BP 1: Protein Structure and Dynamics

Time: Monday 9:30–13:00 Location: H 1028

Invited Talk BP 1.1 Mon 9:30 H 1028

Thermodynamics and kinetics of protein aggregation from atomistic simulations — •Birgit Strodel — Institute of Complex Systems: Structural Biochemistry, Forschungszentrum Jülich, Germany — Institute of Theoretical and Computational Chemistry, Heinrich Heine University, Düsseldorf

I will present two different techniques developed by my group, which allow the extraction of the thermodynamics and kinetics of protein aggregation from molecular dynamics data. The first technique uses transition networks (TNs) to characterize the aggregation pathways, as will be demonstrated for the formation amyloid β-protein (Aβ) oligomers, which are connected to the development of Alzheimer's disease. The TNs reveal that the oligomers leading to the size distributions observed in experiments originate from metastable compact conformations, while extended oligomers are the ones driving the aggregation process. It is further elucidated how changes in the sequence of Aβ, a pH change or the presence of Cu(II) ions lead to different aggregation pathways, which is of direct relevance to the toxicity of Aβ oligomers. In the other technique, we extended the idea of automated Markov state models (MSM) to protein self-assembly by constructing reaction coordinates from descriptors that are invariant to permutations of the molecular indexing. I will demonstrate the power of this technique for the identification of kinetically relevant aggregation pathways for the KFFE peptide. Both the TN and MSM formalism developed by us are quite general and can therefore be used for the automated analysis of any other self-assembling molecular system.

BP 1.2 Mon 10:00 H 1028

Short-time self-dynamics of immunoglobulin under biomimicking crowding conditions — •Marco Grimaldo[1], Beck Christian[1,2], Felix Roosen-Runge[3], Fajun Zhang[2], Frank Schreiber[2], and Tilo Seydel[1] — [1]Institut Laue-Langevin, Grenoble, France — [2]IAP- Universität Tübingen, Tübingen, Germany — [3]Division for Physical Chemistry, Lund University, Lund, Sweden

Approximately 10-40% of the intra- and extracellular fluids of living organisms are occupied by macromolecules such as proteins. This macromolecular crowding condition was shown to influence reaction rates, and to lead to anomalous diffusion. We present a neutron backscattering study on the pico- to nanosecond self-diffusion and internal motion of the antibody proteins immunoglobulins (Ig) in aqueous environment. To systematically investigate the effect of macromolecular crowding on protein dynamics we vary the concentration of cellular lysate, mimicking a cellular environment. The dynamics of Ig in lysate is then compared with that of Ig in pure (heavy) water as a function of its own concentration (self-crowding) [1]. Despite the high polydispersity and the not easily predictable variance in lysate composition, both the measured diffusion and the localized internal atomic motion of Ig as a function of the overall volume fraction are in rather good agreement with those of Ig in the self-crowded environment at comparable volume fraction, suggesting a crucial role of hydrodynamic interactions on short-time protein dynamics even in a cell-like environment.
[1] Grimaldo M., Roosen-Runge F., Zhang F., Seydel T., Schreiber F. JPCB 118 (2014): 7203.

BP 1.3 Mon 10:15 H 1028

Dramatic influence of anisotropic interaction and shape on short-time protein diffusion — Jin Suk Myung[1], •Felix Roosen-Runge[1], Roland G. Winkler[2], Gerhard Gompper[2], Peter Schurtenberger[1], and Anna Stradner[1] — [1]Division of Physical Chemistry, Lund University, Sweden — [2]Theoretical Soft Matter and Biophysics, Forschungszentrum Jülich, Germany

Diffusion of proteins in cells is an essential process, strongly influencing the cellular machinery through numerous processes such as signal transmission or reactions between proteins. We present a combined experimental and computational study on effects of anisotropic interactions and shape on the initial step of structural relaxation on nearest-neighbor distance, i.e. short-time cage diffusion. Using neutron spin echo spectroscopy in crowded solutions of crystallin proteins, cage diffusion for α crystallin follows predictions for hard spheres, while the cage diffusion of the weakly attractive γ crystallin shows a dramatical slowing down at comparably low volume fractions [1]. In mesoscale hydrodynamic simulations employing multiparticle collision dynamics (MPC), we observe a significant dynamical slowing down due to attractions, which is strongly enhanced due to anisotropy in protein interactions [1] and shape [2]. These results particularly demonstrate that simplistic spherical models for globular proteins can be severely misleading when studying effects of crowding on structural relaxation and diffusion.
[1] S Bucciarelli, JS Myung et al. Sci. Adv. (2016) 2:e1601432
[2] JS Myung, F Roosen-Runge et al. in preparation

BP 1.4 Mon 10:30 H 1028

Stochastic modeling of multiprotein complex formation — •Stefanie Förste, Reinhard Lipowsky, and Sophia Rudorf — Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

The formation of a multiprotein complex, arising from the assembly of multiple peptide chains inside the crowded cell environment, is subject of ongoing research. In contrast to the canonical view that protein assembly is a post-translational process, recent experiments show that protein complexes can also assemble co-translationally, i.e., the different chains may assemble before translation has finished. Here, we investigate under which conditions post translational and/or co-translational assembly can occur. We analyze the influence of different parameters - such as the spatial distance of the translation sites - on the assembly dynamics using a combination of Gillespie simulations and analytical Markov modeling. In particular, we study the cross-over from a co-translational to a post-translational assembly regime.

BP 1.5 Mon 10:45 H 1028

Characterisation of binding interaction of the influenza virus proteins Hemagglutinin and Neuraminidase with a synthetic sialic acid receptor by single molecule force spectroscopy — •Valentin Reiter-Scherer[1], Sumati Bhatia[2], Jose Luis Cuellar-Camacho[2], Daniel Lauster[1], Rainer Haag[2], Andreas Herrmann[1], and Jürgen P. Rabe[1] — [1]HU Berlin — [2]FU Berlin

The influenza virus is causing annual epidemics. In the first step of the infection, the virion binds to a host cell through multivalent attachment, mediated by the major virus spike protein hemagglutinin (HA) and sialic acid (SA) receptors of the glycocalyx of epithelial cells of the respiratory tract [1]. Neuraminidase (NA) on the other hand is known to cleave SA from the glycoproteins enabling the release of newly formed virions. A common strategy to inhibit infection, is the use of drugs that bind specifically to the binding pockets of the viral proteins to prevent SA binding [2]. Here we introduce a ligand architecture (LAPEG-SA) ideally synthesized to test the tensile strength between individual SA units and recombinant HA and NA of influenza H1N1. Individual binding strength and affinity at the single molecular level, being of central importance for the development of novel potent inhibitors, are characterized by scanning force microscope based single molecule force spectroscopy. Rupture forces of the SA protein binding are measured for several rates of force loading and the dissociation parameters off-rate as well rupture length are derived from the single barrier model [3]. - [1] Sieben et al., PNAS 2012. [2] Bhatia et al., J. Am. Chem. Soc. 2016. [3] Evans et al., Biophys. Journal 1997.

15 min. break

BP 1.6 Mon 11:15 H 1028

Exploring protein structure with cryogenic optical localization in three dimensions — Daniel Boening, •Franz Ferdinand Wieser, and Vahid Sandoghdar — Max Planck Institute for the Science of Light, Erlangen, Germany

Super-resolution optical microscopy has considerably advanced the study of cellular processes, but optical access to the molecular structure of proteins and biomolecular assemblies remains very limited. We have recently exploited the enhanced photostability of fluorophores at cryogenic temperatures to increase the number of detected photons, thus reaching a significantly higher signal-to-noise ratio compared to room-temperature measurements. Using this approach, cryogenic optical localization in three dimensions (COLD) is capable of determining the positions of several fluorescent sites within a single protein at Angstrom resolution [1]. We present results on imaging DNA Origami, the four binding sites of streptavidin and the conformational state of

the Per-ARNT-Sim domain of the histidine kinase CitA. With its high spatial resolution COLD opens new possibilities for obtaining quantitative structure information from small to medium sized biomolecules and for correlative measurements with established imaging methods.

[1] S. Weisenburger et al., Nature Methods 14, 141-144 (2017).

BP 1.7 Mon 11:30 H 1028

The relevance of conformational entropy for ligand-protein interactions: The case of biotin and streptavidin — •Mona Sarter[1,2], Andreas Stadler[1], Doreen Niether[1], Bernd König[1], Simone Wiegand[1,3], Jörg Fitter[1,2], Michaela Zamponi[1], Wiebke Lohstroh[4], and Niina Jalarvo[5] — [1]FZJ Jülich — [2]RWTH Aachen — [3]Universität zu Köln — [4]TUM München — [5]SNS Oak Ridge

Molecular dynamics play a vital role for the biological function of proteins. For protein ligand interactions changes of conformational entropy of the protein and the hydration layer are relevant for the binding process. In an experimental study we investigated the relevance of conformational entropy for the binding of biotin to the protein streptavidin. In order to investigate the proteins conformational entropy and dynamics quasi elastic neutron scattering (QENS) was used, for the protein and hydration layer isothermal titration caliometry (ITC) was used and for the hydration layer thermodiffusion (TDFRS) was used.

QENS results show that the conformational entropy of streptavidin is reduced upon biotin binding, while ITC results show that the conformational entropy of the hydration layer increases upon biotin binding. TDFRS results also indicate an increased entropy of the hydration layer. This leads to the conclusion that the hydration layer plays an important role in stabilising the binding of biotin to streptavidin. The internal streptavidin dynamics before and after biotin binding were compared. This showed that the flexibility of streptavidin is greatly reduced upon biotin binding leading to the complex being more rigid.

BP 1.8 Mon 11:45 H 1028

Hydration behaviour of collagen — •Philip Loche[1], Lise Thornfeldt Hansen[1], Lorena Ruiz[2], Jan Daldrop[1], Alexander Schlaich[1], Emanuel Schneck[2], Luca Bertinetti[2], Kerstin G. Blank[2], and Roland R. Netz[1] — [1]Department of Physics, Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany — [2]Max Planck Institute of Colloids and Interfaces, Am Mühlenberg, 1 OT Golm, Germany

Collagen is a key protein in the extraceullar matrix of connective tissues such as bones, skin, cartilage, tendons and muscles. Materials such as leather or parchment are known to shrink upon dehydration. An important component in these materials is collagen. The fact that water has an effect on the properties of collagen is well known, but the reasons for this are not. Here we use classical molecular dynamics simulations to obtain insights into the structure and behaviour of collagen. By comparison with x-ray scattering experiments, we show that the choice of the forcefield used in simulations is crucial to reproduce correct hydration effects. From our simulations we reproduce the experimental scattering intensities as well a experimental osmotic pressures. We also calculate the energetic and entropic contributions to the osmotic pressure for different collagen types.

BP 1.9 Mon 12:00 H 1028

Protein assemblies of hGBP1 studied with Time Resolved-Small Angle Scattering — •Charlotte Lorenz and Andreas Stadler — Jülich Centre for Neutron Science (JCNS-1), Forschungszentrum Jülich

The human Guanylate Binding Protein 1 belongs to the family of dynamin-like proteins and is activated by addition of nucleotides which lead to protein oligomerization and stimulated GTPase activity. Standard protein expression and purification from bacterial E.coli cells leads to hGBP1 without the posttranslational attachment of farnesyl. With an integrative approach using analytical ultracentrifugation (AUC), dynamic light scattering (DLS) and on-line size exclusion chromatography (SEC-SAXS) we investigated intermediate states during the hydrolysis cycle of hGBP1. We were able to show that farnesylation prevents hGBP1 in the inactive monomeric form in nucleotide free solution, whereas the unmodified hGBP1 (nf-hGBP1) consists of monomers and dimers in nucleotide free solution. Furthermore, the nf-hGBP1 assembles to mostly dimers and tetramers after nucleotide induction. Contrary, the farnesylated hGBP1 assembles after nucleotide addition to large macromolecular structures. The polymer growth and composition is analyzed in solution using time resolved SAXS (TR-SAXS). This study shows the importance of posttranslational modifications regarding the signaling regulation and controlled growth of macromolecular complexes.

BP 1.10 Mon 12:15 H 1028

High spatial and temporal resolution study of biological processes in a live cell via interferometric scattering microscopy (iSCAT). — Richard Taylor[1], Reza Gholami[1], Verena Rauschenberger[2], •Anna Kashkanova[1], Alexandra Schambony[2], and Vahid Sandoghdar[1] — [1]Max Planck Institute for the Science of Light, Erlangen, 91058, Germany — [2]Developmental Biology Unit, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, 91058, Germany

Transmembrane proteins on a live cell exhibit a variety of dynamic behaviors, such as diffusion on the cell membrane, transport into the cell and trafficking along filaments. Those processes have been studied using standard technologies, however real-time visualization with nanoscopic resolution is a challenge, with fluorescent microscopy as the primary workhorse.

However, fluorescent microscopy has a critical limitation: fluorophores emit a limited number of photons in their lifetime, which limits spatial and temporal resolution, and the observation time. Interferometric scattering microscopy (iSCAT), uses gold nanoparticles in place of fluorophores. The scattered light is imaged interferometrically and the measurement can be performed indefinitely with spatial (temporal) resolution of several nanometers (microseconds).

We present an experiment in which the epidermal growth factor receptor (EGFR) protein in a live HeLa cell was labeled with a 50 nm gold nanoparticle and its life cycle observed. Our precise 3D information provides exciting new insights into the dynamics of the receptor.

BP 1.11 Mon 12:30 H 1028

Validation of reaction coordinates describing protein functional motion: combining equilibrium and non-equilibrium MD methods — •Matthias Ernst, Steffen Wolf, and Gerhard Stock — Biomolecular Dynamics, University of Freiburg

Finding low-dimensional reaction coordinates that concisely describe mechanistic details of protein motion is a fundamental and crucial step to understand (and, at a later stage, to manipulate) protein dynamics. Statistical methods like Principal Component Analysis are often used and well understood, but usually not able to causally disentangle local rearrangements that drive some motion and others that are merely correlated or follow it. In my presentation, I will outline a strategy to combine non-equilibrium methods with equilibrium results to challenge and validate reaction coordinates: we use Targeted MD[1] as "molecular tweezer" to induce local rearrangements and explain causal relations between and the overall functional motion. Investigating the prominent PacMan-like hinge-bending motion of T4 Lysozyme, with 2600 atoms a rather small but extensively studied protein, we could show[2] that a so far unrecognized reorientation of actually one single side chain acts as a lock to stabilize and distinguish the open from the closed state and is the cause of the rather long ($\approx 10\mu$s) timescale. We propose and verify a 4-state model for the hinge-bending motion of T4 Lysozyme, which is supported by mutation studies and higher temperature runs.

[1] J.Schlitter, M.Engels, P.Krüger, *J.Mol Graph.* **1994**, *12*, 84.
[2]M.Ernst,S.Wolf,G.Stock,*J.Chem.Theory Comput.***2017**,*13*(10),5076.

BP 1.12 Mon 12:45 H 1028

Elucidation of light-induced structural changes of aureochrome and its recovery kinetics by small-angle X-ray scattering — •Saskia Bannister, Elena Herman, Thomas Hellweg, and Tilman Kottke — Bielefeld University, Germany

Aureochromes function as blue-light-regulated transcription factors in algae. Their basic region leucine zipper (bZIP) effector domain binds DNA specifically while a light-, oxygen-, or voltage-sensitive (LOV) domain acts as the sensor. Due to the inversed arrangement of sensor and effector, aureochromes are interesting for studying their mechanism and for the engineering of new optogenetic tools.

By applying small-angle X-ray scattering (SAXS) we pursue two main targets, namely the elucidation of light-induced structural changes of the receptor in solution and the analysis of the recovery kinetics from its light state back to its dark state. However, SAXS on photoreceptors is challenging. First, dark conditions need to be absolutely strict to avoid conversion of the highly sensitive receptor. Second, the analysis under illumination needs to ensure full conversion. Therefore we have established SAXS experiments under rigorous

control of light. Here, we reveal light-induced structural changes of the photoreceptor and its recovery kinetics.

Banerjee, A., Herman, E., Serif, M., Maestre-Reyna, M., Hepp, S., Pokorny, R., Kroth, P. G., Essen, L.-O., Kottke, T. (2016), *Nucleic Acids Res.* 44(12), 5957-5970.