Topical Talk

BP 28.1 Wed 15:00 HÜL 386

Single Molecule Mechanics of Proteins — **MATTHIAS REEF** — 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**, **TOM HOFFMANN**, **DAVID BROCKWELL**, and **LORNA DOUGAN** — 1Molecular and Nanoscale Physics Group, School of Physics and Astronomy, University of Leeds, LS2 9JT, UK — 2Astbury 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 advanttages 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 force 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].


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 mass spectrometry (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 VOLGEL** — Institut für Festkörperfysik, 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 internal whether these steps result from to a true dynamical onset or from local (β-) [2] or structural (α-) [3] relaxations entering the time window. A promising approach to clarify these issues is to combine NS 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 an unusual 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.


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**, **MEIKE LUCK**, **TILO MATHEIS**, and **PETER HEGERMANN** — 1Fakultät für Physik, Universität Regensburg, Universitätsstraße 31, D-93053 Regensburg, Germany — 2Institut für Biologie/Experimentelle Biophysik, Humboldt Universität zu Berlin, Invalidenstraße 42, D-10115 Berlin, Germany — 3Department 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±0.005 and 0.405±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 approtein ground-state equilibration on a millisecond timescale.

Genetically Encoded Spin Labelled Artificial Amino Acids —

MALTE DRESCHER, MORITZ SCHMIDT, and DANIEL SUMMERMER — Konstanz Research School Chemical Biology and Department of Chemistry, University of Konstanz, Germany

Recent publications demonstrate the ability of electron paramagnetic resonance spectroscopy (EPR) for 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 modifications. However, so far, these approaches require microinjection of spin-labelled macromolecules.

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

Structure and dynamics of interfacial water associated with Fröhlich energy. Studies using e. g. ellipsometry or X-ray reflectometry observe a surface chemistry and is dominating the strength of the interfacial energy. 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. Atomicistic 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.

its aggregation properties[1].

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