria in the standard Gram staining. We performed also genome sequencing and transcriptome analysis to establish genetic origin of changes in phenotype. This is the first example of a real-time experiment where the bacteria undergo evolution upon instantaneous, acute physical stress without any obviously available time for gradual adaptation.

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NON-UNIFORM DIFFUSION IN *E. COLI* AS A FUNCTION OF A POSITION IN A CELL

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Knowledge about the processes occurring in lliving organisms is to characterize the mechanism of protein transport through biopolymer solutions such as DNA in the cytoplasm. Over several decades there were done many investigations, but still our knowledge about the transport of particles through concentrated polymers is incomplete. We recently shown that the transport properties of the complex liquids, including polymer, surfactant or colloidal solutions are length-scale-dependent (1–3). The same applies to the bio-complex liquids the cytoplasm of living cells. Based on proposed models there was created an unique database of diffusion coefficients of all proteins of strain K12 of E. coli, their oligomers and their potential complexes with translocation proteins (4). Now, we want to show that the diffusion coefficients of proteins inside *E. coli* strongly depend on the DNA concentration. For the first time, we measured in vivo the motion of EGFP as a function of highly concentrated DNA. We also proposed the model that describes two dimensional distributions of DNA inside *E. coli* and distributions of diffusion coefficients of EGFP in *E. coli*.

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NON-STANDARD BEHAVIOR OF STANDARD DYES IN FCS EXPERIMENTS

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Quantum dots (QD) are 2 - 100 nm diameter luminescent semiconductors. Compared with the organic fluorescent dyes they have long fluorescence lifetime, high photostability, broad excitation profile and narrow emission profile (both are size–dependent). Quantum dots can be covered with a hydrophilic shell and posses variety of bioconjugates, and are a promising tool for studying biological interactions. They have also been used in a variety of other applications such as photonic devices, diodes, lasers, materials for sensors. However, the quantum dots show a strong blinking effect, which could be a limitation in single molecule measurements. One of the methods used for exploring properties of the QD is FCS (Fluorescence Correlation Spectroscopy). To carry out the FCS measurement correctly, it is crutial to optimize the system using a standard dye. Very commonly used standard dyes are Alexas. We were excitind Alexa 488 and Alexa 430 using the 440 nm laser. W belive that the results we obtained prove that, under this conditions, both Alexa 488 and Alexa 430 photobleach, and the photobleaching influences the shape of the measured correlation curves in different ways fot both Alexas.

BIOCHEMICAL AND BIOPHYSICAL CHARACTERISTICS OF THE C-TERMINAL REGION OF ECDYSTEROID RECEPTOR FROM AEDES AEGYPTI

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Five distinctive domains in the structure of nuclear receptors can be distinguished: the N-terminal domain with AF-1 transactivation site, the DNA binding domain, the hinge region, the ligand binding domain and the F domain. Ecdysteroid receptor (EcR), responsible for the reproduction of, dengue and Zika fever vectors, *Aedes aegypti* mosquitoes, exhibits the same canonical structure. However, the knowledge about the structure and function of the F domain from *A. aegypti* (AaFEcR) is poor. In order to plan successful strategies of mosquitoes population control, full understanding of mechanism of action of this domain needs to be investigated.

Our research on AaFEcR began with establishing optimal overexpression conditions and reproducible purification procedure. *In silico* analyses indicated high probability of disordered structures occurrence. Further circular dichroism (CD) spectra recording and their subsequent deconvolution confirmed that. Size-exclusion chromatography (SEC) and analytical ultracentrifugation (AUC) experiments revealed inflated value of the hydrodynamic radius of AaFEcR compared to proteins with the same molecular weight. Further sequence *in silico* analyses demonstrated presence of amino-acid motif resembling one that is responsible for metal ions binding among histidine-proline-rich glycoproteins. Therefore, series of CD, SEC and AUC experiments in the presence of zinc, copper and calcium ions were conducted. Obtained results were ambiguous: the hydrodynamic radius of AaFEcR decreased in the increasing ions concentration, but the content of secondary structures remained the same. The ability of AaFEcR to bind aforementioned ions was definitively confirmed by complexes observed in ESI-TOF mass spectrometry.

ACKNOWLEDGEMENTS

This work was supported by Wrocław Centre of Biotechnology, programme The Leading National Research Centre (KNOW) for years 2014-2018 and partially supported by a statutory activity subsidy from the Polish Ministry of Science and Higher Education for the Faculty of Chemistry of Wrocław University of Science and Technology.

NEXT NEIGHBOR ORDER AND THE PHASE TRANSITIONS IN YUKAWA COLLOIDS

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The short- and long-range order and the phase transitions of Yukawa colloid were studied by means of the Rogers-Young (RY) model calculations with the Hansen-Verlet (HV) freezing criterion, MC simulations and small angle X-ray scattering (SAXS) using synchrotron radiation. First the Yukawa system and the measures of the short-range (next neighbor) order: the radial pair correlation function g(r), the number of next neighbors n_n and the mean next neighbor distance r_n as well as the measure of the long range order – the structure factor S(q) will be defined. The freezing lines and the short- and long-range order parameters obtained from the RY calculations for a broad range of $\lambda=\kappa\langle r\rangle$ (where $1/\kappa$ is the Debye screening length and $\langle r\rangle$ is the mean interparticle distance) will be discussed [1, 2]. It will be shown that for low λ value the system, deep in the fluid regime, exhibits short range order (in terms of n_n and r_n) although no long range order

(measured by S(q)) can be seen.

MC simulations performed for the same Yukawa system were used (i) to explain the intermediate unphysical values of the short-range order parameters on the freezing line in the intermediate λ-range and (ii) to illustrate the structure of the of the Yukawa system on both sides of the liquid – bcc crystal, liquid –fcc crystal and bcc – fcc crystal phase transition lines. Using the simulation results also the bond angles in colloidal system and the relationship between the short- and long-range order will be discussed. Experimental results showing the phase transitions in the same Yukawa system measured by means of the scattering of synchrotron radiation (SAXS) will be also presented.

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FLUORESCENCE CORRELATION SPECTROSCOPY ANALYSIS OF LENGTH-SCALE DEPENDENT VISCOSITY IN NUCLEI OF CANCEROUS CELLS *IN VIVO*

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A series of fluorescent nanoparticles having hydrodynamic radii from 1 to 9 nm were microinjected into nuclei of living human cancerous cells (HeLa cell line). By means of fluorescence correlation spectroscopy (FCS) we were able to noninvasively measure diffusion coefficients of introduced probes. The obtained *in vivo* autocorrelation curves were fitted with one component anomalous model due to the polydispersity of probes [1]. The results revealed that, as recently showed also for the cytoplasm [2,3], effective viscosity in the nucleosol increases exponentially with the probe size. It is in contrast to reciprocal dependence of the probe radius expected from the Stokes–Sutherland–Einstein (SSE) relation (i.e. $D = kT/6\pi\eta r_p$). Understanding how intranuclear diffusional mobility within chromatin network depends on probe size is of great importance for the fields such as gene expression, mechanism of proliferation, cancer treatment and assessing kinetics of chemical reaction inside nuclei. In this presentation, we will discuss recent results as well as technicalities regarding the microinjection, and FCS measurements in nuclei of living cells.

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CELL CYCLE-DEPENDENT FLUCTUATIONS OF PROTEIN MOBILITY IN CYTOPLASM OF HELA CELL

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Great deal of life-sustaining processes are diffusion-limited and viscosity of cytoplasm may influence the diffusion rates. Up to date no one attempted to measure how viscosity of cytoplasm changes throughout the cell cycle. Current research focuses rather on short time-stamps in cell life. Our research aims to measure fluctuations of rate of diffusion of example protein during cell cycle and to give possible explanations of any changes observed.

We studied diffusion of enhanced green fluorescent protein (EGFP) in cell cytoplasm of HeLa eukaryotic cell line using fluorescence correlation spectroscopy (FCS). Monomeric EGFP was chosen as well-known and biologically-neutral probe which has size comparable to cell cytoplasm content. Observations made on this basis could be easily transferred to other cytoplasmic proteins. To ensure uniform progress through cell cycle, cells were first synchronized at the G1/S phase transition using aphidicolin and then allowed to grow.

Our results show values of diffusion coefficients of EGFP in cytoplasm of cells travelling through the cell cycle (as compared to diffusion of EGFP in buffer). After short lag-phase in which cells resume synthesis of DNA, there is a slight increase in viscosity of cytoplasm (most probably connected to S phase). After that the viscosity of cytoplasm remains on lower, constant level. Our results could hint at possible not yet studied mechanism of sustaining physiological viscosity of cytosol. This observation seems to be plausible since rates of cellular activities strongly depend on changes in diffusions rates of substrates. This equilibrium exist in spite of cellular volume fluctuations.

GENERAL METHOD FOR QUANTIFICATION OF OLIGOMERIZATION OF PROTEINS IN LIVING CELLS

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Nowadays, life-science researchers utilize plenty of methods aiming in quantification of protein-protein interactions. These methods range from simple biochemical experiments, through molecular biology methods, towards advanced proteomic analysis. In this way, myriads of valuable data for biology, medicine and pharmacology were provided. However, majority of experiments was performed on fixed cells or extracted proteins. Therefore, detailed information about *in vivo* dynamics of protein-protein interactions is still missing, but substantially needed. We present a fluorescence correlation spectroscopy method of protein oligomerization analysis. As a protein of interest, we chose dynamin-related protein 1 (Drp1) which is involved in mitochondrial fission process. Our method base on precise determination of length-scale dependent hydrodynamic drag of cytoplasm. It was proved, that cytoplasmic hydrodynamic drag (d_h, also interpreted as viscosity) depends on a probe's size. Therefore, first step of our research was determination of diffusion coefficients (D_{diff}) of probes of known sizes (GFP, Calcein-AM, dextrans) in cytoplasm of HeLa cells. These results were utilized for evaluation of a scaling equation, and, subsequently, for determination of D_{diff} expected for certain oligomers of Drp1. Next, D_{diff} of GFP-fused Drp1 was measured by FCS in HeLa. Different Drp1 mutants were investigated (monomer, dimer, wild type). Results indicate that there is an equilibrium between dimeric and tetrameric form of wild type Drp1 in cytoplasm. Length-scale dependence of d_h enabled separation of D_{diff} of these two forms (D_{diif} of dimer was 1.5 fold bigger than D_{diff} of tetramer, in contrast to constant viscosity conditions). Thus, quantity of dimer and tetramer forms could have been determined. Moreover, dissociation equilibrium constant of tetramer could have been calculated and it was 0.7 μM for wild type Drp1.

ROLE OF UVRD PROTEIN IN NUCLEOTIDE EXCISION REPAIR

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UvrD protein is involved in genome maintenance processes in bacteria such as DNA repair, replication and recombination. In Nucleotide Excision Repair (NER) pathway in bacteria UvrD's role is related to displacing the oligonucleotide sequence excised by UvrC in order to allow DNA polymerase I to fill the gap and finish DNA repair process. Interestingly, it is also suggested that UvrD is specifically involved in Transcription Coupled Repair (TCR) by backtracking RNAP from the DNA lesion and facilitating NER at the same time [1]. Since according to other authors [2] this mechanism is controversial, we decided to shed more light on UvrD role in NER.

In our research we use single-molecule fluorescence imaging (Photoactivated Localisation Microscoy –PALM) and single-particle tracking. Our preliminary results suggest that UvrD's role in DNA repair is rather specific to Global Repair (GR) pathway than TCR. We hypothesize that UvrD may be involved in TCR, but only in high stress conditions. The goal of the project is to mechanistically characterise UvrD behaviour by studying its intracellular mobility and positioning in living bacteria.

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IN VITRO STUDIES OF THE INITIAL STEPS OF NUCLEOTIDE EXCISION REPAIR IN *E. COLI*

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Nucleotide excision repair deals with a variety of DNA lesions including bulky DNA and chemical damage. In *E. coli*, the process is governed by UvrABCD proteins, which recognise damage and excise part of the DNA strand containing lesion. I am working on understanding the molecular mechanism behind the pathway currently focusing mainly on damage recognition and verification steps. Preliminary results indicate that UvrB recruitment *in vitro* is UvrA dependent and DNA damage dependent. Based on that we would like to see how stable the complexes are and eventually reconstitute the whole NER pathway on a surface to study every step individually

ANTISENSE TRANSCRIPTION REGULATION IN *A. THALIANA*

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Antisense transcription is pervasive phenomenon in many genomes that often leads to convergent transcription. In my talk I will tell about new role of Elongin C, protein known to be involved in DNA damage repair, in regulation of protein-coding gene by its antisense transcript. We showed that in plants, like in other organisms, Elongin C can facilitate DNA repair by promoting poly-ubiquitylation of RNA polymerase II stalled on DNA lesion. We also uncovered new role of Elongin C, which is expression regulation of major seed dormancy regulator in Arabidopsis – DOG1 gene and showed that this regulation is dependent on the presence of DOG1 antisense transcript.

MEIBOMIAN GLAND DYSFUNCTION

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Meibomian gland dysfunction is one of the most common disease in ophthalmic practice and a major cause of the evaporative type of dry eye [1]. Meibomian glands are a type of a large sebaceous glands located vertically inside the tarsal plates of the eyelids. These glands consist of excretory part acini connected with long central ducts via short ductules [2-3]. Meibomian oil produced by the meibomian glands is a major component of the tear film lipid layer. The outer surface of the tear film prevents the evaporation of the aqueous portion of the tear film and provides a clear and smooth optical surface [2-4]. Meibomian gland dysfunction (MGD) can be characterized as terminal duct obstruction and/or qualitative/quantitative changes in glandular serection [3,5-6]. Meibography is an infrared (IR) technique that allows observation of the morphological structure of meibomian glands. A non-contact equipment yields information on the meibomian glands by analyzing their silhouette through retroillumination of the everted eyelids from the skin side [7]. A lot of studies have used a subjective grading scales of MGD [8]. The analysis of meibography images by now based on the calculation of area of gland loss [9]. The other algorithm based on the image features such as gland lengths and widths. The method can only differentiate between healthy and unhealthy images [10].There is a need to develop an objective and automatic analysis of MGD based on the obtained photographs of the meibomian gland area. The analysis should take into account not only the area of gland loss but also the morphological features in terms of spatial widths, dilatation and distortion. Meibomian gland dysfunction leads to visible structural irregularities that help in the diagnosis and prognosis of the disease. What is more, the purpose of the developed method is to enable to find a subclinical changes in the area of meibomian gland. The proposed system based on the Fourier analysis, which provides the quantitative information about the morphological features and allows images to be classified. The standardized protocol should be proposed and adopted in clinical trials.

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RANDOM PHASE APPROXIMATION FOR MULTIBLOCK COPOLYMERS: THEORY AND EXPERIMENT

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Random Phase Approximation (RPA) is used to study incipient microphase separation as observed by wide

angle X-ray scattering (WAXS) in short-chain multiblock copolymers consisting of perfluoropolyether (PFPE) and poly(ethylene oxide) (PEO) segments. Despite having a low degree of polymerization, these materials exhibited significant scattering intensity, due to disordered concentration fluctuations between their PFPE-rich and PEO-rich domains. The disordered scattering intensity was fit to the RPA equations to determine the value of the interaction parameter, χ, and the radius of gyration, $R_{g.}$ A detailed derivation of the RPA equations for multiblock copolymers is shown in this presentation.

STATISTICAL ANALYSIS OF PARTICLE TRACKING EXPERIMENT

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In a particle tracking experiment a movie is recorded with images of tracked particles at a constant frame rate. Positions of individual particles are estimated in every frame and a dedicated algorithm calculates trajectories of all of them. In the analysis of such data two major approaches are used: i) all particles are assumed to be identical and ensemble averaging is applied to calculate the mean square displacement (MSD); ii) a distribution of particle mobilities (sizes) is assumed and the MSD values are calculated for each trajectory individually as a time average. In the former case a single value of diffusion coefficient is obtained with a relatively small standard deviation already after a single time step if the ensemble is large enough. In the latter case a time average of a single step square displacement along the trajectory results in a much larger deviation of the mean value even for a monodisperse sample. Moreover, for short trajectories the distribution of such obtained apparent diffusion coefficients is also asymmetric. Proper analysis of MSD data for short trajectories should be based on the superposition of distributions corresponding to particles of different mobilities. Analytical form of such distributions of apparent diffusion coefficients have been derived for single time step MSD values. Comparison with simple simulations of Brownian motion will be shown for the case of a bimodal suspension. Finally, consequences for short FCS measurements will be shortly discussed.