BP 12: Single Molecules

Time: Wednesday 9:30–13:15 Location: HÜL 186

Invited Talk BP 12.1 Wed 9:30 HÜL 186

Conformational Mechanics of Single Protein Molecules — •Matthias Rief — Physikdepartment der TU München, Lehrstuhl für Biophysik E22, 85748 Garching

The development of novel ultrasensitive force probes with high spatial resolution, like AFM and optical tweezers, has allowed us to use mechanical force as a control parameter for bio-molecular conformation. Such single molecule experiments offer new possibilities for understanding the self-organization as well as the mechanical function of bio-molecules. In the talk I will discuss how mechanical forces can be used to explore the complex energy landscape of proteins. Examples will include equilibrium and non-equilibrium folding/unfolding of proteins, as well as force-induced conformational changes of protein-protein complexes.

Invited Talk BP 12.2 Wed 10:00 HÜL 186

Illuminating the way Kinesin-1 walks using FRET between the motor domains — •Erwin Peterman — VU University, Amsterdam, the Netherlands

Kinesin-1 is a motor protein that walks processively along microtubules in a hand-over-hand manner driving intracellular transport of vesicles and organelles. Each step of 8 nm requires the hydrolysis of one ATP and takes about 10 ms at cellular ATP concentrations. Key aspects of kinesin*s walking mechanism are not fully understood. One important question concerns the configuration of the two motor domains during processive motion.

Here, we use a novel assay based on single-molecule confocal fluorescence microscopy to characterize Kinesin-1*s stepping mechanism in vitro. A key advantage of our approach over conventional wide-field methods is that our time resolution is far better, less than 0.1 ms. We apply this approach to kinesin constructs that are labeled with a donor fluorophore on the one motor domain and an acceptor on the other. We follow the distance between the motor domains during stepping with Förster Resonance Energy Transfer. We use four different homodimeric kinesin constructs with dye molecules attached to different sites of the motor domain. With this approach, we can identify an intermediate state in the stepping process that lasts 2-3 ms at saturating ATP concentration. In this intermediate state one motor domain is bound to the microtubule and the other is rotated and substantially less than 8 nm away.

BP 12.3 Wed 10:30 HÜL 186

Single-molecule measurement of protein friction between kinesin and the microtubule surface and its relation to lattice diffusion — Volker Bormuth[1], Vladimir Varga[1], Jonathon Howard[1], and •Erik Schäffer[2] — [1]MPI of Molecular Cell Biology and Genetics, Pfotenhauerstraße 108, 01307 Dresden, Germany — [2]Biotechnology Center, TU Dresden, Tatzberg 47-51, 01307 Dresden, Germany

Friction within an engine or between a vehicle and its track plays a crucial role in the operation of macroscopic machines. Biological machines such as muscle are also subject to frictional forces. The concept of protein friction has been used in theoretical studies, but experimental studies are scarce. We have developed techniques based on optical tweezers to measure the friction between individual kinesin-8 molecules and microtubules in the presence of ADP. At low speeds we find a friction coefficient of 700 ± 300 nNs/m, which is in good agreement with the diffusion coefficient measured under identical conditions. This confirms the fundamental connection between friction and diffusion. We measured a non-linear dependence of friction on velocity, allowing us to estimate the distance between diffusional hopping steps of 8.0 ± 0.6 nm. This step size was confirmed by direct resolution of step-wise motions as well as a fluctuation analysis; thus kinesin-8 steps between adjacent tubulin dimers. Our experiments therefore confirm the presence of protein friction—an important parameter for active protein locomotion limiting the efficiency.

BP 12.4 Wed 10:45 HÜL 186

Reversible Affinity Switching of a Single Supramolecular Receptor Molecule — •Volker Walhorn[1], Christian Schäfer[2], Tobias Schröder[3], Jochen Mattay[3], and Dario Anselmetti[1] — [1]Experimental Biophysics and Applied Nanosciences, Bielefeld University — [2]Organic Nanosciences University of Bordeaux 1 — [3]Organic Chemisty Bielefeld University

Photoactivation of single molecules is a common concept in nature. In order to investigate such mechanisms we synthesized a bistable supramolecular complex consisting of a Resorc[4]arene receptor cavity modified with two anthracene moieties. These can be switched between two isoforms either by UV-light or heat [1]. Using atomic force microscope based single molecule force spectroscopy (AFM-SMFS) we investigated the conformation dependent receptor affinity to different ammonium derivates. Our results show that this system can be reversely and repeatedly switched between two different isomeric conformations accompanied by a drastic change of affinity to ammonium ligands. For the "open"high affinity state we could also demonstrate the specificity by competition experiments and estimate associated binding properties like reaction lengths (x_β) and thermal off-rate constants (k_{off}). Robust bistable molecular systems are potential candidates for novel concepts in bio-medical, analytics, directed molecular assembly or controlled drug delivery.

[1] C. Schäfer et. al. J. Am. Chem. Soc. 2007, 129, 1488-1489

BP 12.5 Wed 11:00 HÜL 186

Dual-focus flow detection: Exposing biological heterogeneity one molecule at a time — •Tyler Arbour, Anastasia Loman, and Jörg Enderlein — III. Institut für Physik, Georg-August-Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen

The ability to distinguish between multiple fluorescent species or states in solution at the single-molecule level is an attractive concept. To realize this level of analysis in confocal detection, we must eliminate the uncertainty introduced by random diffusion of the molecule. In other words, we must know the path a molecule takes through the confocal detection volume. In a conventional detection scheme, this translates into directing the fluorescent species through the confocal volume's center using, for example, a microinjection sample capillary surrounded by a continuous sheath fluid flow. This has been successfully demonstrated in the past, first by Richard Keller et al in the sizing of individual dye-labeled DNA fragments.[1] However, such a setup is difficult to build, and the nanometer-scale components are very prone to clogging as well as unwanted fluorophore-capillary interactions; as a result, this idea has been largely abandoned. Here we present a much simpler setup that takes advantage of dual-focus detection in a net fluid flow to achieve precise knowledge of a molecule's path.

1. Goodwin, P.M., et al., Nucleic Acids Research, 1993. 21(4): p. 803-806

BP 12.6 Wed 11:15 HÜL 186

Time resolved three-dimensional orientation of eGFP — •Richard Börner — Universität zu Lübeck, Institut für Physik, Ratzeburger Allee 160, 23568 Lübeck, Germany

Confocal microscopy is a powerful method for single molecule investigation of fluorescent macromolecules. Beside the translatory movement of labeled or autofluorescent molecules rotational dynamics reflect the properties of the macromolecule and its surrounding. In principal the determination of the molecular orientation bases on the defined orientation of the absorption/emission dipole with respect to the molecular frame. Using a method which has been recently proposed by Hohlbein & Hübner [1,2] we demonstrate the time resolved three-dimensional orientation determination for the well known and biological relevant molecule eGFP. By using adapted FCS and TCSPC measurments we observe the orientation on a millisecond down to a nanosecond time scale.

[1] J. Hohlbein & C. G. Hübner, APL, 86, 121104 (2005)
[2] J. Hohlbein & C. G. Hübner, JCP, 129, 094703 (2008)

15 min. break

BP 12.7 Wed 11:45 HÜL 186

Determining the hydrodynamic size and shape of biomolecules by probing single-molecule Brownian motion — •Sandeep Pallikkuth and Andreas Volkmer — 3rd Institute of Physics, University of Stuttgart, Germany

Information regarding the hydrodynamic volume of a fluorescent biomolecule is obtained by monitoring its Brownian motion in solu-

tion. While the translational diffusion of a fluorescent biomolecule, occurring on the micro- to millisecond time scale, is conveniently obtained from a conventional fluorescence correlation spectroscopy experiment, the more size-sensitive Brownian rotational dynamics of the molecule, occurring on the pico- and nanosecond time scale, is generally obtained from the measurement of its time-resolved fluorescence anisotropy upon pulsed excitation. The application of the latter technique, however, is limited by its fluorescence lifetime, preventing the accurate measurement of rotational diffusion time when in the order of tens of nanoseconds. Based on recent experimental advances allowing the calculation of second-order correlation function from distinct photon arrival times with picosecond time resolution and applying an exact theoretical model, we demonstrate probing of Brownian rotational diffusion of a biomolecule in free solution at time scales between a picosecond and hundreds of nanoseconds without the need for pulsed excitation. Moreover, the simultaneous measurement of both the translational and rotational diffusion of a biological macromolecule with this technique allows the determination of the hydrodynamic size and shape of the biomolecule.

BP 12.8 Wed 12:00 HÜL 186

Stretching and unfolding titin: Metastability and survival of the fittest — •Douglas B. Staple[1,2], Stephen H. Payne[1], Andrew L. C. Reddin[1], and Hans Jürgen Kreuzer[1] — [1]Dalhousie University, Halifax, Canada — [2]Max-Planck-Institut für Physik komplexer Systeme, Dresden, Germany

Single-molecule manipulation has allowed the forced unfolding of multidomain proteins. Here we outline a theory that not only explains these experiments but also points out a number of difficulties in their interpretation and makes suggestions for further experiments. For titin we reproduce force-extension curves, the dependence of break-force on pulling speed, and break-force distributions and also validate two common experimental views: unfolding titin Ig domains can be explained as stepwise increases in contour length, and increasing force peaks in native Ig sequences represent a hierarchy of bond strengths. Our theory is valid for essentially any molecule that can be unfolded in atomic force microscopy; as a further example, we present force-extension curves for the unfolding of RNA hairpins.

BP 12.9 Wed 12:15 HÜL 186

Dual-Focus Correlation Spectroscopy: Advantages and applications — •Anastasia Loman and Jörg Enderlein — III. Institut für Physik, Georg-August-Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen

Fluorescence correlation spectroscopy (FCS) is a powerful technique for measuring diffusion coefficients of fluorescent molecules at pico- to nanomolar concentrations. A modified version of FCS, dual-focus FCS (2fFCS) shows significantly improvement in the reliability and accuracy of FCS measurements and allows for obtaining not relative but absolute values of diffusion coefficients [1].

The high precision of 2fFCS (absolute accuracy is shown to be better than 5 %) and the simple Stokes-Einstein relation directly coupled hydrodynamic radius and diffusion coefficient allow to monitor interactions of biomolecules - in particular proteins, RNA, DNA - with their environment or reacting to changes in environmental parameters such as pH, temperature, or chemical composition (e.g. protein unfolding) or performing biologically important functions (e.g. enzymatic catalysis).

We demonstrate that this method is sensitive enough to resolve length changes in small peptides of only one amino acis, and size changes of hydrodynamic radii as small as 0.5 nanometers. We used 2fFSC to study conformational changes of proteins such as phosphoglycerate kinase (PGK), α-amylase, and MHC class I complex under different conditions.

1. Dertinger, T. at al., ChemPhysChem 8 (2007) 433.

BP 12.10 Wed 12:30 HÜL 186

Calcium mediated carbohydrate-carbohydrate interactions investigated with single molecule force spectroscopy — •André Körnig[1,2], Iwona Bucior[3], Max M Burger[4], Xavier Fernandèz-Busquets[2], and Dario Anselmetti[1] — [1]Experimental Biophysics, Bielefeld University — [2]Nanobioengineering Group, University of Barcelona — [3]University of California, San Francisco — [4]Friedrich-Miescher-Institute, Basel

Marine sponges associate through species-specific, calcium-mediated carbohydrate-carbohydrate interactions of the g200 glycan found on sponge proteoglycans. A detailed and quantitative single molecule force spectroscopy analysis of g200-g200 binding in Microciona prolifera reveals adhesion forces in the range of 100-250 pN. However, our results indicate that calcium-dependent, macroscopic sponge cell self-association is only little influenced by the absolute single molecule binding forces but critically rely on the kinetic reaction properties that manifest themselves in Ca^{2+}-mediated bond lifetimes (10 mM Ca^{2+}/ 0 mM Ca^{2+}: 680 s / 3 s) and bond reaction lengths (10 mM Ca^{2+}/ 0 mM Ca^{2+}: 3.47 Å / 2.27 Å). Since cellular association in sponges is a polyvalent process, the observed binding phenomenon has to be analysed with a cooperative adhesion cluster model that distinctively supports the macroscopic observations of mean dissociation lifetimes for sponge multicell integrity in low and high calcium. A potential relation to a more generalized picture of the mid-cambrian explosion of metazoan evolution will be discussed.

BP 12.11 Wed 12:45 HÜL 186

Microfluidic device for polarizability-quantification and fast DNA-separation on single molecule scales — •Lukas Bogunovic[1], Jan Regtmeier[1], Ralf Eichhorn[2], Alexandra Ros[3], and Dario Anselmetti[1] — [1]Experimental Biophysics & Applied Nanoscience, Bielefeld University, Germany — [2]Condensed Matter Theory, Bielefeld University, Germany — [3]Department of Chemistry and Biochemistry, Arizona State University, Tempe, USA

We present a simple and easy to fabricate poly(dimethylsiloxane) (PDMS) microfluidic device, capable of quantifying DNA polarizabilities on a single molecule level. The same system is able to separate long DNA molecules as well as biotechnologically relevant supercoiled plasmid DNA via electrodeless dielectrophoresis in less than 240 seconds.

The device consists of a cross injector and an analysis channel. The latter is structured with a series of conducting posts creating dielectrophoretic (DEP) traps, when a voltage is applied. The quantificaton as well as the separation is consequently based on the size dependent thermally induced esacpes from those DEP traps.

The observed polarizabilities of linear dsDNA demonstrate a length dependence, which has been discussed controversially. This dependence can be described with a simple power law and an exponent for linear DNA from 6 kbp to 164 kbp close to the Flory exponent. In separation mode, separation times of 200s for λ (48.5 kbp)- and T2 (164 kbp)- DNA were achieved with baseline resolution.

BP 12.12 Wed 13:00 HÜL 186

Transport through OmpF channels simulated using molecular dynamics — •Soroosh Pezeshki, Mathias Winterhalter, and Ulrich Kleinekathöfer — Jacobs University Bremen, Campus Ring 1, 28759 Bremen, Germany

The outer membrane porin OmpF is one of the major porins in E. coli and is the main entrance for small molecules like beta-lactam antibiotics. The narrowest part of the pore, the constriction zone, includes charged residues which have a high but still not well understood influence on the ion permeation. Here different mutations of OmpF are used to study the influence of charged residues on the conductance [1]. The selectivity of the pore can be changed over wide ranges by mutations. In a second step the translocation of antibiotics molecules through the pore is simulated. To speed up the translocation steered molecular dynamics (SMD) is employed. Different pulling strategies are used to get a better understanding of the interaction of pore and antibiotics. The SMD simulations are compared to so-called meta-dynamics simulations for calculating the potential of mean force (PMF) and at the same time used to analyze in vitro experiments [2].

[1] C. Chimerel and L. Movileanu and S. Pezeshki and M. Winterhalter and U. Kleinekathöfer, 2008, Eur. Biophys. J., **38**, 121-125
[2] C. Danelon and E. M. Nestorovich and M. Winterhalter and M. Ceccarelli and S. M. Bezrukov, 2006, Biophys. J., **90**,1617-1617