Session 13: Biophysics in life sciences

Lectures

L.13.1

Liquid-Liquid Phase Separation in ATP-Incorporating Amyloidogenic Peptides

Wojciech Dzwolak

Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Poland
Wojciech Dzwolak <wdzwolak@chem.uw.edu.pl>

Interplay of transition pathways leading to liquid-liquid phase separation (LLPS) and amyloid fibrils often underlies a disease-associated protein dysfunction. Selection criteria of LLPS-dependent / LLPS-independent protein aggregation pathways remain unclear. Here, we report the LLPS/fibrillization properties of a family of chimeric peptides, $ACC_{1\text{-}13}K_n$, in which the highly amyloidogenic fragment of insulin ($ACC_{1\text{-}13}$) is merged with oligolysine segments of various lengths (K_n, n = 8, 16, 24, 32, 40). Fibrillization of these peptides is conditioned on the presence of ATP which is stoichiometrically encapsulated within the forming amyloid. The incorporation of ATP is an emergent property of $ACC_{1–13}K_n$ not observed for $ACC_{1–13}$ and K_n segments separately. The LLPS phase preceding the actual fibrillization is observed only for the peptides with the longest K_n segments, i.e. when Coulombic interactions between charged lysine side chains and ATP anions are mostly involved in the self-assembly process. The kinetics of the phase transition and the stability of mature co-aggregates are highly sensitive to ionic strength indicating that electrostatics play a pivotal role in selecting the LPPS-fibrillization transition pathway. The multicomponent / multiphase $ACC_{1–13}K_n$-ATP system constitutes an excellent model of complex intracellular assemblies involving non-proteinaceous components within which protein misfolding takes place.

L.13.2

Mechanomarkers of various diseases

Małgorzata Lekka

Institute of Nuclear Physics Polish Academy of Sciences, Krakow, Poland
Malgorzata Lekka <malgorzata.lekka@ifj.edu.pl>

In recent decades, evidence shows that mechanical and rheological measurements of cells and tissues have become essential to determine how these changes contribute to disease development and progression. They can distinguish healthy/diseased states through quantitative measurements at the nano- and microscales that are reproducible, label-free, and require small samples (single cells, tissues, or even proteins). Alterations in mechanics/rheology frequently are identified before other markers such as changes in protein expression, inflammation level, or disease symptoms occur. Thus, mechanical/rheological measurements can be a promising tool or become part of the new procedures applied to diagnosse various diseases, including cancers, multiple sclerosis, or muscular dystrophy. Mechanomarkers are gaining attentions in the medical practice thanks to the technical development observed in the past decades. However, still research towards straightforward and fast measurements is needed to improve robustness and their applications in clinical practice.

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L.13.3

Analytical ultracentrifugation in the study of biological macromolecules

Beata Wielgus-Kutrowska

Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw; Warsaw, Poland
Beata Wielgus-Kutrowska <Beata.Wielgus-Kutrowska@fuw.edu.pl>

Analytical ultracentrifugation (AUC) is a powerful research tool used in biophysical studies of the properties and behavior of biological macromolecules in solution. It enables the determination of their interactions, size, shape, and stoichiometry.
Using the analytical ultracentrifuge, two experiments can be conducted: sedimentation velocity and sedimentation equilibrium, using different detection systems: absorption, interference, or fluorescence. This method's scope, advantages, limitations, and specific applications of each technique will be presented, demonstrating how AUC can provide valuable insights into complex biological systems in the fields of molecular biology, biochemistry, drug discovery, and biotechnology.
Several examples from our laboratory employing the analytical centrifugation technique will also be presented. These include studies on EGFP and DNA aggregation monitored by fluorescence and absorption detection, evaluation of the oligomeric structure of wild-type and mutant purine nucleoside phosphorylases, and the examination of the impact of environmental conditions on the quaternary structure of proteins.

Oral presentations

O.13.1

New aspects of the pathophysiology of lipid droplets in inflammation of an isolated blood vessel

Marta Z. Pacia[1], Natalia Chorazy[1,2], Kamila Wojnar-Lason[1,3], Magdalena Sternak[1], Stefan Chlopicki[1,3]

[1]Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics, Krakow, Poland; [2]Jagiellonian University, Doctoral School of Exact and Natural Sciences, Krakow, Poland; [3]Jagiellonian University, Chair of Pharmacology, Krakow, Poland
Marta Zofia Pacia <marta.z.pacia@gmail.com>

Lipid droplets (LDs) play a crucial role in cellular metabolism, buffering toxic lipid species, and are implicated in diseases like diabetes and fatty liver. Despite growing knowledge of endothelial LDs, their precise function and biogenesis pathway remain unclear. This was the motivation for the microscopic and spectroscopic characterization of LDs formed in vascular inflammation and in the response to excess lipids.
The results demonstrate that activation of inflammation in endothelial cells of the isolated aorta, induced by various factors such as tumor necrosis factor (TNF), lipopolysaccharides (LPS), angiotensin II (AngII), hypoxia, or excess oleic acid (OA), leads to the formation of LDs and enhanced generation of prostacyclin (PGI_2). Blocking the adipose triglyceride lipase (ATGL) increases the number of LDs in endothelial cells while inhibiting PGI_2 generation. Furthermore, the Rac1 protein has been identified as a central factor linking LDs formation in endothelial cells with other markers of the inflammatory response, including increased endothelial stiffness, cytoskeletal reorganization, and elevated secretion of adhesion molecules.
In conclusion, the results suggest that the formation of vascular LDs in dysfunctional endothelium is dynamically regulated by both ATGL and the Rac1 protein, shedding light on new aspects of the pathophysiology of LDs.

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Posters

P.13.1

Platinum nanoparticles as cisplatin modulators – direct interactions and biological effects

Patrycja Bełdzińska[1], Kamila Butowska[1,2], Grzegorz Gołuński[1], Marcin Zakrzewski[1], Barbara Galikowska-Bogut[3], Rafał Sądej[3], Augustyn Moliński[4,5], Jacek Piosik[1]

[1]Laboratory of Biophysics, Intercollegiate Faculty of Biotechnology UG&MUG, Gdańsk, Poland; [2]Department of Cellular and Molecular Medicine, University of California San Diego, USA; [3]Department of Molecular Enzymology and Oncology, Intercollegiate Faculty of Biotechnology UG&MUG, Gdańsk, Poland; [4]Department of Biomedical Physics, Adam Mickiewicz University in Poznań, Poland; [5]NanoBioMedical Centre, Adam Mickiewicz University in Poznań, Poland

Patrycja Bełdzińska <patrycja.beldzinska@phdstud.ug.edu.pl>

In the recent years, nanoparticles, as structures diversified in terms of size, shape or surface functionalization, have aroused great interest. Interestingly, some of them, such as platinum nanoparticles (PtNPs) possess anticancer activity and can possibly act as drug delivery platforms which make them promising candidate to investigate.
Cisplatin (CDDP), as a first generation platinum anticancer drug, is widely used in treatment of various malignancies such as breast, ovarian or head and neck cancers. Nevertheless, CDDP causes serious side effects, especially severe nephrotoxicity, but also triggers drug resistance. Consequently, in our research we investigated whether PtNPs could interact directly with CDDP and influence the activity of the tested drug.
The possible interactions between 5 nm and 50 nm PtNPs and CDDP were studied by Atomic Force Microscopy (AFM) and Dynamic Light Scattering (DLS). Moreover, to examine the biological effects of PtNPs on CDDP activity, mutagenicity Ames test on *Salmonella typhimurium TA102* and MTT assay on the selected breast cancer cell lines were exploited.
The AFM revealed that CDDP triggers PtNPs' aggregation. Moreover, the DLS results indicated the changes in hydrodynamic diameter of 5 nm PtNPs after adding CDDP, as opposed to 50 nm where the diameter change was negligible. Furthermore, the biological results imply PtNPs influence on CDDP activity by decreasing the mutagenicity and increasing the cytotoxicity in the selected models.

P.13.2

Platinum nanoparticles and their interactions with daunorubicin

Marcin Zakrzewski, Patrycja Bełdzińska, Grzegorz Gołuński, Jacek Piosik

Laboratory of Biophysics, Intercollegiate Faculty of Biotechnology UG&MUG, Gdańsk, Poland

Marcin Zakrzewski <marcin.zakrzewski@phdstud.ug.edu.pl>

Nanoparticles have been widely studied over the last decades. They gained interest mainly due to their effects on cells, dependent on their size, composition and possible surface functionalization. Among them, platinum nanoparticles (PtNPs) gained particular interest. They are considered safe to human health, and, at the same time, exhibit anticancer effects. What is more, a few sources suggest they could be exploited as drug carriers. Herein, we test the effects of various sizes of platinum nanoparticles on daunorubicin (DAU), an antibiotic used in leukaemia treatment.
To assess their interaction we first conducted DLS (*Dynamic Light Scattering*) to look for changes in size distribution dependent on the drug concentration. Furthermore, we performed fluorescence spectroscopy of DAU titrated with increasing volumes of PtNPs. Finally, we performed dialysis experiments, in which we measured the velocity of the drug release through a porous membrane at three distinct pH.
The results of the DLS analysis suggest that all sizes of PtNPs interact with DAU. What is more, the fluorescence of the drug decreases with addition of nanoparticles, suggesting their direct interactions. The release experiment shows that the amount of released drug depends on both the size of PtNPs and the pH of the solution, suggesting once more that there is an interaction between DAU and PtNPs and that it may be pH-dependent, making PtNPs possible drug carriers.

P.13.3

Dynamics of *S*-adenosyl-L-homocysteine hydrolase from *Pseudomonas aeruginosa* revealed by frequency-domain measurements of fluorescence anisotropy

Monika Moskwa[1,2], Katarzyna Woźniak[1], Gotard Burdziński[3], Tomasz Pędziński[4], Krzysztof Brzeziński[1]

[1]Department of Structural Biology of Prokaryotic Organisms, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland; [2]Faculty of Biology, Adam Mickiewicz University, Poznan, Poland; [3]Faculty of Physics, Adam Mickiewicz University, Poznan, Poland; [4]Faculty of Chemistry, Adam Mickiewicz University, Poznan, Poland.
Monika Moskwa <monmos3@st.amu.edu.pl>

Pseudomonas aeruginosa is an opportunistic bacterium responsible for severe infections in hospitalized patients with compromised immune systems. In *P. aeruginosa*, *S*-adenosyl-L-homocysteine hydrolase (PaSAHase) regulates numerous methylation processes crucial for the bacterium's metabolism. PaSAHase is active as a tetramer, with each subunit folded into three domains. Two major domains (substrate- and cofactor-binding) are connected by a hinge element. During the hydrolytic cycle, the enzyme oscillates between closed (substrate-bound) and open (with a product released) conformations. Recent discoveries indicate that the activity of PaSAHases strongly depends on the frequency of domain oscillations, which must be synchronized with a time interval required to complete the catalytic cycle. This study aimed to define amino acid residues involved in PaSAHase dynamics and analyze their influence on the frequency of domain oscillations. For this purpose, series of PaSAHase point mutants were generated, expressed in *Escherichia coli* and purified. Next, selected variants were covalently and selectively modified with a fluorescent probe (N-(1-pyrenyl)maleimide, PMal) to analyze protein dynamics with frequency-domain measurements of the rotational dynamics of PMal-PaSAHase conjugates using measurements of the fluorescence lifetimes.

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P.13.4

Changes in human erythrocyte morphology under the influence of non-functionalized polystyrene nanoparticles with different diameters

Kamil Płuciennik, Paulina Sicińska, Bożena Bukowska

Department of Biophysics of Environmental Pollution, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland
Kamil Płuciennik <kamil.pluciennik@edu.uni.lodz.pl>

Plastic production is still at high levels. In the environment plastic exposed to physicochemical factors (e.g. biodegradation, UV-radiation) is disintegrated into smaller microplastics (MPs) (< 5000 nm), and then to nanoplastics (NPs) (< 1000nm). MPs and NPs can enter the human organisms through ingestion, inhalation, and dermal exposure. As a result of the detection of plastic particles in blood, there is a potential risk of transferring them to other organs and tissues, which could affect the body's homeostasis.

Among the plastic particles detected in blood there were particles from polystyrene (PS), commonly used as packaging, especially for food. Taking into consideration the lack of data, we began to evaluate the effects of non-functionalized polystyrene nanoparticles (PS-NPs) of different diameters on the shape of human erythrocytes.

The erythrocytes were incubated with PS-NPs with different diameters of ~30 nm, ~45 nm and ~ 70 nm in the concentrations range of 0,001 to 10 μg/mL for 24 h. After incubation, the erythrocytes were washed and fixed with 2% glutaraldehyde.

There was a statistically significant decrease in the number of discocytes, the level of echinocytes was not changed, whereas the percentage of stomatocytes increased after incubation with PS-NPs in all tested diameters. The most intensive formation of stomatocytes was observed under the influence of the largest tested NPs.

P.13.5

Anchoring of the Respiratory Syncytial Virus (RSV) fusion peptide depends on the membrane composition.

Helena Cichocka[1,2], Bartłomiej Garbacz[2], Remigiusz Worch[2]

[1]Department of Physics, University of Warsaw, , 5 Pasteur Street, 02-093 Warsaw, Poland; [2]Nencki Institute of Experimental Biology PAS, Laboratory of Cell Biophysics, 3 Pasteur Street, 02-093 Warsaw, Poland
Remigiusz Worch <r.worch@nencki.edu.pl>

Respiratory Syncytial Virus (RSV) is one of the pathogens causing respiratory infections. RSV is particularly dangerous for infants and can be fatal for premature babies. Viral entry requires fusion of the envelope with the target host cell membrane. The N-terminal fragment of the F1 subunit is considered as the fusion peptide. However, it has not been experimentally confirmed. Our research aims to study the behavior of the RSV fusion peptide at the membrane interface. We used a synthetic fragment of the N-terminal 30 amino acids of the F1 subunit ($F1_{1-30}$). To visualize binding, we performed confocal imaging of giant unilamellar vesicles and fluorescently-labeled $F1_{1-30.}$ We observed binding to the membrane composed of POPC, however it was not the case in the presence of cholesterol. Interestingly, we noticed a more pronounced staining in the presence of phosphatidylserine (POPS), accompanied by membrane deformations. To characterize the binding quantitatively, we determined the partition coefficients (K_x) by means of a series of fluorescence spectroscopy with the use of a single tryptophan-mutated $F1_{1-30.}$ In agreement with microscopic observations, the K_x were in the following order: POPC/POPS (6/4 mol%), POPC, POPC/chol (6/4 mol%). Our research contributes to better understanding of the molecular mechanism of protein-mediated membrane fusion occurring in the early steps of RSV replication.

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