BP 31: Protein Structure and Dynamics

Time: Thursday 15:00–18:00 Location: H 0112

BP 31.1 Thu 15:00 H 0112

BCL11B CCHC Zinc Finger Domain and Its Potential for Cancer Therapy — •Anne Susemihl[1,3], Lukas Schulig[2], Norman Geist[1], Piotr Grabarczyk[3], Felix Nagel[1], Christian Andreas Schmidt[3], and Mihaela Delcea[1] — [1]Institute of Biochemistry, Greifswald, Germany — [2]Institute of Pharmacy, Greifswald, Germany — [3]University Medicine, Greifswald, Germany

B Cell Lymphoma/Leukemia 11B (BCL11B) is a transcription factor, exerting a bi-directional role in cancer. BCL11B KO in vitro lead to T cells acquiring properties of natural killer cells, suggesting BCL11B*s role as an emerging cancer target. Previous FACS-FRET experiments and extensive enhanced sampling simulations indicated that BCL11B forms dimers, with this being a prerequisite for its functionality. New simulations and size exclusion chromatography data suggest the formation of not only dimers but tetramers. Multimerization is mediated by an atypical CCHC zinc finger (ZF) motif within the N-terminal region of the protein. Understanding the nature of BCL11B*s multimerization and its zinc binding properties may enable the use of BCL11B in targeted cancer therapy. We show here that zinc coordination is essential for the oligomerization of the N-terminal domain. Zinc binding properties of the CCHC ZF domain were determined using UV-Vis spectroscopy and isothermal titration calorimetry. BCL11B mutations within the tetramer interface led to insufficient zinc binding and incomplete ZF formation, resulting in monomerization. Other mutants showed monomerization but tetramer formation with the wild type zinc finger, with the physiological relevance yet to be elucidated.

BP 31.2 Thu 15:15 H 0112

Electric-field induced ultrafast protein changes — •Karsten Heyne, Clark Zahn, and Ramona Schlesinger — Freie Universität Berlin, Fachbereich Physik, Berlin, Deutschland

Light absorption activates photoreceptors and triggers a cascade of structural changes leading to the biological function. The time span of these processes ranges from femtoseconds to seconds. The fastest process is the absorption of a photon on a time-scale of a few fs. It is a long-standing debate whether excitation of the chromophore impacts the protein structure immediately or if the protein response is delayed until the photoproduct is formed. Here, we show direct response of the protein prior to chromophore isomerization. The vibrational fingerprint region of bacteriorhodopsin (bR) studied with polarization-resolved fs VIS-pump IR-probe spectroscopy reveals protein restructuration before and after photoisomerization. Transient protein signals were identified prior to and after photoisomerization with 500 fs. A proton continuum band, amide I band, and carboxylic acid groups, essential elements for the function of the bR proton pump, are altered by the excited chromophore. These groups have distances up to 10 Å to the chromophore. Therefore, we propose that an impulsive electric field change at the chromophore in the excited state perturbs polar groups throughout the protein. The ensuing reorganization prepares the protein for the down-stream processes.

BP 31.3 Thu 15:30 H 0112

New insights into the structural dynamics of intrinsically disordered proteins by high-field NMR relaxation experiments — Tobias Stief[1,2], Katharina Vormann[1,2], and •Nils-Alexander Lakomek[1,2] — [1]Forschungszentrum Jülich, Structural Biochemistry (IBI-7), 52425 Jülich, Germany — [2]Heinrich Heine University Düsseldorf, Institute of Physical Biology, 40225 Düsseldorf Germany

Intrinsically disordered proteins (IDPs) compose about 30% of the human proteome and are highly dynamic entities. Nuclear magnetic resonance (NMR) spectroscopy can provide insights into their structural dynamics at residue-specific resolution. Recently available higher magnetic field strengths, up to 28 Tesla (corresponding to 1.2 GHz 1H Larmor frequency), offer substantially improved resolution, with particular benefits for studying IDPs. We have derived an improved set of 15N NMR relaxation experiments suited for operation at high-field magnets and applicable to fully protonated proteins. Here, we used SNARE proteins as a model system. SNARE proteins play a crucial role during neuronal exocytosis by eliciting the fusion of the synaptic vesicle membrane with the presynaptic plasma membrane. In their pre-fusion state, the membrane-anchored SNARE proteins are disordered. To assess the internal dynamics of the SNARE protein SNAP25, we recorded NMR relaxation experiments at different magnetic field strengths, between 14 and 28T. The field-dependent NMR measurements reveal novel insights into IDP dynamics at the ps-ns timescale.

Invited Talk BP 31.4 Thu 15:45 H 0112

Polarizing nuclear spins at the interface between ESR and NMR spectroscopy — •Marina Bennati — Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany — Institute of Physical Chemistry, University of Göttingen, Göttingen, Germany

Latest developments in magnetic resonance spectroscopy are aimed at increasing sensitivity for nuclear spin detection, which is limited by the small energy splitting at the available polarizing magnetic fields. A powerful approach is taking advantage of the larger magnetic moment of unpaired electrons and uses hyperfine couplings to transfer their polarization to nuclear spins.

The talk will illustrate recent progress in polarization transfer experiments using electron-nuclear double resonance techniques, either with ESR or NMR detection. In ESR spectroscopy, we detect nuclear spins up to the second ligation sphere of a paramagnetic center. We have recently demonstrated that at high electron Larmor frequencies (263 GHz) nuclear chemical shift tensors as well as quadrupolar nuclei can be resolved. In conjunction with 19F spin labelling, our techniques can be employed for measuring inter-spin distances in biomolecules in the angstrom to nanometer range. Moreover, we are developing dynamic nuclear polarization for enhanced NMR detection in the liquid state. Progress and strategies for future applications will be discussed.

15 min. break

BP 31.5 Thu 16:30 H 0112

NAP-XPS analysis of X-ray radiation damage 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 the single-stranded DNA-binding protein Gene-V Protein (G5P/GVP) and its most abundant amino acids. This protein plays a crucial role in maintaining the DNA metabolism, especially DNA replication, recombination and repair. XPS vacuum measurements were combined with near-ambient pressure (NAP) XPS measurements under water atmosphere to detect both direct and indirect radiation damage and to identify 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.

BP 31.6 Thu 16:45 H 0112

What is the structure of a biomolecular condensate? — •Charlotta Lorenz[1,2], Takumi Matsuzawa[1], Etienne Jambon-Puillet[3], Teagan Bate[1], Kaarthik Varma[1], Harsha Koganti[1], Gianna Wolfisberg[1], Aleksander R. Rebane[4], and Eric R. Dufresne[1] — [1]Cornell University, Ithaca, NY, US — [2]ETH Zürich, CH — [3]LadHyX, CNRS, Ecole Polytechnique, Paris, FR — [4]New York University Abu Dhabi, AE

Biomolecular condensates are important for a variety of cellular functions, such as biochemical regulation, structural organization, and RNA metabolism. While the properties and physiology of these condensates depend on their structure, this important aspect has received little experimental consideration. On the other hand, recent simulations of disordered proteins with interactions based on the sticker-and-spacer suggest fascinating structures in the bulk and surface of condensates. We aim to reveal the structure of biomolecular condensates using X-ray scattering. Here, we will present results for a simple model system consisting of a stable protein that forms a condensate due to crowding by addition of small molecules. With these results,

we aim to establish methods to probe the structure of a wide variety of biomolecular condensates made of intrinsically disordered proteins.

BP 31.7 Thu 17:00 H 0112

Enzymatic phosphorylation of intrinsically disordered proteins in coarse-grained simulations — •Emanuele Zippo[1], Lukas Stelzl[1,2], Thomas Speck[3], and Friederike Schmid[1] — [1]Institute of Physics, Johannes Gutenberg University Mainz, Mainz, Germany — [2]Institute of Molecular Biology (IMB), Mainz, Germany — [3]Institüt für Theoretische Physik IV, Universität Stuttgart, Stuttgart, Germany

Understanding the condensation and aggregation of intrinsically disordered proteins (IDPs) in a non-equilibrium environment is crucial for unraveling many biological mechanism. We can now address this with residue-level coarse-grained Molecular Dynamics simulations, integrating Metropolis Monte Carlo steps to model chemical reactions. We investigate TDP-43 phosphorylation by CK1d enzyme in simulations, examining patterns of phosphorylation and assessing its preventive role in chain aggregation, possibly associated with neurodegenerative diseases. We find that the degree of residue phosphorylation is determined by sequence preference and charges, rather than the position in the chain. Depending on the sequence context, phosphorylation stabilizes or de-stabilizes condensates. For TDP-43, our simulations show condensates dissolution through phosphorylation, in accordance with experiments. The disordered tail of the kinase Ck1d drives recruitment to the condensates. To further explore the dynamics of non-equilibrium steady-state systems, like our target system, we apply Markov state modelling (MSM). We used MSM to verify the thermodynamic consistency of the phosphorylation step.

BP 31.8 Thu 17:15 H 0112

Memory-dependent friction in protein folding — •Benjamin Dalton and Roland Netz — Freie Universität Berlin, Fachbereich Physik, Arnimallee 14 14195 Berlin, Germany

When described by a low-dimensional reaction coordinate, the folding rates of many proteins are determined by an interplay between free-energy barriers, which separate folded and unfolded states, and friction. While it is commonplace to extract free-energy profiles from molecular trajectories, a direct evaluation of friction is far more elusive and typically relies on fits of measured reaction rates to memoryless reaction-rate theories. Here, using memory-kernel extraction methods founded on a generalized Langevin equation (GLE) formalism, we directly calculate the time-dependent friction acting on a variety of well-known reaction coordinates for eight fast-folding proteins, taken from a published set of large-scale molecular dynamics protein simulations. We show that memory decay times are typically of the same order as folding times and are much longer than transition-path times. Furthermore, we show that folding times are significantly faster than predictions made by standard Markovian models. This memory-induced reaction speed-up effect is a hallmark of non-Markovian systems, confirming that non-Markovian models are, in general, suitable for describing protein folding dynamics.

BP 31.9 Thu 17:30 H 0112

Log-periodic oscillations as real-time signatures of hierarchical dynamics in proteins — •Emanuel Dorbath, Adnan Gulzar, and Gerhard Stock — Institute of Physics, University of Freiburg, 79104 Freiburg, Germany

The time-dependent relaxation of a dynamical system may follow a power law that is superimposed by log-periodic oscillations. This behavior can by explained by a discrete scale invariance of the system, which is associated with discrete and equidistant timescales on a logarithmic scale. Recent time-resolved experiments and molecular dynamics simulations suggest that this also holds for hierarchical dynamics in proteins, where several fast local conformational changes are a prerequisite for a slow global transition to occur.

An entropy-based timescale analysis and Markov state modeling is applied to a simple one-dimensional hierarchical model and biomolecular simulation data of the achiral peptide helix Aib9, showing that hierarchical systems in fact give rise to logarithmically spaced discrete time scales [arXiv:2311.11839 (2023)]. Introducing a one-dimensional reaction coordinate, the free energy landscape exhibits a characteristic staircase shape with two metastable states, which causes the observed log-periodic time evolution of the system. The period of these oscillations reflects the effective roughness of the energy landscape and can in simple cases be interpreted as the energy barriers of the staircase.

BP 31.10 Thu 17:45 H 0112

IP_3 affinity of Tubby reveals cooperativity mechanism for membrane binding — •Sebastian Thallmair — Frankfurt Institute for Advanced Studies (FIAS), Germany

We recently showed by means of coarse-grained (CG) molecular dynamics (MD) simulations and life cell experiments that two cooperative binding sites of the C-terminal domain of the Tubby protein determine its $PI(4,5)P_2$ affinity. Notably, the $PI(4,5)P_2$ concentration sensitivity of the Tubby protein is more pronounced than the one of the well-known $PI(4,5)P_2$ binding Pleckstrin homology (PH) domain of phospholipase C (PLC)-δ1.

Here, I will show that surprisingly the IP_3 affinity of Tubby is comparably to the one of the PLCδ1-PH domain using CG MD simulations with the Martini 3 force field. This is in contrast to the pronounced affinity difference between both proteins to a single $PI(4,5)P_2$ lipid embedded in a POPC membrane. In addition, I will compare both affinities to a single $PI(4,5)P_2$ lipid in water. Taken together, my results indicate that the recently discovered second $PI(4,5)P_2$ binding site of Tubby not only preferably interacts with $PI(4,5)P_2$, but also disfavors the interaction with zwitterionic lipids such as POPC. I will discuss how this increases the $PI(4,5)P_2$ concentration sensitivity of Tubby compared to the PLCδ1-PH domain.