BP 30: Protein Structure and Dynamics

Time: Thursday 15:00–18:00 Location: H46

BP 30.1 Thu 15:00 H46

A protein sensor for plasma membrane lipid composition – insights from coarse-grained simulations — Saara Lautala and •Sebastian Thallmair — Frankfurt Institute for Advanced Studies, Frankfurt a.M., Germany

Extended synaptotagmins (E-Syts) are tethering proteins, which keep the plasma membrane (PM) and the endoplasmic reticulum (ER) membrane in close proximity at ER-PM contact sites. C2 domains are responsible for the binding of E-Syts to the PM. After depletion of phosphatidylinositol 4,5-bisphosphate $PI(4,5)P_2$, resynthesis of $PI(4,5)P_2$ takes place at ER-PM contact sites and thus, requires their integrity. The terminal C2C domain of E-Syt3 is known to bind $PI(4,5)P_2$. This results in an apparent paradox as the membrane binding and thus the tethered ER-PM contact site potentially become instable upon $PI(4,5)P_2$ depletion.

Here, we applied coarse-grained molecular dynamics simulations with the Martini 3 force field to investigate the membrane binding of the E-Syt3 C2C domain. Our simulations show that the C2C domain not only exhibits a binding hotspot for $PI(4,5)P_2$, but an additional binding hotspot for phosphatidylserine (PS) as well as a region binding to the membrane core. We will discuss that binding to PS results in a reorientation of the protein on the membrane surface and compare the different binding strengths. Overall, the PS binding site not only contributes to the ER-PM contact site integrity upon $PI(4,5)P_2$ depletion, but might also play a role in sensing low $PI(4,5)P_2$ levels.

BP 30.2 Thu 15:15 H46

Cross correlations in the Fluctuation-Dissipation Relation Reveal Solvent Friction in Hydrophobic Folding Transition — •Niklas Wolf, Viktor Klippenstein, Madhusmita Tripathy, and Nico F. A. van der Vegt — TU Darmstadt, Darmstadt, Germany

The Generalized Langevin Equation is a powerful tool for modeling and understanding the conformational dynamics of molecules in solution. However, recent works[1] have demonstrated that for these kinds of applications, the usual fluctuation-dissipation relation connecting the statistics of the random force to the memory kernel could contain a cross-correlation term. This raises the question of how the memory kernel should be extracted from simulation data and if a naive approach via the Volterra equations even gives a kernel related to a Markovian friction coefficient. We propose an approximation[2] to account for the cross-correlation term and show in a systematic study[3] that this approximation leads to an improved description of long-time dynamics and transition rates. Finally, we show that cross-correlations play an important role in the coil-to-globule transition of a hydrophobic polymer under various solvent conditions, where a naive approach would predict a significant violation of the Stokes-Einstein relation and give a poor description of barrier crossing times with rate theories.

[1] H. Vroylandt 2022 EPL 140 62003

[2] V. Klippenstein N. F. A. van der Vegt 2021 J. Chem. Phys. 154 191102

[3] N. Wolf et al. J. Chem. Phys. (under Review)

BP 30.3 Thu 15:30 H46

Multiscale simulation of protein phase separation — •Supriyo Naskar, Kurt Kremer, and Oleksandra Kukharenko — Max Planck Institute for Polymer Research, Mainz, Germany

The post-translational modifiers such as mono and poly ubiquitins and SUMOs are known for their ability to modulate protein-protein interactions by becoming covalently attached to other target proteins. Despite the high similarity in the tertiary structure and sequence, they differentially influence the target protein properties. In this work, we employed a multiscale simulation approach that encompasses atomistic to different level coarse-grained modelling techniques with data-driven machine-learning methods to explore the structural differences and multidimensional energy landscape of ubiquitin and SUMO and their conjugates. We finally study the influence of distinct features of the targets and modifiers on protein phase separation and aggregation, providing molecular-level insight into the corresponding in vitro measurements and instructing further experiments through adjustment of relevant parameters.

BP 30.4 Thu 15:45 H46

Sequence specificity and polymer physics — •Martin Girard — Max-Planck Institute for Polymer Research, Mainz, Germany

Sequence properties of disordered proteins in the context of phase separation has led to development of molecular grammar. So far, this has led to the development of empirical parameters tied to protein sequences.

Using surrogate models for low-complexity sequences, I will show that sequence-property relations are tied to the polymer collapse transition. I will further discuss implications for biological systems.

Invited Talk BP 30.5 Thu 16:00 H46

Topology in biological matter - are there double knots in proteins or maybe even more complicated knots? Prediction and in vitro verification. — •Joanna I Sulkowska — University of Warsaw, Banacha 2C, 02-097, Poland

We have been aware of the existence of knotted proteins for over 30 years-but it is challenging to predict what is the most complicated not that can be formed in proteins. Recently, based on AlphaFold (AF) method we predicted new and the most complex knotted topologies recorded to date - double trefoil knots (see AlphaKnot database). We found five domain arrangements that result in a doubly knotted structure in almost a thousand proteins. The double knot topology is found in knotted membrane proteins from the CaCA family, that functions as ion transporters, in the group of carbonic anhydrases that catalyze the hydration of carbon dioxide, and in the proteins from the SPOUT superfamily that gathers 31 knotted methyltransferases with the active site-forming knot.

Herein, I will present the first crystal structure of a double knotted protein TrmD-Tm1570 from Calditerrivibrio nitroreducens from SPOUT superfamily. The protein consists of two domains TrmD and Tm1570, each embedding a single trefoil knot, which can function on their own. We show that it folds in vitro and is biologically active.

I will also explain how AF and AI methods can be used to design artificially knotted proteins that can be obtained in vitro. This shows that AF, while predicting structure, also takes into account folding and overcoming a non-trivial looping pathway.

15 min. break

BP 30.6 Thu 16:45 H46

Single molecule FRET studies on folding properties of multidomain protein fragments — •Alida Meyer[1], Alexandros Katranidis[2], Nuno Bustorff[2], and Jörg Fitter[1,2] — [1]RWTH Aachen University, I. Physikalisches Institut (IA), AG Biophysik, Aachen, Germany — [2]Forschungszentrum Jülich, ER-C-3 Structural Biology, Jülich, Germany

Protein folding and unfolding are crucial for cellular function and stability. This study emloys single-molecule Förster resonance energy transfer (smFRET) to investigate structural transitions in yeast phosphoglycerate kinase (yPGK). We focus on its two-domain structure and the relationship between the Rossmann-fold topology and folding intermediates. Earlier studies with full-length yPGK labelled with fluorescent dyes at multiple different positions allowed to map several different intra-molecular distances during unfolding transitions [1,2]. To mimic co-translational folding properties, we performed smFRET measurements with truncated yPGK fragments. The results are compared with those of full-length proteins, including whether the same type of unfolding transition occurs as in the full-length protein (e.g., two state transitions or compact intermediates). In addition, the results from truncated fragments are also compared with nascent-chain folding in ribosome-nascent chain complexes (RNCs), analyzed via cryo-electron microscopy. These methods provide insights into how domain topology and neighboring structural elements influence multidomain protein folding. [1] Cerminara et al., Biophysical Journal, 2020, 118, 688 [2] Bustorff et al., Biomolecules, 2023, 13, 1280

BP 30.7 Thu 17:00 H46

Probing the dynamics of small unilamellar vesicles inside Synapsin pools using X-ray photon correlation spectroscopy — •Titus Czajka[1], András Major[1], Hendrik Bruns[1], Christian Hoffmann[2], Dragomir Milovanovic[2], and Tim Salditt[1] —

[1]Georg-August-Universität Göttingen — [2]Deutsches Zentrum für Neurodegenerative Erkrankungen, Berlin

The dynamics of many subcellular biological processes are difficult to access directly with microscopic techniques due to the resolution limit. Length and time scales beyond those accessible by conventional light microscopy can be probed via X-ray photon correlation spectroscopy (XPCS), even in dense media, by analysing the intensity autocorrelation function at different scattering vectors. However, the low scattering cross section of dilute biological samples and the sensitivity to radiation damage complicate the application of XPCS to biological systems. We have coated silica nanoparticles with a lipid bilayer to improve the scattering strength and overcome these challenges. Using such colloid-supported lipid bilayers (CSLBs), we have studied the dynamics of small unilamellar vesicles within synapsin protein pools, a system that exhibits evidence of both liquid-like and network-like phases. Our results show distinct diffusion constants at varying protein concentrations and provide evidence for non-diffusive behaviour within the pools.

BP 30.8 Thu 17:15 H46

Novel sample delivery for small nanoparticles and biomolecules for cryo-em — •Kevin Janson[1], Armando D. Estillore[1], Jiri Wald[4,5,6], Madeline Memovich[1], Thomas Marlovits[4,5,6], Amit K. Samanta[1,3], and Jochen Küpper[1,2,3] — [1]Center for Free-Electron Laser Science, Deutsches Elektronen-Synchrotron DESY, Hamburg, Germany — [2]Department of Physics, Universität Hamburg, Hamburg, Germany — [3]Center for Ultrafast Imaging, Universität Hamburg, Hamburg, Germany — [4]Centre for Structural Systems Biology, Hamburg, Germany — [5]Institute of Structural and Systems Biology, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany — [6]Deutsches Elektronen-Synchrotron DESY, Hamburg, Germany

Cryo-electron microscopy (Cryo-EM) is one of the key techniques in the field of structural biology. Recent years brought considerable improvements both on the software and hardware of the microscopes, and resolving high-resolution structures of proteins has become a standard procedure. However, most cryo-EM grids are still prepared by plunge freezing, a technique developed about ~40 years ago. During this process, proteins can be exposed to the air-water interface, possibly causing a preferential orientation or damaging their structure. We present the novel freeze-and-deposit sample delivery approach to deposit particles for cryo-EM using cryogenic shockfreezing technology. The cooling process produces cold high-density beams of nanoparticles. In this process, nanoparticles and macromolecules are aerosolized and rapidly cooled in the gas phase using a cryogenic buffer-gas cell.

BP 30.9 Thu 17:30 H46

Laser flash melting restores native protein conformation after cryoEM preparation by soft-landing, native electrospray ion beam deposition. — Sarah V. Barrass[1], Tim K. Esser[2], Nathan J. Mowry[1], Lukas Eriksson[2], Jakub Hruby[1], Laurence Seeley[3], Marcel Drabbels[1], Lindsay Baker[3], •Stephan Rauschenbach[2], and Ulrich J. Lorenz[1] — [1]EPFL Lausanne — [2]Univ. of Oxford, Dept. of Chem.. — [3]Univ. of Oxford, Dept. of Biochem.

Electron cryo microscopy (cryoEM) is today the dominating method for protein structure determination. Samples for cryoEM consist of thin, freestanding layers of amorphous ice in which proteins are embedded. Conventionally, these samples are prepared by shock-freezing of thin water films held in grid holes.

Alternative sample preparation methods are being developed, as the plunge-freezing method is not compatible with all types of protein sample. One of these methods is electrospray ion beam deposition (ESIBD) where mass-selected proteins from the gas-phase are landed on a thin amorphous carbon film in vacuum and embedded in ice grown from the gas phase for imaging. Recently it was shown that this method yields atomically resolved protein structures characterised by small changes in ternary structure due to dehydration.

Here we show that the dehydration can be reversed by irradiating the sample with short laser pulses, effectively melting the ice for a short time, allowing the protein to recover the native conformation, before the ice rapidly re-vitrifies.

BP 30.10 Thu 17:45 H46

Native Electrospray Ion Beam Deposition for Atomic-level Structure Analysis of Membrane Protein — •Jingjin Fan, Tim Esser, Clare De'Ath, Lukas Eriksson, Abdul Aziz Qureshi, Abraham Abraham, Laurence Seeley, Lindsay Baker, Carol Robinson, and Stephan Rauschenbach — The Kavli Institute for Nanoscience Discovery, University of Oxford, Oxford OX1 3QU, United Kingdom

Membrane proteins play vital roles in cellular physiology, but their structural analysis remains challenging due to heterogeneity, flexible conformations, and demanding native conditions. To address these challenges, we established electrospray ion beam deposition (ESIBD) to directly couple native mass spectrometry (MS) with cryogenic electron microscopy (cryo-EM) for studying membrane protein structures.

Standard membrane proteins, including aquaporin Z (AqpZ) and ammonium transporter B (AmtB), were selected as testing models. By optimizing surfactant, the ion transfer in vacuum and the embedding of the proteins after landing we successfully manipulated membrane protein particles and achieved soft-landing on grids, evidenced by high-resolution imaging in cryoEM.

Our results demonstrate that the membrane structures can be preserved even in the absence of visible micelle.This molecular-level structural analysis captured by ESIBD in vacuum provides new insights into the correct folding of membrane proteins and understanding fundamental questions in structural biology.