BP 24: Posters - Protein Structure and Dynamics

Time: Tuesday 14:00–16:00 Location: P1A

BP 24.1 Tue 14:00 P1A

NMR Investigation of Protein Aggregation in Concentrated Solutions of Eye Lens Crystallins. — •Maria Camilles, Susanne Link, Alexey Krushelnitsky, Jochen Balbach, and Kay Saalwächter — Institute of Physics, Faculty of Natural Sciences II, Betty-Heimann-Str. 7, 06120 Halle/Saale, GERMANY

Crystallins are the major vision-related (i.e. refractive) proteins found in the eye lens. The mammalian lens consist of three classes of proteins, i.e alpha-, beta- and gamma-crystallins, which are structural proteins. The former also acts as chaperone. Commonly, proteins are subject to a continuous degradation and replacement process, but the eye lens proteins have to remain stable and soluble for a lifetime. Heat, shock or other stressors can cause aggregation and lead to cataract, thus the major chaperone function is to prevent aggregation. The conventionally used methods to study aggregation include observations by optical techniques applied mostly to dilute solutions. Here we demonstrate the use of 1H pulsed field gradient (PFG) NMR as an alternative to study the aggregation kinetics of crystallin proteins in highly concentrated protein solutions. PFG-NMR provides self-diffusion coefficients and is thus sensitive to aggregate size. We have studied the thermal denaturation and aggregation kinetics of gammaB-crystallin in the absence and presence of alphaB-crystallin. The components can be easily distinguished by their rather different sizes, thus their self diffusion coefficients. Our data demonstrate qualitative changes in the thermal degradation of gammaB-crystallin in the presence of alphaB-crystallin.

BP 24.2 Tue 14:00 P1A

Molecular dynamics study of the mechanical stability of dimeric coiled-coils under strain — •Chuanfu Luo, Ana Vila Verde, and Reinhard Lipowsky — Max Planck Institute of Colloids and Interfaces, 14476 Potsdam, Germany

Coiled-coils (CCs) are ubiquitous folding motifs found in proteins. Dimeric CCs consist of two alpha-helices wrapped around each other in a super helix configuration. In biological systems, CCs are highly versatile: they play an important role in various intracellular regulation processes as well as in membrane fusion. Their unusual structure suggests that it may also be possible to use them as biological force sensors to detect forces involved in biochemical processes *in vivo*. We investigated this possibility by carrying out Steered Molecular Dynamics to simulate the shear pulling of *de novo* designed coiled-coils with different lengths. We found that the pulling force at slow pull appears to be independent of either the initial length or the contact length of the coiled-coils. Analysis shows that there are two pathways when pulling CCs: "opening of helices" at fast pulling speeds, and "step-wise sliding" at slower speeds.

BP 24.3 Tue 14:00 P1A

Characterization of open and closed beta 2 glycoprotein I conformation — •Ina Buchholz, Peter Nestler, Florian Berg, and Mihaela Delcea — University of Greifswald, ZIK HIKE, Fleischmannstr. 42, 17498 Greifswald, Germany

The antiphospholipid syndrome (APS) is an autoimmune disease characterized by the presence of anti-beta 2 glycoprotein I (b2GPI) antibodies circulating in blood of patients. b2GPI exists in at least two different conformations: the closed form which is found circulating in blood and the open form which exposes a cryptic binding site (i.e. epitope). This antigenic conformation leads to formation of antibody-protein complexes which induce thrombotic events and recurring pregnancy loss. To identify the conditions that direct to the development of APS, a conversion protocol is used to prepare the open and closed forms of b2GPI. Therefore, the structures of b2GPI are studied by circular dichroism spectroscopy, fluorescence spectroscopy and atomic force microscopy in varying buffer systems. First results indicate inherent differences between open and closed conformation. Some experimental conditions also provide mixed conformational populations. These findings will be handled as a basic reference for further experiments aiming for the alteration of b2GPI protein structure. For this, the influence of different stress conditions like pH shift, temperature change or ionic strength, as well as the binding of various ligands to b2GPI will be investigated and will be further correlated with APS antibody binding to b2GPI.

BP 24.4 Tue 14:00 P1A

Biophysical characterization of integrin alpha IIb-beta3 under physiological and stress conditions — •Una Janke, Peter Nestler, and Mihaela Delcea — University of Greifswald, ZIK HIKE, Fleischmannstr. 42, 17498 Greifswald, Germany

The heterodimeric transmembrane platelet receptor integrin alpha IIb-beta 3 (αIIbβ3) plays a crucial role in haemostasis and is involved in the autoimmune disease Immune Thrombocytopenia (ITP). ITP patients develop a higher bleeding risk due to autoantibody mediated platelet destruction. The immunogenicity (i.e. capacity of the immune system to induce an immune response) depends strongly on the conformation of the αIIbβ3. Thus, we aim to study the influence of closed, opened and intermediate conformations on the binding properties of αIIbβ3 to ITP patient autoantibodies. Here we present a strategy to investigate the effect of mutations, external stress factors and binding partners on the αIIbβ3 conformation. The interaction under physiological conditions, e.g. in a membrane environment, is investigated using surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) after incorporating αIIbβ3 into liposomes. Different incorporation protocols are compared with regards to liposome-αIIbβ3 conjunction. First results indicate that pH, manganese ions and temperature are important influence factors.

BP 24.5 Tue 14:00 P1A

Identification of reaction coordinates for functional dynamics: understanding the molecular pacman T4 Lysozyme — •Matthias Ernst, Steffen Wolf, and Gerhard Stock — Biomolecular Dynamics, University of Freiburg, Germany

T4 Lysozyme is a model system for both experimental and computational investigations of functional protein dynamics. The major functional dynamics is a hinge-bending motion between its two domains which occurs on a timescale of 10-20μs[1]. Yet, the Free energy landscape based on widely applied reaction coordinates like the radius of gyration or the root mean square deviation to the crystal structure shows a barrier that is much too small to explain this timescale. Hence, there must be mechanistic details that require other reaction coordinates.

Using a combination of distance-based principal component analysis[2] on a 50us long equilibrium trajectory of T4L and inverse targeted molecular dynamics simulations[3], we could identify new reaction coordinates describing local structural rearrangements which act as a locking mechanism. We find two intermediate states on the path between "open" and "closed" which slow down the process. Thus, we propose a hierarchical four-state model that can explain the observed timescales semi-quantitatively.

[1] R.B. Yirdaw and H.S. Mchaourab, *Biophys.J.* **2012**, *103(7)*, 1525.
[2] M. Ernst, F. Sittel and G. Stock, *J.Chem.Phys.* **2015**, *143*, 244114.
[3] J. Schlitter, M. Engels and P. Krüger, *J.Mol.Graph.* **1994**, *12*, 84.

BP 24.6 Tue 14:00 P1A

Fast liquid DSC - a novel tool to study protein solutions — •Jana Rüdel, Patrizia Kruse, and Maria Ott — Institute of Physics, Martin-Luther-University Halle-Wittenberg, 06120 Halle, Germany

Fast scanning calorimetry with heating rates up to 10000 K/s allows to study rapid non-equilibrium processes which remain invisible in standard DSC experiments. The available sensor chips, however, which were successfully used in polymer science [1] restricted the use to dry or highly viscious samples. If this technique could be extended to liquid samples, fast scanning calorimetry would give a unique access to study the time dependencies of processes in biology.

We extensively tested novel, recently introduced liquid Flash DSC sensors [2] by studying aqueous protein solutions with scanning rates up to 500K/s. We will illustrate the potential of using fast scanning calorimetry and discuss current challenges and limitations.

[1] V. Mathot et al., Thermochim Acta 522 (2011), 36-45
[2] R. Splinter et al., Thermochim Acta 603 (2015), 162-171

BP 24.7 Tue 14:00 P1A

Thermophoretic trapping of single molecules — Tobias Thalheim, Marco Braun, Andreas Bregulla, and •Frank Cichos —

Molecular Nanophotonics Group, Institute of Experimental Physics I, University of Leipzig, Germany

We present a force-free trapping method with is capable of confining the Brownian motion of single molecules by applying the actual fuel of Brownian motion: temperature. A focused laser beam is used to optically heat a plasmonic structure generating dynamical temperature gradients. Single molecules migrate in these temperature gradients typically to colder regions due to an effect known as thermophoresis or Ludwig-Soret effect. An optical feedback algorithm utilizes this behavior to reposition the focused laser beam and, hence, restricts the Brownian motion of the single molecule. As a first biological model system, double-stranded lambda-DNA is investigated in the thermophoretic trap. The macromolecular conformational changes due to the inhomogeneous temperature gradients are evaluated with the help of a principal component analysis. Furthermore, the Soret coefficient of single 6-helix bundle DNA origami structures has been measured in the trap.

BP 24.8 Tue 14:00 P1A

Small-angle X-ray scattering study of aqueous Trypsin solutions and the influence of pressure on interaction potential — •Jan Latarius, Julian Schulze, Michael Paulus, Christian Sternemann, Jan Nysar, Göran Surmeier, and Metin Tolan — Fakultät Physik/DELTA, Technische Universität Dortmund, D-44221 Dortmund

Proteins as outmost important components of life show complex and non-intuitive behaviour regarding the interaction with themselves and each other under high pressure. Using the method of small-angle X-ray scattering we explore the properties of soluted Trypsin exposed to hydrostatic pressure and compare the findings to studies on aqueous solutions of Lysozyme and RNase. By altering the pressure in a range from 1 to 4000 bar with constant pH and adding co-solvents like TMAO and TMACl to differently concentrated protein solutions we hope to reveal the influence of co-solvents and pressure on the interaction potential.

BP 24.9 Tue 14:00 P1A

MD Simulation Studies of Protein Dynamics in Neutral Confinement — •Timothy Wohlfromm, Matthias Bartelmess, Tatjana Thiel, and Michael Vogel — Institut für Festkörperphysik, TU Darmstadt, Hochschulstraße 6, 64289 Darmstadt, Germany

We report on recent findings regarding dynamics of the elastin like protein model $(VPGVG)_{50}$ and its surrounding hydration shell in a neutral pore. Despite recent progress we lack understanding of dynamics of complex systems, such as proteins, specifically when studied in confinement. To isolate direct effects of confining geometries on protein dynamics the pore consists of the same atom type as the solvent, in most cases water, restrained in position by harmonic potentials of varying restoring forces to simulate confining surfaces with differing rigidity. Varying the hydration level of the confined protein we find that the minimal degree of hydration as ratio of water mass to protein mass for the protein to show bulk behaviour is 1 g/g. Moreover, we observe in spatially resolved analyses that with decreasing distance to the pore wall the correlation times of water increase by more than one order of magnitude. This effect is drastically reduced with reduced rigidity of the pore wall. In addition the protein acts as a soft confinement to water in vicinity to its surface. Finally, we systematically investigate changes in the dynamics of the elastin model when water is replaced by other solvents.

BP 24.10 Tue 14:00 P1A

Chiral effects in CH3->CF3 mutations in amino acids determine hydrophobicity — Joao Robalo[1], Susanne Huhmann[2], Beate Koksch[2], and •Ana Vila Verde[1] — [1]MPIKG, Theory and Bio-Systems Dept., Am Mühlenberg 1 OT Golm , 14476 Potsdam, Germany — [2]Freie Universität Berlin, Institute of Chemistry and Biochemistry, Takustr. 3, 14195 Berlin

Protein fluorination is a promising avenue to modify protein properties. Predicting the impact of protein fluorination on protein stability based on simple heuristics - e.g., changes in amino acid apolar surface area or polarity - has proven impossible because of the interplay between the fluorinated site and its neighboring environment. Ultimately understanding and predicting how fluorination impacts proteins can best be done using molecular simulations and classical, atomistic models. Here we present such a model for fluorinated amino acids. We apply this force field to investigate how CH3->CF3 mutations alter the hydrophobicity of apolar amino acids. Our results show that these mutations increase the hydrophobicity of the amino acid directly, by increasing the apolar surface area, and indirectly, by decreasing the number of backbone-water hydrogen bonds. Strikingly, stereoisomeric effects strongly impact the conformational orientation and the flexibility of the amino acid side chain and ultimately determine the magnitude of changes in hydrophobicity. We demonstrate that the commonly accepted notion that CH3->CF3 mutations alter protein stability only via changes in apolar surface area is incorrect, and show that different fluorinated stereoisomers may be exploited for particular purposes.

BP 24.11 Tue 14:00 P1A

Metadynamics Simulations of the Fibrinogen Protomer — •Timo Schäfer[1,2], Lorenz Ripka[1], Friederike Schmid[1], and Giovanni Settanni[1,3] — [1]Johannes Gutenberg-University Mainz — [2]Graduate School Materials Science in Mainz — [3]Max Planck Graduate Center with the Johannes Gutenberg-University Mainz

Fibrinogen is a dimeric multi-chain serum protein that polymerizes into fibrin when activated by thrombin as part of the coagulation cascade. Fibrinolysis, the cleavage of fibrin by the enzyme plasmin, controls the dissolution of blood clots. While the major factors contributing to fibrin formation and dissolution have been identified, the atomistic details of these mechanisms are largely unknown.

Here, the connection between structure and function of fibrinogen is studied using classical atomistic molecular dynamics simulations coupled to metadynamics, a technique that allows for a thorough exploration of the important degrees of freedom of the system. Based on our previous characterization of a hinge along the coiled-coil region of the fibrinogen protomer, we used metadynamics to explore the major degrees of freedom related to this hinge, represented by the two largest principal components of motion. The simulations reveal the presence of two specifically distinct modes of bending, characterized by a different loss of secondary structure and exposure of plasmin cleavage sites.

BP 24.12 Tue 14:00 P1A

Chemical Selective Preparation of Native Proteins on Surfaces by Mass Spectrometry for High Resolution, Single Molecule Imaging — •Stephan Rauschenbach[1], Jean-Nicolas Longchamp[1], Joseph Gault[3], Sabine Abb[2], Hans-Werner Fink[2], and Klaus Kern[1] — [1]Max Planck Institute for Solid State Res., Stuttgart, Germany — [2]Univ. of Zuerich, Switzerland — [3]Oxford Univ., UK

The structural characterization of proteins relies on extensive preparation efforts; once to obtain and purify the molecule, and second to bring it in the required form and environment. With free electron lasers or low energy electron holography/1/ new single molecule methods for structural analysis are in sight. They, too, require matching preparation methods, in particular ensuring chemical purity, specificity and compatibility with the vacuum environment. Preparative mass spectrometry, specifically soft-landing electrospray ion beam deposition/2/ is in the unique position to fulfill these requirements. We can can generate intense beams of native proteins, protein complexes, and even membrane proteins and deposit them on solid surfaces. The imaging by scanning tunneling microscopy on metal surfaces confirms the protein is deposited as a three-dimensional objects. In the future, relevant structural information is to be expected by using free-standing graphene/3/ as a substrate and low-energy electron holography/4/ for imaging. /1/ PRL 110,255501(2013) /2/ Annu. Rev. Anal. Chem. 9, 16.1 (2016) /3/ J. Vac. Sci. Technol. B31, 020605 (2013) /4/ arXiv:1512.08958 (2015)

BP 24.13 Tue 14:00 P1A

Polarization anisotropy of IR spectra reveals geometry of a protonated water cluster — •Jan Daldrop[1], Mattia Saita[1], Matthias Heyden[2], Joachim Heberle[1], and Roland Netz[1] — [1]Fachbereich Physik, Freie Universität Berlin, 14195 Berlin, Germany — [2]MPI für Kohlenforschung, 45470 Mülheim an der Ruhr

Infrared spectra for protonated and unprotonated water chains, water slabs and water drops are calculated from ab initio Molecular Dynamics trajectories. For all three water cluster geometries we obtain a pronounced IR continuum band over a wide frequency range in the presence of an excess proton. This continuum band exhibits a strong polarization anisotropy for chains and slabs with maximal adsorption for IR polarization along the water cluster long axes. The continuum band for protonated water chains is shown to be due to charge fluctuation dynamics of the Zundel state ensemble linked to pronounced friction memory effects that decay over 100fs. For proton-conducting

proteins, where a water chain traverses the membrane-spanning protein, this anisotropy allows to distinguish proton motion along the water chain from proton motion along the protein or membrane surfaces by the use of polarization and time-resolved IR adsorption spectra. We use the results to interpret our experimental data for the continuum band around $\nu = 1900$ cm*1 of aligned bacteriorhodopsin proteins in membranes during laser-flash initiated proton transfer. Polarization-resolved IR spectroscopy thus allows for the interpretation of IR continuum bands and in particular furthers the microscopic understanding of water-mediated proton-transfer processes.

BP 24.14 Tue 14:00 P1A

Global Langevin model of multidimensional biomolecular dynamics — •Benjamin Lickert, Norbert Schaudinnus, Mithun Biswas, and Gerhard Stock — Albert Ludwigs University, 79104 Freiburg, Germany

Biomolecular processes, recorded by molecular dynamics simulations, are often treated as diffusive motions on low-dimensional free energy landscapes $F(\vec{x})$. A theoretical basis of such an interpretation is provided by Zwanzig's system-bath Hamiltonian approach which allows to derive a memory-free Langevin equation describing the motion of the system $\vec{x}$ on the free energy landscape $F(\vec{x})$.

While the theoretical framework of Zwanzig generally assumes a highly idealised form of the bath Hamiltonian and the system-bath coupling, it would be desirable to apply the approach to realistic data-based biomolecular systems. Here, we propose a practical method to construct an analytically defined global model of structural dynamics. On basis of molecular dynamics simulations and suitable system coordinates our approach uses an "empirical valence bond"-type model to represent the multidimensional free energy and an approximate description of the friction. We show for several systems that the resulting Langevin models reproduce the results of the underlying all-atom simulations. Since the Langevin equation can also be shown to satisfy the underlying assumptions of the theory (e.g., delta-correlated Gaussian noise), the analytically defined model provides a correct realisation of Zwanzig's formulation.

BP 24.15 Tue 14:00 P1A

Temperature dependence of the self-diffusion of BSA in solution with trivalent ions — •Christian Beck[1,2], Michal Braun[1], Marco Grimaldo[2], Niina H. Jalavaro[3], Felix Roosen-Runge[2], Fajun Zhang[1], Tilo Seydel[2], and Frank Schreiber[1] — [1]Institut für Angewandte Physik, Universität Tübingen, 72076 Tübingen — [2]Institut Laue-Langevin, Grenoble, France — [3]Neutron Sciences Directorate, Oak Ridge National Laboratory, USA

Recent studies focused on the salt-induced slowing down of the short-time self-diffusion of bovine serum albumin (BSA) in aqueous solutions (D_2O) at constant temperature [1] using quasi-elastic neutron scattering. The diffusion coefficients of the clusters, induced by the presence of trivalent yttrium ions, can be described independently of the protein concentration as a function of the ratio of salt ions per protein.

With experiments at BASIS (SNS, ONRL, Oak Ridge, TN) we determined the temperature-dependence of the system. For each temperature, a master curve is observed. In samples with trivalent salts, the diffusion coefficients increase less with increasing temperature than predicted by the Stokes-Einstein relation. The different master curves were used to determine the temperature dependent binding probabilities between the proteins using the Flory-Stockmeyer theory. An increasing binding probability with increasing salt concentration and increasing temperature was found. This observation on the microscopic scale is in agreement with the observed lower critical solution temperature (LCST) on macroscopic scale.

[1] M.Grimaldo et al. J. Phys. Chem. Letters, **6** (2015) 2577

BP 24.16 Tue 14:00 P1A

Effect of disulfide bridges on denatured protein dynamics investigated by neutron spin-echo spectroscopy — •Felix Ameseder[1], Aurel Radulescu[2], Peter Falus[3], Andreas Stadler[1], and Dieter Richter[1] — [1]Forschungszentrum Jülich GmbH, JCNS-1/ICS-1, Germany — [2]Forschungszentrum Jülich GmbH, JCNS Outstation at MLZ, Germany — [3]Institut Laue-Langevin, France

The dynamics of proteins in solution is highly dependent on the presence of covalent bonds acting as internal crosslinks between different domains. Here, we investigate the denatured Bovine Serum Albumin (BSA) protein in solution at 6 molar guanidine hydrochloride first with active disulfide bridges, and secondly with reduced disulfide bridges using β-mercaptoethanol as additional chemical denaturant. The results are interpreted with common polymer models that include hydrodynamic interactions like the Zimm model.
The protein structure was investigate beforehand with small angle neutron scattering SANS and small angle x-ray scattering SAXS. A distinct power law scaling behavior could be retrieved for both cases. The dynamics of the protein was investigated with dynamic light scattering, and neutron spin-echo spectroscopy NSE. The NSE results reveal distinct differences of the internal dynamics between the both cases that will be discussed in detail in the talk.

BP 24.17 Tue 14:00 P1A

Principal Component Analysis of Circular Data: Theory and Application — •Florian Sittel, Thomas Filk, and Gerhard Stock — Uni Freiburg/Brsg.

Principal Component Analysis (PCA) is a widely adopted technique for dimensionality reduction. However, being a linear transform it is not directly applicable to circular data, like the dynamics of protein backbone dihedral angles. There have been several attempts already in modifying PCA to circular data (Dihedral angle-based PCA, GeoPCA, Principal Geodesic Analysis), yet none addressed the special geometry of the underlying space (N-dimensional Tori) to full extent, resulting in projection errors. Here we present a theoretical analysis of this geometry and identify the pitfalls given by the periodicity of the data. Based on our analysis, we derive a new formulation of PCA of circular data and demonstrate its performance in the context of protein dynamics.

BP 24.18 Tue 14:00 P1A

The influence of non-Markovian effects on reaction coordinate quality — •Florian Brünig, Jan Daldrop, and Roland Netz — Freie Universität Berlin, Germany

Defining appropriate low-dimensional reaction coordinates remains a crucial task in molecular-dynamics data analysis. Quantification of reaction-coordinate quality by analyzing transition-path probabilities is a commonly applied method, but it is based on Markovian theory. However, non-Markvian effects, arising from inertia, friction memory or orthogonal degrees of freedom, cannot be neglected in relevant biophysical systems.

We investigate the applicability of this method to non-Markovian processes by two model systems that allow to continuously introduce non-Markovian effects: Langevin dynamics in a two dimensional potential and generalized Langevin dynamics with a friction memory kernel in a doublewell potential. Results are discussed with respect to data obtained from molecular-dynamics simulations.