BP 11: Posters: Single-Molecule Biophysics

Time: Monday 17:15–20:00 Location: P3

BP 11.1 Mon 17:15 P3

High Resolution Optical Tweezers for Single-Molecule Studies of Eukaryotic Transcription — •Korbinian Paul, Adam Muschielok, Noemi Maria Porcellato, and Jens Michaelis — Department für Chemie LMU München, Butenandstr. 5-13

Investigating mechanical aspects of single RNA polymerases will further our understanding of the molecular mechanism of transcription elongation. For these single-molecule experiments we establish a high resolution optical tweezers setup in the dual trap design, where one trap is moveable by a piezo-driven mirror. Experiments with 5 kbp DNA tethers attached to trapped beads have shown that our current resolution is about 0.5 nm for forces measured around 15 pN at a time resolution of approximately 0.1s. This is sufficient to study single base pair steps of RNA Polymerase II on DNA. In further experiments we will investigate the behavior of RNA Polymerases I and II from a mechanical perspective. In addition, we will study transcription regulation by performing experiments in presence of different transcription factors such as TFIIS and TFIIF.

BP 11.2 Mon 17:15 P3

Dynamics of a Single DNA-bound Protein Translocating through a Nanopore — •Andre Spiering, Andy Sischka, Katja Toensing, Karsten Rott, Sebastian Getfert, Peter Reimann, and Dario Anselmetti — Faculty of Physics, Bielefeld University, 33615 Bielefeld, Germany

In order to investigate the physical behaviour of DNA-bound ligands translocating through a nanopore, we threaded single DNA-protein complexes into a solid-state nanopore while simultaneously measuring the electrostatic forces and ionic currents through the pore. This controlled translocation was examined with pN force sensitivity, ms time resolution and pA ionic current sensitivity by a high precision 3D optical tweezers setup with backscattered light detection. We found that each ligand (RecA, EcoRI or 2-Cysteine-Peroxiredoxin-A) causes a reproducible and individual change of both the electrostatic force and the ionic current while dynamically threading and unthreading the complex [1]. Detailed studies of these charge-dependent translocation processes revealed a hopping between two states in the nanopore potential and a small hysteresis between threading and unthreading cycles. All experimental force response curves and the corresponding effects can be theoretically modelled and verified within a framework of thermally activated transitions in a time-dependent nanopore potential (Kramers theory) and reflect the stochastic nature of such nanopore translocation events [2].

[1] A. Sischka et al.: J. Phys.: Condens. Matter 22, 454121 (2010)
[2] A. Spiering et al.: submitted (2010)

BP 11.3 Mon 17:15 P3

Nano-Mechanics of Homologous Recombination — •Marcel Ander and Erik Schäffer — Biotec TU Dresden, Tatzberg 47-51, Dresden

Homologous recombination is the key biological process for exchanging DNA segments between two DNA molecules. It serves to repair DNA double strand breaks, re-launch stalled replication forks, and maintains genetic diversity by mediating horizontal gene transfer mechanisms such as conjugation and meiotic recombination. In all of these processes, a segment of DNA is stably integrated into the recipient DNA. Utilizing optical tweezers we analyzed the DNA single-strand annealing mechanism of homologous recombination studying the phage lambda protein Redβ. Redβ is the key actor in a technique termed recombineering ensuring efficiency of the recombination process. We discovered that Redβ can actually block annealing of complementary DNA strands, and is active towards the 3' end of a single-stranded DNA. If sufficient complementarity is given, Redβ holds complementary DNA strands together. This sheds light onto the mechanism of DNA single-strand annealing and highlights force as a crucial item in molecular genetics.

BP 11.4 Mon 17:15 P3

Analysis of multivalent effects using single molecule force spectroscopy (SMFS) on pyridine coordination compounds — •Manuel Gensler[1], Artur Galstyan[2], Ernst-Walter Knapp[2], and Jürgen P. Rabe[1] — [1]Institut für Physik, Humboldt-Universität zu Berlin, Newtonstr. 15, 12489 Berlin — [2]Institut für Chemie und Biochemie, Freie Universität Berlin, Fabeckstr. 36a, 14195 Berlin

Multivalent interactions are of great importance in chemistry, nanotechnology and biochemistry. They strongly increase binding free energies and association kinetics between partners of appropriate geometry [1]. Thus it is important to obtain a deeper understanding of the basic factors influencing multivalent interactions.

We used SFM based SMFS [2] to measure interaction forces between mono- and multivalent coordination compounds of pyridine nanorods with different metal salts such as $Zn(NO_3)_2$ and $CuSO_4$in aqueous solutions. Force-distance measurements were performed over a broad range of loading rates to estimate associated binding properties according to the Bell-Evans model [3]. In combination with computational calculations of the bond dissociation under force we propose different rupture mechanisms of the divalent complexes with Copper and Zinc. Our model system can be extended to various geometries and therefore provides essential knowledge about geometrical factors influencing multivalency.

[1] M. Mammen et al. *Angew. Chem. Int. Ed.* **1998**, 37, 20, 2754-2794. [2] M.I. Gianotti et al. *ChemPhysChem* **2007**, 8, 2290-2307. [3] S. Guo et al. *Biophys. J.* **2008**, 95, 3964-3976.

BP 11.5 Mon 17:15 P3

High-Resolution Scanning Near-Field Optical Microscopy of Dye Labelled Single Tobacco Mosaic Viruses — •Alexander Harder[1], Sven Degenhard[2], Fabian Eber[2], Fania Geiger[3], Joachim Spatz[2], Holger Jeske[2], Christina Wege[2], and Dario Anselmetti[1] — [1]Experimental Biophysics & Applied Nanoscience, Bielefeld University, Germany — [2]Molecular Biology and Virology of Plant, Stuttgart University, Germany — [3]Max Planck Institute for Metals Research, Stuttgart, Germany

Scanning near-field optical microscopy (SNOM) is a fluorescence microscopy technique achieving optical resolution of better than 20 nm by means of strongly confined non-propagating electromagnetic fields. We investigated dye-labelled single Tobacco mosaic viruses (TMV) with apertureless SNOM by using standard Si-AFM tips illuminating their apex with a focused laser beam. Our home-built SNOM device system additionally allows simultaneous atomic force microscopy (AFM) tapping topographic measurements [1]. In the future concurrent high structural and optical resolution will allow the investigation of virus orientation as well as site-specific immobilization that is prerequisite for possible bioengineering applications of TMV based channels.

BP 11.6 Mon 17:15 P3

Friction dynamics of peptides at polar and non-polar surfaces — •Aykut Erbas, Dominik Horinek, and Roland R. Netz — Technische Universitaet Muenchen, Physik Department, Garcing, Germany

The friction forces and mobilities for the C_{16} spider silk and various peptides on polar and non-polar surfaces are investigated using molecular dynamics simulations. For both surfaces, the velocity dependence of the monomer mobility is determined and interpreted with non-linear analytical models. The obtained diffusion coefficients are in good agreement with experiments. It is concluded that the reason for the high friction forces on polar surfaces is hydrogen bonding. It is further shown that each hydrogen bond contributes equally to the total friction force, independent of the concentration of surface-polar groups or the type of amino acid.

BP 11.7 Mon 17:15 P3

Single-Molecule Force Spectroscopy Binding Studies of DNA Recognition by Transcription Factor Epitopes — •Adeline Bieker[1], Volker Walhorn[1], Gesa Niemann[2], Markus Ritzefeld[2], Norbert Sewald[2], and Dario Anselmetti[1] — [1]Experimental Biophysics and Applied Nanoscience, Universität Bielefeld, Deutschland — [2]Organic and Bioorganic Chemistry, Universität Bielefeld, Deutschland

Interactions between proteins and DNA are essential for the regulation of cellular processes in all living organisms. In this context, it is of special interest to investigate and quantify the sequence-specific molecular recognition between peptidic transcription factors and their cognate DNA sequences [1].

We investigated protein epitopes and peptides originating from the DNA-binding domain (DBD) of the *Escherichia coli* transcription factor PhoB. By means of AFM-based Single Molecule Force Spectroscopy (SMFS) we investigated the specific binding forces and molecular elasticities to elucidate the DNA-protein complex stability. Based on the Bell-Evans-Model [2] we estimated the thermal dissociation rate constants (k_{off}) an the molecular interaction length (x_β), that allowed a structure related interpretation of the physical binding mechanisms involved.

BP 11.8 Mon 17:15 P3

Permeation through nanochannels: Revealing fast kinetics — Kozhinjampara R. Mahendran, Pratik Raj Singh, Ulrich Kleinekathöfer, and •Mathias Winterhalter — Jacobs University Bremen, Campusring 1, D-28759 Bremen

The permeation of water soluble molecules across cell membranes is controlled by channel forming proteins and particularly the channel surface determines the selectivity. An adequate method to study properties of these channels is electrophysiology and in particular analysing the ion current fluctuation in the presence of permeating solutes provides information on possible interactions with the channel surface. Due to the limited time resolution, fast permeation events are not visible. Here we demonstrate that miniaturization of the lipid bilayer, varying the temperature or changing the solvent may enhance the resolution. Although electrophysiology is considered as a single molecule technique, it does not provide atomic resolution. Molecular details of solute permeation can be revealed by combining experiments and computer modelling.

K.R. Mahendran et al. J Phys. Condensed Matter 22 (2010) 454131; E. Hajjar et al. Biochemistry 49 (2010) 6928-35; I. Biro et al. Biophys J 98 (2010) 1830-9.

BP 11.9 Mon 17:15 P3

Stochastic reconstruction of interactions within protein complexes from single-molecule force spectroscopy — •Magnus Schwörer and Ulrich Gerland — Arnold Sommerfeld Center for Theoretical Physics, LMU München, Munich, Germany

Dynamic Force Spectroscopy is a well-established technique where a pulling force is applied with a certain rate of loading to probe the (un)folding of biomolecules or the interaction between two biomolecules. The technique typically permits to extract information such as the barrier height and distance to the transition state, and ideally even the entire free energy landscape along the reaction coordinate of this process. Here, we explore theoretically which information could be obtained when this technique is applied to macromolecular complexes. Specifically, we consider the sequential application of dynamic force spectroscopy to all pairs of constituents within such a complex, and test to which extent the interactions between the constituents can be reconstructed. Our analysis is based on a simple toy model.

BP 11.10 Mon 17:15 P3

Stochastic enzymatic reactions with spatially arranged enzymes — •Fabienna Arends, Alexander Buchner, and Ulrich Gerland — Arnold Sommerfeld Center for Theoretical Physics (ASC) and Center for NanoScience (CeNS), Department of Physics, Ludwig-Maximilians-Universität München

To efficiently catalyze multi-step biochemical reaction pathways, cells have optimized the synergistic action of a multitude of enzymes. Not only do they carefully control the concentrations and activities of enzymes as a function of the external conditions, but in many known cases cells also coordinate the enzymes that catalyze different steps in the same biochemical reaction pathway by arranging them in self-assembled multi-enzyme complexes. In these complexes, single enzymes of several types are localized on well-defined spots. So far, the theoretical study of these systems has focused on the deterministic level. Here, we investigate the behaviour of spatially arranged enzymes in different configurations including stochastic effects.

BP 11.11 Mon 17:15 P3

Coronavirus nsp7-nsp8 complex formation investigated by single-molecule methods — •Henning Seidel[1], Yibei Xiao[2], Rolf Hilgenfeld[2], and Christian G. Hübner[1] — [1]Institute of Physics, Ratzeburger Allee 160, 23562 Lübeck, Germany — [2]Institute of Biochemistry, Ratzeburger Allee 160, 23562 Lübeck, Germany

The self-organized structure building capabilities of proteins are fascinating biophysicists since decades. With the advent of single-molecule methods, namely fluorescence correlation spectroscopy (FCS) and fluorescence resonance energy transfer (FRET), the process of complex formation is becoming accessible to direct observation.

Coronaviruses are enveloped positive-stranded RNA viruses. For SARS-CoV, it was shown that coronaviruses encode a RNA-dependent RNA-polymerase (RdRp) build from non-structural protein 7 (nsp7) and non-structural protein 8 (nsp8). This hexadecameric nsp7-nsp8 complex is a hollow, cylinder-like structure assembled from eight copies of nsp8 and held together by eight nsp7 molecules. We are aiming at understanding the assembly process and conformational changes of the complex for the related Feline Coronavirus. The structural and functional examination of the nsp7-nsp8 complex formation should help in understanding the replication and transcription mechanisms of Fe-CoV and other coronaviruses like SARS-CoV.

BP 11.12 Mon 17:15 P3

Hydrodynamic interaction destabilizes soft bonds. — Suman Das[1], Dimitri Pescia[1], Mithun Biswas[1], and •Anirban Sain[1,2] — [1]Physics Department, IIT Bombay. Powai, Mumbai 400076, India. — [2]MPI-PKS, Nothnitzer Str. 38, 01187, Dresden.

Weak bonds are ubiquitous in biological structures. They often act as adhesive contacts within an extended structure, for example, the internal bonds in a folded protein or a DNA/RNA loop. They also act as linkers between two structures, for example, a protein grafted in a cell membrane or a protein linking the cell membranes of two neighboring cells. Typically, the breakage of a bond depends on the strength of the binding potential and viscosity of the medium. But when extended structures couple to the bond, as in the above examples, the dynamics of the structure also has to be considered in order to understand the bond breakage phenomenon. Here we consider a generic model, a stretched polymer an extended structure tethered to a soft bond and study how the dynamics of the polymer, in addition to thermal noise, influences bond breakage. We also explore how the hydrodynamic interaction due to the fluid, which couples the distant parts of the polymer, change the bond breakage rate. We find that breakage rate is enhanced and also the motion becomes more coherent.

BP 11.13 Mon 17:15 P3

A theoretical description of the 3D orientation determination of dipoles near interfaces — •Richard Börner and Christian G. Hübner — Insitute of Physics, University of Lübeck, Ratzeburger Allee 160, 23562 Lübeck, Germany

One of the unique features of single molecule absorption/emission is their anisotropy due to the well-defined transition dipoles for both processes allowing the determination of the molecules 3d orientation. Therefore, several techniques have been proposed in order to determine the full three-dimensional orientation of dipole emitters on a single molecule level. One of these techniques proposed by Hohlbein and Hübner in 2005 combines emission distribution and polarization detection. The theoretical background of this approach assumes an optical homogenous surrounding. In order to overcome this limitation we want to indicate an extension of the theory for dipole emitters near optical interfaces e.g. glass/water. Moreover, we implement the dipole excitation probability. In conclusion, we present extensive simulations, which allow for the evaluation of the capabilities of this extended method.

BP 11.14 Mon 17:15 P3

The strong excitonic coupling is not an important factor in the fast excitation energy transfer in phycocyanin of A.marina. — •albert collins nganou assonkeng — Institute of Optics and Max-Volmer-Laboratory for Biophysical Chemistry, Technical University Berlin, Str. 17 Juni 135, 10623 Berlin, Germany

The Cyanobacterium Acaryochloris Marina (A.marina) is unique in nature because it contains Chl d instead of Chl a as major pigment. In addition to the Chl containing light harvesting antennas A.marina has also a Phycobiliprotein (PBP) antenna as a light harvesting complex that shows a more simple structure than phycobilisomes of other typical cyanobacteria. This PBP-antenna is a rod shaped complex consisting of three homo-hexamers containing Phycocyanin (PC) and one hetero-hexamer containing PC and Allophycocyanin (APC) absorbing in the spectral range between 560 nm and 630 nm, where the absorption of the chlorophylls is low. In order to get a better insight in the fundamental processes of excitation energy transfer (EET) in this antenna system we performed time resolved absorption studies as well as measurements of the transient anisotropy. The maximum anisotropy value of 0,37 that is close to theoretical limit for weak interaction of 0,4 indicates that strong excitonic coupling is not an important factor in the fast EET in PC of A.marina.