BP 17: Protein Structure and Dynamics

Time: Wednesday 15:00–17:30 Location: BAR 0106

BP 17.1 Wed 15:00 BAR 0106

Computational Approaches to Liquid-Liquid Phase Separation of Partially Disordered RS-Proteins — •Yannick Witzky[1], Stephan Hobe[2], Andreas Wachter[2], Arash Nikoubashman[1], and Friederike Schmid[1] — [1]Institute of Physics, Johannes Gutenberg University — [2]Institute for Molecular Physiology, Johannes Gutenberg University

RS-proteins are a class of proteins that contribute to light-activated gene regulation (via an alternative splicing mechanism) in plant morphogenesis. It has been hypothesized that liquid-liquid phase separation (LLPS) plays an important role for the regulation mechanism. Studying these proteins is challenging because they contain both intrinsically disordered regions (IDRs) - which presumably control the LLPS - as well as folded domains that contain the functionally important RNA binding sites. Here we use and compare different coarse-grained models to study the condensation and phase behavior of RS proteins: Commonly used IDP models [1,2] as well as the structure predictive UNRES model [3]. We specifically focus on the on the single-chain conformations, phase behavior and the accessibility of RNA binding site.

[1] Tesei et al. (2022) Open Research Europe, 2(94), 94.
[2] Rizuan et al. (2022) J Chem Inf Model 62(18), 4474-4485.
[3] Sieradzan et al.(2019) J. Phys. Chem. B, 123, 27, 5721-572

BP 17.2 Wed 15:15 BAR 0106

Key Role of the Solvent in Driving Liquid-Liquid Phase Separation — •Ellen Adams[1,2], Jonas Ahlers[3], Verian Bader[3], Simone Pezzotti[3], Konstanze Winkhofer[3], Jörg Tatzelt[3], and Martina Havenith[3] — [1]Technische Universität Dresden — [2]Hemholtz Zentrum Dresden Rossendorf — [3]Ruhr Universität Bochum

In recent years the importance of the aqueous solvent in influencing protein structure, function, and dynamics has been recognized. Coupling of water molecules to the protein surface creates an interfacial region in which water molecules within this region have distinctly different properties than bulk water. Yet, the structure and dynamics within this interfacial region are still not easy to access experimentally. Terahertz (THz) spectroscopy has been shown to be a powerful tool to investigate solvent dynamics in bulk solutions and is directly sensitive to changes in the low frequency collective intermolecular hydrogen-bonding vibrations of water. Here the role of solvation dynamics in the liquid-liquid phase separation (LLPS) of the intrinsically disordered protein fused in sarcoma (FUS) is probed. Characterization of the hydrogen bonding network reveals that water solvating hydrophobic groups is stripped away in the membrane-less FUS biomolecular condensates. Additionally, water left inside of the biomolecular condensates is highly constrained, indicative of a population of bound hydration water. These results uncover the vital role of hydration water in LLPS: the entropically favorable release of unfavorable hydration water serves as a driving force for LLPS.

BP 17.3 Wed 15:30 BAR 0106

Local structure and dynamics of water molecules in FUS protein molecular condensates. — Daniel Chavez Rojas[1], •Joseph Rudzinski[1,2], and Martin Girard[1] — [1]Max Planck Institute for Polymer Research, Mainz, Germany — [2]Institut für Physik, Humboldt-Universität zu Berlin, Berlin, Germany

There is evidence that molecular condensates of the FUS protein play a role in the development of some neurodegenerative diseases like ALS. For this reason, understanding the molecular mechanism by which these condensates form at an atomistic level is of therapeutic interest. However, the molecular structure and water-protein interactions of these condensates is poorly understood. In this work, we utilize a multi-scale approach to generate FUS condensates at a sufficient scale with a coarse-grained model, followed by investigation of the atomic scale with shorter, fully-atomistic molecular dynamics simulations. As a result, we are able to efficiently characterize water-protein hydrogen bonding interactions, contacts, and water ordering around the individual amino acids of FUS proteins in the condensate versus in solution. The characterization of water-protein and protein-protein structure provides insights about the driving forces that promote the formation of these molecular condensates.

BP 17.4 Wed 15:45 BAR 0106

Structural dynamics of the intrinsically disordered SNARE proteins at the membrane interface: Recent insights by NMR spectroscopy — Tobias Stief[1,2], Mirko Kraus[1,2], Katharina Vormann[1,2], Reinhard Jahn[3], Angel Perez-Lara[4], and •Nils-Alexander Lakomek[1,2] — [1]Forschungszentrum Jülich, Jülich, Germany — [2]Heinrich-Heine-Universität, Düsseldorf, Germany — [3]Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany — [4]University of Granada, Granada, Spain

SNARE proteins play a crucial role during neurotransmitter release by eliciting the fusion of the synaptic vesicle membrane with the presynaptic plasma membrane. In their pre-fusion state, the SNARE proteins are intrinsically disordered. They do not exhibit a well-defined structure and show high internal flexibility, being membrane-anchored. However, the mode of interaction between the SNARE proteins and the lipid membrane needs to be better understood.

We use the SNARE proteins as a model system for developing novel NMR methods to characterize the inner and conformational dynamics of intrinsically disordered proteins interacting with lipid membranes or being membrane-anchored. Therefore, we address a large range of timescales, from pico- to milliseconds, employing both solution NMR and solid-state NMR methods. The aim is to better describe the conformational space of intrinsically disordered proteins at the lipid membrane interface. At the conference, we will present recent (unpublished) insights into the structural dynamics of the SNARE protein synaptobrevin-2 at the lipid membrane interface.

BP 17.5 Wed 16:00 BAR 0106

Single-chain and condensed-state behavior of intrinsically disordered nuclear proteins in bulk and confinement — •Janka Bauer[1], Lukas Stelzl[1,2,3], Dorothee Dormann[2,3], and Arash Nikoubashman[1] — [1]Institute of Physics, JGU Mainz, Germany — [2]Biocenter, Institute of Molecular Physiology, JGU Mainz, Germany — [3]Institute of Molecular Biology, Mainz, Germany

The liquid-liquid phase separation of intrinsically disordered proteins plays an integral part for the formation of membraneless organelles in cells, which in turn have key functional and regulatory roles. To better understand the complex relation between the sequence and self-assembly of these heteropolymers, we perform molecular simulations of the low-complexity domains of heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) and Fused in Sarcoma (FUS). For hnRNPA1, we systematically analyze how the conformation and phase behavior are affected by the number of aromatic residues within the examined sequences in both single-chain and condensed state simulations. Our observations strongly support the hypothesis that aromatic residues play a dominant role for condensation, which is further corroborated by a detailed analysis of the intermolecular contacts. To mimic more closely conditions prevalent in cellular environments, we perform simulations of hnRNPA1 and FUS in spherical confinement, where we systematically vary the fraction of the crowding agent polyethylene glycol.

15 min. break

BP 17.6 Wed 16:30 BAR 0106

Efficiency and selectivity in the self-assembly of SAS-6 rings on a surface — •Santiago Gomez Melo[1], Dennis Wörthmüller[1], Pierre Gönczy[2], Niccolo Banterle[3], and Ulrich Schwarz[1] — [1]Heidelberg University, Heidelberg, Germany — [2]EPFL, Lausanne, Switzerland — [3]EMBL, Heidelberg, Germany

Centrioles are large cylindrical structures that organize various microtubule-based processes in cells, including the formation of cilia and spindles. Their characteristic nine-fold symmetry results from rings that are formed by dimers of the protein SAS-6. Recently it was observed that the self-assembly of SAS-6 rings is strongly facilitated on a surface. Moreover, a fraction of non-canonical symmetries (i.e., different from nine) was observed. To better understand the factors that determine the efficiency and selectivity of this process, we have performed Brownian Dynamics computer simulations with patchy particles, in which we varied interaction energies and angular binding range. For weak interaction energies and large angular range, we find that the assembly kinetics can be described well by the

coagulation-fragmentation equations in the reaction-limited approximation. In contrast, large interaction energies and small angular range lead to kinetic trapping and diffusion-limited assembly. Comparison with experimental data suggests that the SAS-6 system combines a weak binding energy with a small angular range in order to avoid kinetic trapping and favor the desired nine-fold symmetry.

BP 17.7 Wed 16:45 BAR 0106

Alphafold predicts the most complex protein knot and composite protein knots — Maarten Brems[1], Robert Runkel[1], Todd Yeates[2], and •Peter Virnau[1] — [1]Institut für Physik, Staudingerweg 9, JGU Mainz — [2]UCLA-DOE Institute for Genomics and Proteomics, University of California Los Angeles (USA)

The computer artificial intelligence system AlphaFold has recently predicted previously unknown three-dimensional structures of thousands of proteins. Focusing on the subset with high-confidence scores, we algorithmically analyze these predictions for cases where the protein backbone exhibits rare topological complexity, i.e. knotting. Amongst others, we discovered a 7_1-knot, the most topologically complex knot ever found in a protein, as well as several 6-crossing composite knots comprised of two methyltransferase or carbonic anhydrase domains, each containing a simple trefoil knot [1]. These deeply embedded composite knots occur evidently by gene duplication and interconnection of knotted dimers. Finally, we report two new five-crossing knots including the first 5_1-knot. Our list of analyzed structures forms the basis for future experimental studies to confirm these novel knotted topologies and to explore their complex folding mechanisms.

[1] M. Brems et al, Protein Science 31(8), e4380 (2022).

BP 17.8 Wed 17:00 BAR 0106

Multi-state Unfolding Processes: Discrimination of protein domains by urea-induced thermal shift — •Ji Young Yang[1,2], Oliver Burkert[2], Boris Mizaikoff[1], and Jens Smiatek[3,4] — [1]Institute for Analytical and Bioanalytical Chemistry, University of Ulm, Ulm, Germany — [2]Boehringer Ingelheim Pharma GmbH & Co. KG, Analytical Development Biologicals, Biberach(Riss), Germany — [3]Boehringer Ingelheim Pharma GmbH & Co. KG, Development NCE, Biberach (Riss), Germany — [4]Institute for Computational Physics, University of Stuttgart, Stuttgart, Germany

Co-solute induced molecular denaturation and aggregation mechanisms related to stability changes for multi-domain proteins like mAbs are often hard to monitor experimentally. In addition, a thorough theoretical explanation is often missing. We performed intrinsic fluorescence (IF) measurements of monoclonal antibody (mAb) samples for different aqueous urea concentrations under thermal denaturation. Our results show that the denaturating effect of urea on individual mAb domains can be explained by linear mapping of the thermal shifting curve to the actual urea concentration. Notably, the achieved thermal shifting curves can be assigned to certain protein domains, which enables discrimination of overlapping denaturation processes. Our approach highlights the benefits of direct monitoring of co-solute effects on the conformational stability of mAb domains and its colloidal stability. We will discuss the experimental approach and present the corresponding outcomes in terms of the underlying molecular mechanisms.

BP 17.9 Wed 17:15 BAR 0106

X-ray damage to Gene-V Protein: NAP-XPS analysis of Chemical changes to Proteins in Water — •Dorothea C Hallier[1,2,3], Jörg Radnik[2], Paul M Dietrich[4], Harald Seitz[1,3], and Marc Benjamin Hahn[2] — [1]Fraunhofer Insitute for Cell Therapy and Immunology, Branch Bioanalytics and Bioprocesses, Potsdam, Germany — [2]Federal Insitute for Materials Research and Testing BAM Berlin, Berlin, Germany — [3]Univerity of Potsdam, Institute for Biochemistry and Biology, Potsdam Germany — [4]SPECS Surface Nano Analysis GmbH, Berlin, Germany

X-ray photoelectron spectroscopy (XPS) was used to analyze the chemical damage of ionizing radiation to a single-stranded DNA-binding protein: Gene-V Protein (G5P/GVP) and its most abundant amino acids (Alanine, Arginine, Cysteine, Glycine, Lysine, Methionine, Tyrosine). This protein plays a crucial role in maintaining the DNA metabolism, especially DNA replication, recombination and repair. Vacuum measurements were combined with near-ambient pressure (NAP) XPS measurements under water and nitrogen atmosphere to detect both direct and indirect radiation damage and corresponding damage pathways. The exposure of proteins and aminoacids to x-rays leads to degradation i.e. via dehydrogenation, decarboxylation, dehydration and deamination. A strong increase of protein damage was observed in water as compared to vacuum.