BP 1: Protein structure and dynamics

Time: Monday 9:30–13:00 Location: H4

Invited Talk BP 1.1 Mon 9:30 H4

Structural dynamics of active membrane transporters as seen by single-molecule techniques — •Thorben Cordes — Physical and Synthetic Biology, LMU Munich, Germany — Molecular Microscopy Research Group, University of Groningen, The Netherlands

Membrane transporters are vital to any living system and are involved in the translocation of a wide variety of different substrates. Despite their importance, all proposed molecular models for transport are based on indirect evidence due to the inability of classical biophysical and biochemical techniques to visualize dynamic structural changes. We recently started to use single-molecule fluorescence microscopy to characterize conformational states and changes in active membrane transporters in vitro to directly observe how different steps in transport are coordinated.[1-4] In my contribution I will first introduce our methodological approach to visualize structural dynamics[5] and accurate distances in biomacromolecules[6] using single-molecule spectroscopy and microscopy. Secondly, I will provide an overview of our mechanistic contributions to the field of primary[1,2,4] and secondary active transporters[3]. These involve various prokaryotic transporters, i.e., ABC importer-related periplasmic binding proteins[1,2], the ABC exporter McjD[4] and the sodium-symporter BetP[3].

[1] Gouridis et al., NSMB 22 (2015) 57-64. [2] van der Velde et al., Nature Communications 7:10144 (2016). [3] Jazi et al., Biochemistry 56 (2017) 2031-2041. [4] Husada et al., EMBO Journal (2018) e100056. [5] Lerner et al., Science 359 (2018) eaan1133. [6] Hellenkamp et al., Nature Methods 15 (2018) 669-676.

BP 1.2 Mon 10:00 H4

A Coarse-Grained Network Description of Protein Tertiary Structure — •Nora Sophie Martin[1,2] and Sebastian Edmund Ahnert[1,2] — [1]Theory of Condensed Matter Group, Cavendish Laboratory, University of Cambridge, Cambridge, UK — [2]Sainsbury Laboratory, University of Cambridge, Cambridge, UK

Due to the complexity of protein tertiary structure, many methods of describing, classifying and comparing solved structures have been proposed. Network-based descriptions, known as protein structure networks, amino acid networks or protein contact maps, have been used successfully. There is some regularity and redundancy in protein structure networks: two segments of the polypeptide chain, which are close in the native structure, give rise to a group of edges in the contact network. We identify these groups of edges to coarse-grain and simplify protein structure networks. First, the network is compressed: a shorter encoding of a protein structure network is found by sorting network edges into groups. Then, a minimal network is constructed by choosing all nodes of the full network, but only one edge per group. In this contribution, we will present the construction of these coarse-grained networks and how they can be used to compare protein structures.

BP 1.3 Mon 10:15 H4

Imaging single proteins with Low Energy Electron Holography — •Hannah Ochner[1], Sven Szilagyi[1], Sabine Abb[1], Stephan Rauschenbach[1,2], and Klaus Kern[1,3] — [1]Max-Planck-Institut für Festkörperforschung, Stuttgart — [2]Chemistry Research Laboratory, Department of Chemistry, University of Oxford — [3]École Polytechnique Fédérale de Lausanne

Recently, Low Energy Electron Holography (LEEH) has been shown to be able to image proteins at the single molecule level (without averaging), while avoiding radiation damage [1]. LEEH [2] is a lens-free imaging method in which the sample is radiated by coherent low energy electrons (50-200eV) [3] to form holograms that in principle contain full 3D information of the object. Basic Kirchhoff-Fresnel propagation-based numerical reconstruction of the holograms yields the shapes of the investigated proteins, as well as some internal contrast that already hints at 3D information. Thus, this technique can serve as a complementary method for protein structure determination, especially for types of proteins that are hard to access using other methods such as Cryo-EM or X-ray crystallography. We present the current state of the experiment and the reconstruction process along with future plans to enhance resolution and to improve the reconstruction towards including 3D information.

[1] PNAS 114, 1474-1479 (2017)
[2] Phy. Rev. Lett, 1990, 65(10), 1204-1206.
[3] Phys. Scr., 1988, 38, 260

BP 1.4 Mon 10:30 H4

Single Amyloid Fibrils Studied in a Thermophoretic Trap — •Martin Fränzl[1], Tobias Thalheim[1], Juliane Adler[2], Daniel Huster[2], and Frank Cichos[1] — [1]Peter Debye Institute for Soft Matter Physics, Molecular Nanophotonics Group, Universität Leipzig, Linnéstr. 5, 04103 Leipzig, Germany — [2]Institute for Medical Physics and Biophysics, Universität Leipzig, Härtelstr. 16-18, 04107 Leipzig, Germany

The aggregation of soluble proteins into highly ordered, insoluble amyloid fibrils is characteristic for a range of neurodegenerative disorders. While many different techniques have been applied to the investigation of fibril formation, almost all of them address the average properties of the ensemble. Here, we present a method that removes the ensemble average observing single fibrils freely dispersed in solution enabling to detect events commonly hidden in the ensemble average. The trapping scheme is based on the thermophoretic drift of nano-objects in temperature gradients allowing to probe the dynamics of a single fibril at various stages of its growth, e.g., the time evolution of the diffusion coefficients. It is shown that the rotational diffusion coefficient provides a unique measure to follow the growth of single fibrils with a precision below the optical resolution. Fibril growth of a few 10 nm can be identified providing a promising platform for studies of molecular interactions and in particular of protein and macromolecular aggregation processes at the single fibril level.

BP 1.5 Mon 10:45 H4

Compression of Single DNA Molecules in a Thermophoretic Trap — •Tobias Thalheim and Frank Cichos — Peter Debye Institute for Soft Matter Physics, Leipzig University, 04103 Leipzig, Germany

The Brownian motion of single DNA molecules, which are suspended in liquid, can be counter-acted by inhomogeneous temperature gradients which are generated by an optically heated metal structure of a thermophoretic trap. The trap [1] relying on thermophoresis, also known as Soret effect, consists of a focused laser beam which rotates on the ring-like metal nano-structure in a continuous fashion thereby generating the inhomogeneous temperature profile which, in turn, induces thermophoretic drift velocities preventing the free fluctuations of the DNA strand. Drift velocities in outer regions of the trap are due to the inhomogeneity of the temperature landscape larger than those closer to the trapping center. An elongated soft molecule like DNA therefore experiences different drift velocities at different parts of the molecule in the trap leading to a compression of the DNA strand which is reflected in a decreased radius of gyration. The influence of the trap on the conformation dynamics of the DNA molecule will be studied with a model-free statistical tool which is called principal-components analysis applied as introduced by Cohen and Moerner [2].

References

[1] M. Braun, A. P. Bregulla, K. Günther, M. Mertig, and F. Cichos, Nano Lett 15, 5499–5505 (2015)
[2] A. E. Cohen, and W. E. Moerner, PNAS 104, 12622–12627 (2007)

30 minutes break.

BP 1.6 Mon 11:30 H4

LOVely aureochromes: Time-resolved small-angle X-ray scattering reveals the global structure recovery time of the multidomain photoreceptor — •Saskia Bannister, Elena Herman, Thomas Hellweg, and Tilman Kottke — Bielefeld University, Germany

Aureochromes (AUREO) function as blue-light-regulated transcription factors in algae.[1] Their basic region/leucine zipper (bZIP) effector domain binds DNA with a specific sequence while a light-, oxygen-, or voltage-sensitive (LOV) domain acts as the C-terminal sensor. In the dark, LOV binds flavin non-covalently while upon illumination an adduct is formed. Due to their unusual domain arrangement AUREOs are versatile candidates for new synthetic optogenetic tools. We therefore characterized a full-length AUREO1c variant (38 kDa) and found that its quantum yield of adduct formation is exceptionally low. Furthermore, we applied time-resolved small-angle X-ray scat-

tering (SAXS) on an inhouse setup to investigate the recovery of the overall structure of AUREO1c in the dark. Additionally, the recovery kinetics of the flavin was determined by UV/vis spectroscopy under similar conditions. These studies revealed a discrepancy between the lifetime of the flavin adduct and the global structural recovery time of the multidomain photoreceptor. We therefore conclude that an additional spectrally silent intermediate exists that significantly prolongs the lifetime of the signalling state.

[1] Takahashi et al. (2007), *PNAS 104*, 19625-19630.

BP 1.7 Mon 11:45 H4

Protein-ligand interaction and hierarchical complex dynamics of Hsp90 — •Steffen Wolf[1], Benedikt Sohmen[2], Björn Hellenkamp[2], Thorsten Hugel[2], and Gerhard Stock[1] — [1]Biomolecular Dynamics, Institute of Physics, Albert Ludwigs University Freiburg, Germany — [2]Institute of Physical Chemistry, livMatS and CIBSS, University of Freiburg, Germany

Ligand binding to proteins and subsequent functional control by the appearing structural changes is at the heart of regulation of protein function. As these processes take place on timescales from μs (ligand binding) to hours (ligand off-binding), accessing them via molecular dynamics simulations is challenging, and requires state-of-art unbiased simulations. Here, we report on extensive unbiased all-atom molecular dynamics simulations with the full 1300 amino acid Hsp90 dimer on the order of 25 μs simulated time, and compare these results to recent single molecule FRET experiments. We show how external energy input in the form of ATP puts the protein under strain, and forces the protein into an energetically disfavored active folding confirmation. Interestingly, only few amino acids appears to be responsible to mediate this conformational shift between nucleotide binding pocket and the full protein dimer. Hydrolysis of ATP to ADP+P_i removes this strain from the protein, causing a relaxed, inactive confirmation. The transition of structural information from the nucleotide binding site to the full dimer structure follows hierarchical dynamics, from initial nucleotide/amino acid contact loss on a ns time scale to complex structure changes on the order of $0.1 - 1$ μs.

BP 1.8 Mon 12:00 H4

Dynamical Coring of Markov State Models — •Anna Weber, Daniel Nagel, Benjamin Lickert, and Gerhard Stock — Albert Ludwigs University, 79104 Freiburg

The construction of suitable metastable conformational states is fundamental for the description of protein dynamics through Markov state models. These microstates can be generated via density-based clustering algorithms as, e.g., presented by Sittel et al. [J. Chem. Theory Comput. 38, 152 (2017)], resulting in clusters that are cut at the energy barriers. However, the lack of sampling in the transition region combined with the inevitable projection from high dimension onto a low dimensional space, typically leads to a misclassification of points in the transition region. This often causes intrastate fluctuations to be misinterpreted as interstate transitions, causing artificially short life times of the microstates and spoiling calculations of MSM transition rates.

Dynamical coring represents an effective and simple remedy for those problems by requiring the trajectory to spend a minimum time in the new state for a transition to be counted. Adopting molecular dynamics simulations of a well-established biomolecular system (villin headpiece), we demonstrate that coring immensely improves the Markovianity and metastability of the microstates. Providing high structural and temporal resolution, the combination of density-based clustering and dynamical coring is particularly well suited to describe the complex structural dynamics of unfolded biomolecules.

BP 1.9 Mon 12:15 H4

MD Simulation Studies of Protein Dynamics in Molecule-Shaped Confinement — •Timothy Wohlfromm and Michael Vogel — TU Darmstadt, Institut für Festkörperphysik, Hochschulstr. 6, 64289, Darmstadt, Germany

We report on findings regarding dynamics of the elastin-like polypeptide $(VPGVG)_{50}$ and its surrounding hydration shell in confinement. An understanding of confinement effects on protein dynamics is of utmost importance to improve our still limited knowledge about protein functions in the crowded interior of cells. To isolate pure geometrical effects of the confinement on protein dynamics, we generated the pores consisting of the same type of molecules as the solvent, in our case water, but restrained in position by harmonic potentials of varying restoring forces to simulate confining surfaces with differing rigidity. Moreover, we use a pore shape which is consistent with the protein shape such that the thickness of the hydration layer is well defined. Varying the thickness of hydration layers we find that the minimal hydration level for the confined protein to show bulk behaviour is 1 g/g, defined as ratio of water mass to protein mass. We observe in spatially resolved analyses that the correlation times of water vary by more than one order of magnitude across the confinement depending on the rigidity of the pore wall. We find a correlation between the slowdown of water molecules in vicinity of the protein surface and the protein dynamics. This effect is not caused by the reduced center of mass motion of the protein in confinement, but related to internal flexibility. These results shed new light on protein-solvent couplings.

BP 1.10 Mon 12:30 H4

Drug-induced changes in protein secondary structure and its relation to major globular rearrangements and activation of integrin alphaIIb beta3 — •Una Janke[1,2], Martin Kulke[1], Walter Langel[1], and Mihaela Delcea[1,2] — [1]Institute for Biochemistry, University of Greifswald, Felix-Hausdorff-Straße 4, 17489 Greifswald, Germany — [2]ZIK HIKE, University of Greifswald, Fleischmannstraße 42, 17489 Greifswald, Germany

The heterodimeric platelet receptor integrin αIIbβ3 is involved in hemostasis and clot formation. αIIbβ3 exists in three different conformations: the bent (resting) state; the intermediate extended state; and the ligand-occupied active state. The dramatic rearrangements of the overall structure due to αIIbβ3 activation, is possibly related to changes in the secondary structure of the protein. Here, we use a combination of biophysical methods and molecular dynamics simulations (MDS) to investigate whether clinically relevant drugs induce changes in protein structure and lead to its activation in a membrane environment (e.g. liposomes). By QCM and CD spectroscopy we show Mn2+-induced binding of the active-conformation specific antibody PAC-1. However, no major secondary structural changes of αIIbβ3 were found. We also show that treatment with drugs (e.g. quinine) causes activation of αIIbβ3 without any significant changes in secondary structure. MDS studies confirmed the idea of a hinge motion in the extracellular part of the integrin. The combination of biophysical tools and MDS can be applied to study other transmembrane proteins in a membrane environment.

BP 1.11 Mon 12:45 H4

Quantum Mechanics of Proteins in Water: The role of Plasmon-like Solute-Solvent Interactions — •Martin Stöhr and Alexandre Tkatchenko — Physics and Materials Science Research Unit, University of Luxembourg, Luxembourg

van der Waals dispersion interactions form a major component of both intra-protein and protein-water interactions. As such, they play an essential role for the spontaneous folding of proteins in aqueous environments. van der Waals forces arise from long-range electron correlation and are thus inherently quantum-mechanical and many-body in nature. Nevertheless, they are typically only treated in a phenomenological manner via pairwise potentials. Here, we employ an explicit quantum-mechanical framework based on the many-body dispersion formalism, which allows us to highlight the importance of the many-body character of dispersion interactions for protein energetics and protein-water interactions. As such, our study provides unexplored insights into the fundamental quantum-mechanics of proteins in water. In contrast to commonly used pairwise approaches, many-body dispersion effects significantly affect relative stabilities during protein folding in the gas-phase. Embedding in an aqueous environment leads to a quenching of such effects and stabilizes native conformations. Remarkably, this quenching arises from a high degree of delocalization and collectivity of protein-water dispersion interactions, which hints at an unexpected persistence of electron correlation through aqueous environments. Our findings are exemplified on several prototypical proteins, emphasizing their broad validity in the biomolecular context.