

## BP 28: Protein structure and dynamics II

Time: Wednesday 15:00–18:30

Location: HÜL 386

**Topical Talk**

BP 28.1 Wed 15:00 HÜL 386

**Single Molecule Mechanics of Proteins** — ●MATTHIAS RIEF — Physikdepartment der TUM, James-Franck-Str., 85748 Garching, Germany

The development of nano-mechanical tools like Atomic Force Microscopy and optical traps has made it possible to address individual biomolecules and study their response to mechanical forces. In my talk, I will show how single molecule mechanical methods can be used to study the folding and interaction of proteins. Examples include the folding of calmodulin as well as the interaction of the cytoskeletal protein filamin with transmembrane proteins.

BP 28.2 Wed 15:30 HÜL 386

**Variable Temperature Single Molecule Force Spectroscopy of an Extremophilic Protein** — ●KATARZYNA TYCH<sup>1,2</sup>, TONI HOFFMANN<sup>1,2</sup>, DAVID BROCKWELL<sup>2</sup>, and LORNA DOUGAN<sup>1,2</sup> — <sup>1</sup>Molecular and Nanoscale Physics Group, School of Physics and Astronomy, University of Leeds, LS2 9JT, UK — <sup>2</sup>Astbury Centre for Structural Molecular Biology and Institute of Molecular and Cellular Biology, University of Leeds, LS2 9JT, UK

Extremophiles (organisms which survive and thrive in the most extreme chemical and physical conditions on Earth) exhibit a range of fascinating cellular- and molecular-level adaptations. The flexibility of extremophilic proteins is one of the key determinants of their ability to function at the extremes of environmental temperatures.

We use single molecule force spectroscopy (SMFS) by atomic force microscopy (AFM) to measure the effect of temperature on the mechanical stability and flexibility of a protein derived from a hyperthermophilic organism.

The study was performed using an AFM SMFS instrument with variable temperature capabilities. We study temperature-dependent changes in the unfolding energy landscape of this protein by measuring changes in the unfolding force with temperature in combination with Monte Carlo simulations. We find that the position of the transition state to unfolding shifts away from the native state with increased temperature, reflecting a reduction in the spring constant of the protein and an increase in structural flexibility [1].

[1] K. M. Tych et al. (2013), *Soft Matter* (9): 9016-9025

BP 28.3 Wed 15:45 HÜL 386

**Determining the protein folding core: an experimental and computational approach** — ●JACK HEAL, CLAUDIA BLINDAUER, ROBERT FREEDMAN, and RUDOLF RÖMER — University of Warwick, Coventry, England, CV4 7AL

The protein folding problem has been a prevalent concern of structural biology for more than 50 years. We study the folding process by identifying an experimental 'folding core' through hydrogen-deuterium exchange NMR (HDX) as well as a computationally determined folding core based on a combination of coarse-grained simulations using the software FRODA and rigidity analysis using FIRST. We test whether such rapid methods can reliably predict the results of HDX experiments. Our experimental system is Cyclophilin A (CypA), an enzyme that helps proteins to fold. It also binds to and aids the function of the immunosuppressant drug cyclosporin A (CsA) as well as binding to the HIV-1 capsid protein. We characterise the protein and its interaction with CsA using circular dichroism and fluorescence spectroscopy in addition to HDX experiments. From the set of slowly exchanging residues we establish the HDX folding core for both the unbound CypA and the CypA-CsA complex. We are able to improve upon the prediction from the established method of FIRST by using FRODA in combination with normal mode analysis. To accomplish this, we introduce a method of tracking the surface-exposure of backbone N-H atoms through the simulation. In this way, we are in the process of designing computationally undemanding methods that can predict the results of sophisticated experiments characterising ligand binding.

BP 28.4 Wed 16:00 HÜL 386

**Protein dynamical transition \* Insights from a combination of neutron scattering and MD simulations** — ●KERSTIN KÄMPF and MICHAEL VOGEL — Institut für Festkörperphysik, TU Darmstadt

Evaluating the temperature-dependent mean square displacement (MSD) of proteins with neutron scattering (NS) a non-linear increase

due to anharmonic dynamics is found well below room temperature [1]. It is still under debate whether this phenomenon, denoted as protein dynamical transition, occurs in one or two steps and whether these steps result from a true dynamical onset or from local ( $\beta$ -) [2] or structural ( $\alpha$ -) [3] relaxations entering the time window. A promising approach to clarify these issues is to combine NS data with MD simulations [4]. Application of such combination to hydrated elastin shows that NS data obtained from backscattering experiments are highly consistent with MD results. We find that anomalous internal protein dynamics, leading to a subdiffusive time dependence of the MSD and a power-law or logarithmic-like decay of correlation functions [5], dominates the findings in the time window of the experiments. The increase of the MSD is thus a signature of the onset of complex internal protein motion.

[1] Doster et al, *Nature*, 337, 754, (1989).

[2] Capaccioli et al. *J. Phys. Chem. B*, 111, 8197, (2007).

[3] Doster et al, *J. Non-Cryst. Sol.*, 357, 622, (2011).

[4] Hong et al, *Phys. Rev. Lett.*, 107, 148102, (2011).

[5] Kämpf et al., *J. Chem. Phys.*, 137, 205105, (2012).

BP 28.5 Wed 16:15 HÜL 386

**Bistable retinal Schiff base photo-dynamics of the histidine kinase rhodopsin HKR1 from the green alga *Chlamydomonas reinhardtii*** — ●ALFONS PENZKOFER<sup>1</sup>, MEIKE LUCK<sup>2</sup>, TILO MATHES<sup>2,3</sup>, and PETER HEGEMANN<sup>2</sup> — <sup>1</sup>Fakultät für Physik, Universität Regensburg, Universitätsstrasse 31, D-93053 Regensburg, Germany — <sup>2</sup>Institut für Biologie/Experimentelle Biophysik, Humboldt Universität zu Berlin, Invalidenstrasse 42, D-10115 Berlin, Germany — <sup>3</sup>Department of Exact Sciences / Biophysics, Vrije Universiteit, De Boelelaan 1081A, 1081 HV Amsterdam, The Netherlands

The photo-dynamics of the recombinant rhodopsin fragment of HKR1 [1] was studied. The retinal cofactor of HKR1 exists in two Schiff base forms, RetA (deprotonated 13-*cis* retinal) and RetB (protonated all-*trans* retinal). Blue light exposure converts RetB fully to RetA. UVA light exposure converts RetA to RetB and RetB to RetA giving a mixture of both. The quantum efficiencies of photo-conversion of RetA to RetB and RetB to RetA were determined to be  $0.096 \pm 0.005$  and  $0.405 \pm 0.01$ , respectively. In the dark, thermal equilibration occurs between RetA and RetB with a time constant of about 3 days giving mole fractions of 0.8 RetA and 0.2 RetB. Ground state and excited state potential energy curve schemes for the inter-conversion of RetA and RetB were developed. The photo-induced inter-conversions of RetA and RetB are caused by excited-state isomerization on a picosecond timescale, proton transfer, and retinal Schiff base - rhodopsin apoprotein ground-state equilibration on a millisecond timescale.

[1] M. Luck et al., *J. Biol. Chem.* 287 (2012) 40083.

BP 28.6 Wed 16:30 HÜL 386

**Terahertz spectroscopy on amino acids** — ●SEBASTIAN EMMERT, MARTIN WOLF, PETER LUNKENHEIMER, and ALOIS LOIDL — Experimental Physics V, Center for Electronic Correlations and Magnetism, University of Augsburg, Germany

All known proteins are built up from a set of 23 standard amino acids. Their three-dimensionally folded structure is mainly determined by the non-covalent interactions of the amino acid residues, such as hydrogen bonds. Therefore it is crucial to study the binding abilities and vibrational properties of these basic building blocks, to achieve a better understanding of the dynamics of the biological macromolecules.

With the novel technique of terahertz time-domain spectroscopy the interesting, but only rarely explored spectral region between the dielectric and the optical frequency regime can be covered. The amino acids in their crystalline state show numerous characteristic resonant features in the range 0.2 THz to 5 THz. It is shown that only by a thorough investigation of the temperature evolution of these spectral features and by a comparison with additional experimentally and theoretically obtained data, a complete assignment of all resonances is made possible. For this purpose, spectra of various amino acids were measured in the temperature range 4 K to 300 K. Fits were performed to quantify the temperature-induced shifts and intensity variations. In this way, spectral contributions from intra- and intermolecular vibrations could be separated by means of their anharmonicity and the dynamics of specific functional groups could be studied.

BP 28.7 Wed 16:45 HÜL 386

**Genetically Encoded Spin Labelled Artificial Amino Acids** — ●MALTE DRESCHER, MORITZ SCHMIDT, and DANIEL SUMMERER — Konstanz Research School Chemical Biology and Department of Chemistry, University of Konstanz, Germany

Recent publications demonstrate the ability of electron paramagnetic resonance spectroscopy (EPR) to provide structural, dynamical and functional data on biomacromolecules in cells. Of particular interest are distance measurements in the nanometer range. The advantages of the method are sensitivity, selectivity, the lack of any limitation imposed by the size of the macromolecule, and the possibility to get information on coexisting conformations via analyzing distance distributions. However, so far, these approaches require microinjection of spin-labelled macromolecules. Moreover, the biomolecules transferred to cells by these means have limited access to natural mechanisms of cellular processing like folding, localization, posttranslational modification and natural decay.

Here, we show for the first time the successful incorporation of a genetically encoded modified lysine amino acid containing 2, 2, 5, 5, -tetramethyl-pyrrolin-1-oxyl-label into various positions in GFP and TRX mutants in E.coli. First EPR distance measurements on extracted proteins demonstrate the potential of this novel approach.

15 min. break

BP 28.8 Wed 17:15 HÜL 386

**Structure and dynamics of interfacial water associated with the climate-active ice nucleating proteins probed by sum frequency generation spectroscopy** — ●RAVINDRA PANDEY<sup>1</sup>, JANINE FRÖHLICH<sup>2</sup>, ULRICH PÖSCHL<sup>2</sup>, RUTH LIVINGSTONE<sup>1</sup>, MISCHA BONN<sup>1</sup>, and TOBIAS WEIDNER<sup>1,3</sup> — <sup>1</sup>Max-Planck Institute for Polymer Research, Mainz — <sup>2</sup>Max-Planck Institute for Chemistry, Mainz — <sup>3</sup>Chemical Engineering, University of Washington, USA

Specific Bacteria such as *Pseudomonas syringae* effectively attack plants by using ice-nucleating proteins (INP) anchored to their outer cell surfaces. INP promotes the growth of ice crystals. To understand the ice formation by INP, it is important to understand the molecular mechanisms by which INP interact with water molecules. In this study, we have investigated the interaction of a monolayer of the INP with water molecules at the air-water interface using static and time resolved sum frequency generation spectroscopy. When cooling the monolayer of the INP with water molecules from room temperature to near-freezing temperature, an increase in the structural order of interfacial water molecules was observed. This effect was not observed for water surface or for proteins lacking ice nucleating activity. By using femtosecond pump probe SFG spectroscopy, we found a decrease of the lifetime of the O-D stretch vibrations as a function of temperature. This may be explained by strongly bound O-D groups, which play a decisive role in the ice nucleating activity. The specific binding at lower temperatures could be due to side chain orientations, which emulate the lattice of ice and hence promote the ice formation.

BP 28.9 Wed 17:30 HÜL 386

**Characterizing Protein Adsorption by *In situ* Atomic Force Microscopy at Single Protein Resolution** — ●CHRISTIAN KREIS, JONAS HEPPE, CHRISTIAN SPENGLER, HENDRIK HÄHL, and KARIN JACOBS — Department of Experimental Physics, Saarland University, Saarbrücken, 66041, Germany

The adsorption of proteins to surfaces is governed by the mutual interactions of proteins, solution and substrate. To fully characterize the interactions, we have shown before that also the long range van der Waals forces arising from the subsurface of the substrate have to be taken into account [1,2]. However, the uppermost layer defines the surface chemistry and is dominating the strength of the interfacial energy. Studies using e. g. ellipsometry or X-ray reflectometry observe a strong influence of the surface chemistry on protein adsorption. These studies, however, average over hundred thousands of proteins in the measurement and the spatial arrangements of the proteins remain unknown. To resolve the latter, we applied *in situ* AFM measurements in buffer solution and characterized the different protein distributions on hydrophilic and hydrophobic silicon wafers. Additionally, a strong denaturation of the studied proteins can be observed. These results demonstrate the influence of the surface chemistry on protein adsorption and help to elucidate the differences in adsorption kinetics or the final adsorbed layer.

[1] Y. Schmitt et al. *Biomicrofluidics* 4 (2010) 032201[2] H. Hähl et al. *Langmuir* 28 (2012) 7747–7756

BP 28.10 Wed 17:45 HÜL 386

**Adsorptive Capability and Conformational Efficiency between Lysozyme and Nanosilica/-diamond at pH=7-13** — ●VICTOR WEI-KEH CHAO — Department of Chemical and Materials Engineering, National Kaohsiung University of Applied Sciences, 80782 Kaohsiung, Taiwan. — Victor Basic Research Laboratory, e.V. Gadderbaumer-Str. 22, D-33602 Bielefeld, Germany.

Adsorption dynamics of lysozyme and nanosilica(NS)/-diamond(ND) with diameter 100 nm and 0.25  $\mu\text{g}/\mu\text{L}$ , lysozyme in 0-1000 nM of 7 mM PPBS at pH=7, 9, 11, and 13 have been investigated by Fluorescence spectroscopy. Chem. instead of phys. adsorption, as well as modification of nanosurface may be necessary for the further investigation of nanocarrying of protein or drug through pH-gradients *in vivo*. Surface of ND was acidified and with approx. 7 % of all covered with COOH groups. Because the acidified surface was only a small part, profile or roughness of the nanosurface is still the decisive factor for the comparison of adsorption strength between NS and ND (adsorption reaction constant NS/ND $\approx$ 1/4 at four pH values). The highest adsorption capabilities and conformational efficiencies at pH=13 have been obtained. Lysozyme can be prepared, adsorbed and carried with optimal activity and helicity, with 10 and 2 mg/m<sup>2</sup> on nanosurface, 150 and 130 mg/g in g of nanoparticle, within the linear coverages at 150-250 nM and four pH values for NS and ND, respectively. They can be prepared in the tightest packed form, with 55 and 20 mg/m<sup>2</sup>, 580-1100 and 810-1680 mg/g at adsorption thresholds and four pH for NS and ND, respectively. **Ref.** *Chin. J. Chem. Phys.* 26, 295(2013).

BP 28.11 Wed 18:00 HÜL 386

**Biomolecules at metal interfaces: a novel force field approach including polarization** — ●SIDRO LORENZO<sup>1</sup>, HADI RAMEZANI-DAKHEL<sup>2</sup>, HENDRIK HEINZ<sup>2</sup>, and MARIALORE SULPIZI<sup>1</sup> — <sup>1</sup>Johannes Gutenberg University Mainz, Staudinger Weg 7 55099 Mainz — <sup>2</sup>Department of Polymer Engineering, University of Akron, Ohio 44325

Increasing interest in bio-interfaces for medical and bio-technological applications calls for microscopic understanding and control of protein-surface interactions. In particular here we aim to provide a characterization of peptide / gold interactions at a molecular level in order to explain and interpret recent surface experimental results [1] and to fill the gap between fundamental science and real applications. Atomistic simulations have been performed with the GROMACS package using available force field parameters such as CHARMM27 using 12-6 Lennard-Jones potentials [2] force field. A novel scheme is devised to include the metal polarization (image charge effect) induced by the adsorbed molecules. Extensive tests have been performed for the force field validation and comparisons with quantum mechanics (QM) density functional theory (DFT) are also discussed. Results for the di- and tri-peptide of the insulin-like growth factor on gold are presented.

[1] Anne Vallee, Vincent Humblot, and Claire-Marie Pradier *Acc. Chem. Res.*, 2010, 43 (10), pp 1297\*1306[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

BP 28.12 Wed 18:15 HÜL 386

**Fibrinogen flexibility and adsorption properties investigated using atomistic molecular dynamics simulations** — STEPHAN KÖHLER<sup>1,2</sup>, FRIEDERIKE SCHMID<sup>1</sup>, and ●GIOVANNI SETTANNI<sup>1,3</sup> — <sup>1</sup>Institut für Physik, Johannes Gutenberg-Universität, Mainz, Germany — <sup>2</sup>Graduate School Materials Science in Mainz — <sup>3</sup>Max Planck Graduate Center mit der Johannes Gutenberg-Universität Mainz

Fibrinogen is a multiprotein complex, fundamental for the coagulation of blood. Adsorption of fibrinogen on material surfaces plays an important role in the viability of those materials for medical implants. Here we use molecular dynamics simulations of fibrinogen in solution and adsorbing on inorganic surfaces to evaluate the behavior of fibrinogen on material surfaces and study the initial adsorption stages. The simulations reveal the extraordinary flexibility of fibrinogen and help to explain how fibrinogen's surface electrostatics influence the adsorption patterns observed experimentally on different inorganic surfaces. This, in turn, may have implications for medical applications such as material design for implants. In addition, the simulation data can ultimately be used to build coarse grained models of fibrinogen to study

its aggregation properties[1].

[1] A multiscale model for fibrinogen, S. Köhler, M. McCullagh, F.

Schmid, G. Settanni, DPG meeting '14 abstract BP56