BP 41: Protein structure and dynamics I

Time: Thursday 9:30–13:00 Location: H 1058

Invited Talk BP 41.1 Thu 9:30 H 1058

Probing the downhill folding kinetics of Lambda repressor variants with optical tweezers — Ann Mukhortava, Andreas Hartmann, and •Michael Schlierf — B CUBE - Center for Molecular Bioengineering, TU Dresden, Dresden, Germany

Protein folding is a process of molecular self-assembly during which a disordered polypeptide chain collapses to form a compact and well-defined three-dimensional structure. The process of folding is described as a path on a multi-dimensional energy landscape.

Here, we present a comparative study of single-molecule protein folding using optical tweezers that provide the possibility to measure structural dynamics with sub-millisecond and nanometer resolution. We characterize the folding dynamics of three different lambda repressor variants: a two-state folder LambdaWT* (Y22W) and two downhill folding variants, LambdaYA (Y22W/Q33Y/G46,48A) and LambdaHA (Y22W/Q33H/G46,48A). We show that force perturbation of the energy landscape slowed down the ultrafast kinetics of downhill folders, making them accessible to single-molecule studies. Interestingly, the downhill variants of lambda repressor appeared as two-state folders under load with significantly different folding kinetics and force dependence. A comparison between these variants allowed us to extract fine details of their underlying energy landscape.

BP 41.2 Thu 10:00 H 1058

Insulin at the air water interface: Monomers or dimers? — •Sergio Mauri[1,2], Tobias Weidner[2], and Heike Arnolds[1] — [1]Surface Science Research Centre, University of Liverpool, UK — [2]Max Planck institute for polymer research, Mainz, Germany

The adsorption of insulin at surfaces is a ubiquitous problem of interest in various fields of biotechnology and pharmaceutical applications. Studying protein structure at surfaces is generally though a challenging task: Conventional techniques like IR and Raman spectroscopy, can achieve surface sensitivity, but different states (aggregates) of the same protein have often similar spectral signatures. Here we combine sum frequency spectroscopy (SFG) spectroscopy with spectra calculations to identify specific oligomeric species of insulin at interfaces. In particular we study the air/water interface due to its relevance in the production, storage and delivery of insulin-based medications. Insulin is present in Nature mainly as hexamers, dimers and monomers. While the first two are stable, monomers do denaurate and aggregate under certain conditions. Eventually, monomers further aggregate in amyloid-like structures, which are undesired. We find that only insulin monomers segregate at the air/water interface. This advance helps to solve the long standing puzzle of insulin fibril formation. The versatility of the proposed experimental approach could be used to investigate a large variety of proteins and surfaces.

- Mauri et al., PCCP, 2014, 16, 26722-26724

BP 41.3 Thu 10:15 H 1058

Structural changes of proteins at interfaces — •Lars Schmüser[1], Nadja Hellmann[2], Mischa Bonn[1], and Tobias Weidner[1] — [1]Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany — [2]Institute for Molecular Biophysics, Jakob Welder Weg 26, 55128 Mainz, Germany

Information about the 3D structure of proteins at interfaces is essential for understanding of interfacial biological mechanisms. Structural insight can aid the design of tailored proteins with specific functions, structures or binding sites. However, static snapshots of protein structure are insufficient to understand many protein functions, which arise from protein dynamics: Protein folding, reorientation and motion. A well-established tool to study static protein structures is X-ray crystallography. But with this method it is still challenging to follow protein conformational change, folding or refolding in real time. We use vibrational sum frequency generation spectroscopy (SFG) to follow conformational changes of proteins at interfaces. The aim is to combine time resolved SFG with molecular dynamics simulations to glean information about protein dynamics and intermediate structures at interfaces during folding. For a pump-SFG probe detection scheme, an optical trigger for conformational change is a core requirement. We will discuss the design, preparation and characterization of model protein films with optical triggers.

BP 41.4 Thu 10:30 H 1058

The initial adsorption stages of fibrinogen at mica and graphite — •Stephan Köhler[1,2] and Giovanni Settanni[1,3] — [1]Johannes Gutenberg-Universität Mainz, Institut für Physik, Staudinger Weg 7, D-55128 Mainz — [2]Graduate School Materials Science in Mainz, Staudinger Weg 9, D-55128 Mainz — [3]Max Planck Graduate Center mit der Johannes Gutenberg-Universität Mainz, Staudinger Weg 9, D-55128 Mainz

Fibrinogen is a large glycoprotein in the blood of vertebrates. It is an essential factor in blood clotting where it forms fibrin after being activated by thrombin. Furthermore, adsorbed fibrinogen is known to be an important factor for the biocompatibility of materials. The protein contains binding sites for leukocytes and platelets. Consequently, adsorbed fibrinogen has been implicated as a cause for thrombosis and inflammation at implants. The molecular underpinnings of this have been investigated in many experimental studies. These studies often use model surfaces like mica and graphite to investigate the structure of adsorbed fibrinogen.

Here we present the first fully atomistic simulations of the initial adsorption stages of a fibrinogen protomer at such model surfaces. The simulations reveal a weak adsorption at mica that allows frequent desorption and reorientation events. This adsorption is driven by electrostatic interactions between the protein and the silicate surface as well as the counter ion layer. Preferred adsorption orientations for the globular regions are identified. As a contrast to mica, adsorption at graphite is more permanent and the onset of denaturation is observed.

BP 41.5 Thu 10:45 H 1058

Biomolecules at gold-water interfaces: the role of the metal polarization — •Isidro Lorenzo[1], Hadi RAmezani-Dakhel[2], Hendrik Heinz[2], and Marialore Sulpizi[1] — [1]Johannes Gutenberg University Mainz, Staudinger Weg 7 55099 Mainz — [2]epartament of Polymer Engineering, University of Akron, Ohio 44325

Microscopic understanding and control of protein-surface interactions is gaining an increasing interest due to the new development of bio-interfaces for medical and bio-technological applications. In this contribution we aim to provide a characterization of different peptides / gold interactions at a molecular level in order to explain and interpret recent surface experimental results [1]. We have devised a novel scheme to include the metal polarization (image charge effect) induced by the adsorbed molecules into atomistic simulations. Our scheme can easily complement currently used 12-6 Lennard-Jones potentials [2], as included in simulation packages as GROMACS and LAMMPS. Extensive tests have been performed for the force field validation and comparisons with quantum mechanics (QM) density functional theory (DFT) calculations are also discussed. Results for aminoacids and nucleic acids nano assembly different gold surfaces are presented.

[1] V. Humblot, A. Tejeda, J. Landousi, A. Vallee, A. Naitabdi, A. Taleb, C.-M. Pradier. Surface Science 2014, 628, 24-29.

[2] Heinz H, Vaia RA, Farmer BL, Naik RR J. Phys. Chem. C 2008, 112, 17281 17290; Heinz H, Farmer BL, Pandey RB, Slocik JM, Patnaik SS, Pachter R, Naik RR. J. Am. Chem. Soc. 2009, 131, 9704-9714

30 min break

BP 41.6 Thu 11:30 H 1058

Self-assembled protein nanofibers as basis for novel biomaterials — •Christian Helbing[1], Gang Wei[2], Tanja Deckert-Gaudig[3], and Klaus D. Jandt[1] — [1]Chair of Materials Science (CMS), Otto-Schott-Institute of Materials Research (OSIM), Friedrich Schiller University Jena, Jena, Germany — [2]Hybrid Materials Interfaces Group, University of Bremen, Bremen, Germany — [3]Institute for Photonic Technology, Jena, Germany

Protein nanofibers (PNFs) are promising materials for numerous applications in the field of biomedical engineering. Especially, self-assembled PNFs based on plasma proteins have a high importance due their easy fabrication and high biocompatibility. However, knowledge about the self-assembly mechanism and the properties of such PNFs is limited. The aim of the current study is to deepen the understanding of the formation mechanism. We tested the hypotheses that properties, morphology and inner structure of PNF depends on

environmental conditions. In this work, we present first results of self-assembled PNF structures formed in solution from different plasma proteins and plasma protein combinations. The observed morphology and mechanical properties of the formed PNFs depended strongly on the formation conditions. The structural analysis suggest that a partial denaturation, i.e. a change in the secondary structure, of the plasma proteins is a necessary requirement for the formation of PNFs. The comparison of the secondary structure of the PNFs and the native proteins helps to improve the understanding of the self-assembly mechanism. The current results leads to a better control during the PNF formation.

BP 41.7 Thu 11:45 H 1058

Ultrafast Infrared Spectroscopy Reveals Water-mediated Coherent Dynamics in an Enzyme Active Site — •Katrin Adamczyk[1], Niall Simpson[1], Gregory M. Greetham[2], Andrea Gumiero[3], Martin A. Walsh[3], Michael Towrie[2], Anthony W. Parker[2], and Neil T. Hunt[1] — [1]University of Strathclyde, Glasgow, UK — [2]Central Laser Facility, Rutherford Appleton Laboratory, Didcot, UK — [3]Diamond Light Source, Didcot, UK

Understanding the impact of fast dynamics upon chemical processes occurring within the active sites of proteins and enzymes is a key challenge that continues to attract interest. Similar gaps in our knowledge exist in understanding the role played by water, either as a solvent or as a structural/dynamic component of the active site. In order to investigate further the potential biological roles of water, ultrafast infrared spectroscopy is employed that directly probe the vibrational dynamics of NO bound to the ferric haem of the catalase enzyme from Corynebacterium glutamicum in both H2O and D2O. An isotope dependence of the vibrational relaxation parameters of the NO stretching vibration is observed indicating that water molecules interact directly with the haem ligand. Furthermore, IR pump-probe data feature quantum beats originating from the preparation of a coherent superposition of low-frequency vibrational modes in the active site of catalase that are coupled to the haem ligand stretching vibration. Together, the data establishes a strong interaction between the haem ligand and a water-mediated H-bond network in catalase that is likely to be pivotal to proton transfer events during the enzymatic cycle.

BP 41.8 Thu 12:00 H 1058

Structural mechanics of 2D and 3D lattices from clathrin proteins — •Mitja Platen[1], Philip N. Dannhauser[2], Heike Böning[2], Huberta Ungewickell[2], Ernst Ungewickell[2], and Iwan A.T. Schaap[1] — [1]IIIrd Institute of Physics, Georg August University Göttingen, Germany — [2]Institute of Cell Biology, Centre of Anatomy, Hannover Medical School, Hannover, Germany

In the cell clathrin proteins can form polyhedral scaffolds that facilitate the formation of highly curved vesicles (~100 nm) from lipid bilayers. These vesicles are involved in intracellular transport but also in the transport of compounds in and out the cell. We recently developed the methodology to reconstitute clathrin lattices in a minimal system, with and without lipid bilayers. With AFM imaging we are able to reconstruct the orientation of the clathrin triskelia in a planar lattice and to quantify the mechanical role of the clathrin light chains in the lattice. Our current efforts are aimed to measure the forces that are exerted by clathrin lattice to bend the lipid bilayer into vesicles.

BP 41.9 Thu 12:15 H 1058

Effects of molecular noise on bistable protein distributions in rod-shaped bacteria — •Lukas Wettmann, Mike Bonny, and Karsten Kruse — Theoretische Physik, Universität des Saarlandes, Postfach 151150, 66041 Saarbrücken, Germany

The distributions of many proteins in rod-shaped bacteria are far from homogeneous. Often they accumulate at the cell poles or in the cell centre. At the same time, the copy number of proteins in a single cell is relatively small making the patterns noisy. To explore limits to protein patterns due to molecular noise, we studied a generic mechanism for spontaneous polar protein assemblies in rod-shaped bacteria, which are based on cooperative binding of proteins to the cytoplasmic membrane. For mono-polar assemblies, we find that the switching time between the two poles increases exponentially with the cell length and with the protein number. This feature could be beneficial to organelle maintenance in ageing bacteria.

BP 41.10 Thu 12:30 H 1058

Refractive index regulation of the vertebrate retina. — •Alfonso Garcia-Ulloa, Heike Petzold, Alexandr Dibrov, Kaushikaram Subramanian, and Moritz Kreysing — Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Due to its similarity, the vertebrate retina serves as a model system for the brain, and yet it is different. Whereas brain tissue is opaque on sub-millimeter scale, the retina is of high optical quality and manages to suppress the backscattering of photons on their way to the sensitive photoreceptors.

Although the molecular basis of the retina's optical quality is largely unknown so far, physical optics clearly states that the magnitude of scattering at given particle sizes mostly depends on the refractive index contrast. Also, it has been noted that phase contrast based microscopy technique yields small signals when applied to retinal tissues. These observations raise the question of what regulates the retinal refractive index distribution.

We hypothesize that proteins with housekeeping functions, highly soluble and with high weight-specific refractive indices fulfill the role of refractive index regulators in the retina. In mouse models, the expression of crystallins has been localized in regions of high refractive index contrast like the nuclei, in the inner nuclear layer and in between nuclei, in the outer nuclear layer.

Our study establishes the role of refractive index regulators in specific retinal layers by combining fluorescent localization, quantitative phase-contrast microscopy and quantitative mass spectrometry.

BP 41.11 Thu 12:45 H 1058

Hierarchical nanoscale dynamics of proteins in solution — •Marco Grimaldo[1,2], Felix Roosen-Runge[1], Fajun Zhang[2], Frank Schreiber[2], and Tilo Seydel[1] — [1]Institut Laue-Langevin, Grenoble, France — [2]Institut für Angewandte Physik - Universität Tübingen, Tübingen, Deutschland

The dynamics of proteins in solution is essential for both protein function and cellular processes. The hierarchical protein dynamics, from the global center-of-mass diffusion to the motions of side-chains and chemical groups renders a complete understanding challenging. Profiting from the very high flux of the new backscattering spectrometer IN16B, the translational and rotational diffusion of the proteins can be self-consistently separated from the internal molecular motions. The global protein diffusion on the nanosecond time scale is consistent with predictions for colloidal suspensions of effective hard spheres even when the molecular structure differs considerably from a sphere [1]. The internal motions on nanometer length scales are characterized both geometrically and dynamically, suggesting a picture of methyl rotations and restricted diffusion of side chains. We also systematically explore the temperature-dependence of both the global and internal diffusive motions in protein solutions, including unfolding and aggregation at temperatures beyond the thermal denaturation [2].

[1] M. Grimaldo, F. Roosen-Runge, F. Zhang, T. Seydel, F. Schreiber, J. Phys. Chem. B **118**, 7203 (2014) [2] M. Grimaldo, F. Roosen-Runge, N. Jalarvo, M. Zamponi, F. Zanini, M. Hennig, F. Zhang, F. Schreiber, T. Seydel, submitted