BP 15: Single Molecules

Time: Wednesday 14:00–17:15 Location: C 243

BP 15.1 Wed 14:00 C 243

Single molecule detection of Myosin V in living cell — •Paolo Pierobon[1], Giovanni Cappello[1], Sarra Achouri[1], Sebastien Courty[2], Maxime Dahan[2], Alex Dunn[3], and James Spudich[3] — [1]Institut Curie, Physico-Chimie-Curie, 11 rue P. et M. Curie, 75005 Paris, France — [2]Laboratoire Kastler Brossel, Physics & Biology Department, Ecole Normale Supérieure 24, rue Lhomond 75005 Paris, — [3]Dept. of Biochemistry, Stanford University School of Medicine, Beckman Center B405, Stanford , CA 94305

Single molecule imaging and manipulation provide an irreplaceable tool to isolate each component of the cell and to quantitatively study its dynamics in a perfectly controlled environment. However, the experiments are usually performed out of the physiological context and a priori no indication on the behaviour of the molecule in the cell can be given. To show that this limitation can be overcome, we marked with a quantum dots single myosin V (a processive motor whose physical properties have been largely investigated in vitro). We observed the motion of the motors at sub-pixel resolution directly in living cells. We measured for the first time the processivity, the speed and the step size of the motor in its natural environment and compared the results with the one obtained from in vitro experiments.

BP 15.2 Wed 14:15 C 243

Walking the line: kinesin motors observed with sub-molecular resolution by atomic force microscopy — •Iwan A.T. Schaap[1], Carolina Carrasco[2], Pedro J. de Pablo[2], and Christoph F. Schmidt[3] — [1]National Institute for Medical Research, London, UK — [2]Universidad Autónoma de Madrid, Madrid, Spain — [3]Georg-August-Universität, Göttingen, Germany

Intracellular transport is largely driven by dyneins and kinesins moving on microtubules in complex, but highly coordinated patterns. How exactly a single motor proceeds on the 13 narrow "lanes" or protofilaments of a microtubule remains unknown because the required resolution lies beyond the reach of light microscopy. We have here succeeded to image kinesin-1 dimers immobilized on microtubules with single-head resolution and in addition in their motion along microtubules with nanometer resolution by atomic-force-microscopy. We show that both heads of one dimer are microtubule-bound for the major part of the chemical cycle. Furthermore, we could unambiguously resolve that both heads bind to the same protofilament, instead of straddling two, and remain on this track during processive movement.

BP 15.3 Wed 14:30 C 243

Towards resolving single helicase steps on DNA using magnetic tweezers — •Daniel Klaue and Ralf Seidel — Biotechnology Center, Dresden University of Technology, Germany

Replicative helicases drive processive DNA unwinding during DNA replication, the process during which a copy of the genome is synthesized. They are large hexamers, which encircle DNA. ATP hydrolysis in each of the six monomers drives processive movement of the helicase along DNA, which is coupled to DNA unwinding. However, it still remains elusive, how the six ATPase units are coordinated to achieve directional movement. To address this question, we study Large T antigen, a viral replicative helicase, which serves as an important model system for eukaryotic replication. We apply magnetic tweezers in order to follow the DNA unwinding of a single DNA hairpin in real-time. DNA unwinding by T antigen is comparably slow with 1-2 $\mathrm{bp\,s^{-1}}$. Resolving the bp-sized steps of the helicase along DNA would provide important insight into the coordination of the ATPase units. We therefore tested and improved the resolution limits of the applied magnetic tweezers, where a magnetic microsphere is used to exert force on a single DNA molecule. We achieve sub-nm accuracy in detecting the position of immobilized microspheres. However, we find that DNA bound microspheres can exhibit significant rotational fluctuations thereby limiting the resolution in these experiments. Nonetheless, by carefully selecting the measured microspheres we can obtain nm resolution on a second time scale, which would be sufficient to resolve bp-sized helicase steps.

BP 15.4 Wed 14:45 C 243

Transcriptional pausing and proof reading — •Martin Depken, Stephan Grill, and Eric Galburt — Max-Planck-Institute for the Physics of Complex Systems, Dresden, Germany

RNA polymerases are protein molecular machines that read the genetic information and transcribe it into messenger RNA. The process of adding new bases to the nascent RNA molecule is frequently interrupted by pauses. Here we consider the nature of these pauses, and show that they are well described by a diffusive process. This has implications for the pause time distribution, and the polymerase ability to transcribe through structural barriers such as nucleosomes and other DNA binding proteins. We further consider the possible relation between transcriptional pausing and proof reading, and examine the resulting interplay between fidelity and transcription speed.

BP 15.5 Wed 15:00 C 243

A unified model of transcription elongation — •Dáibhid Ó Maoiléidigh — Max Planck Institute for the Physics of Complex Systems, Dresden, Germany

The copying of DNA into RNA is the first step required for the production of proteins and RNA with a direct function. This process of transcription is highly regulated and is carried out by RNA polymerase (RNAP), a complex multi-functional molecular motor. In this talk we present a model which explains most published single-molecule observations of the elongation of the RNA transcript by RNAP. The model is an extension of previous work where we successfully predicted the sequence dependent positions of pauses during the elongation process [1]. Pauses have many functions, for example, they are associated with the correction of errors during transcription and are required for the termination of transcription. We have proposed previously that the folding of the RNA transcript behind RNAP creates a barrier which restricts the backwards movement of RNAP along DNA during a pause [1]. We now provide an estimate for the barrier position distribution. Furthermore, we present new analytical expressions which describe the dependence of the elongation velocity on force applied in single-molecule experiments. The model resolves many of the inconsistencies in the interpretations of single-molecule experiments on transcription elongation and illuminates mechanisms for its control.

[1] Tadigotla V. R., Ó Maoiléidigh D., Sengupta A. M., Epshtein V., Ebright R. H., Nudler E., Ruckenstein A. E., Proc Natl Acad Sci U S A, 103:4439-44 (2006).

BP 15.6 Wed 15:15 C 243

Peptide adsorption, friction and unfolding: Theoretical approaches — •Roland Netz, Dominik Horinek, Andreas Serr, Hirofumi Wada, Alfredo Alexander-Katz, and Thorsten Hugel — Physik Department, TU München, 85748 Garching

Single-molecule behavior combines the fields of non-equilibrium thermodynamics, elasticity theory and hydrodynamics. Theoretical approaches thus rely on molecular simulations, continuum modeling and scaling approaches. This is demonstrated with a few examples: - Spider silk consists of polypeptides with highly repetitive motives and readily adsorbs on hydrophobic and hydrophilic surfaces. Single molecule AFM studies yield adsorption energies and point to an extremely high mobility on hydrophobic surfaces. The dominant hydrophobic attraction can be quantitatively explained with classical MD simulations including explicit water. Both water structural effects and dispersion interactions contribute to this solvation attraction. - The friction coefficient of bound polymers is very low on hydrophobic substrates, which is traced back to the presence of a vacuum layer between substrate and water, which forms a lubricating cushion on which a polymer can glide. Conversely, friction forces on hydrophilic substrates are large and make determining the equilibrium binding constant in computer simulations impossible. - Shear-flow induced unfolding of proteins plays an important role in starting the coagulation cascade in small blood vessels. In the theoretical modeling the unfolding is initiated by single-chain protrusion-like excitations and leads to a hydrodynamic unfolding transition, which is well captured by a scaling nucleation argument.

15 min. break

BP 15.7 Wed 15:45 C 243

Fluorescent Nanodiamonds for Biological Applications — •Felix Neugart[1], Andrea Zappe[1], Fedor Jelezko[1], Carsten

Tietz[1], Jean Paul Boudou[2], Anke Krüger[3], and Jörg Wrachtrup[1] — [1]3. Physikalisches Institut, Universität Stuttgart, Stuttgart — [2]Université Pierre et Marie Curie, Paris VI — [3]Christian-Albrechts-Universität, Kiel

Nanodiamonds with nitrogene vacancy (NV) defects as colour centre are a promising candidate for a bright, not toxic, not blinking and not bleaching lable for biological aplications. The NV centre is excited in the green (514nm or 532nm wavelength) and emites around 700nm where living cells show a low autofluorescence. We inserted nandiamonds into living cells via microinjection and endocytosis. The diamonds could be detected by fluorescence as well refraction.

Functionalisation of the nanodiamonds surface is important for labelling as well as for the avoidance of aggregation under physiological conditions. The stability of hydrosols strongly depends on the surface potential of the particles, pH, and solvent salt concentration.

The fluorescence and motional dynamics of single diamond nanocrystals in buffer solution and in living cells is investigated. Stable hydrosols of nanodiamonds in buffer solutions are analysed by fluorescence correlation spectroscopy. [1]

[1] Neugart et al., Nano Letters, (2007)

BP 15.8 Wed 16:00 C 243

(Non-) linear deformation of viral shells — •Wouter H. Roos[1], Charlotte Uetrecht[2], Norman Watts[3], Paul Wingfield[3], Alasdair Steven[3], Albert Heck[2], and Gijs J. L. Wuite[1] — [1]Natuur- en Sterrenkunde, Vrije Universiteit, Amsterdam, Niederlande — [2]Bijvoet Instituut, Universiteit Utrecht, Niederlande — [3]NIH, Bethesda, USA

Nanoindentation techniques are increasingly being applied to study the mechanical properties of complex protein assemblies such as viral shells (capsids). Numerical simulations guided by the Föppl- von Kármán (FvK) number γ (a dimensionless number relating the "in-plane" elasticity of the shell to its "out-of-plane" bending rigidity) have been able to explain indentation results on capsids with $\gamma < 150$ (linear response) and $\gamma > 700$ (buckling transition). Yet for shells with a γ between those values a non-linear, but continuous response is expected. Here we report nanoindentation experiments with an atomic force microscope on capsids of the Hepatitis B Virus (HBV) to investigate this intermediate response regime. HBV was chosen as a model system because its capsids can form in a smaller T=3 and a bigger T=4 configuration that have FvK values within our region of interest. We demonstrate that the HBV T=3 capsid shows a subtle non-linear behaviour while the T=4 capsid reacts strongly non-linear, but continuously to deformation. Both non-linear responses can be understood in relation to their FvK values. At large indentations HBV undergoes permanent plastic deformation indicating a rearrangement of capsid proteins. The presented results demonstrate the surprising strength of continuum elastic theory to describe these nanometre sized objects.

BP 15.9 Wed 16:15 C 243

Effect of low pH on the Influenza virus membrane — •Frederic Eghiaian, Iwan A.T. Schaap, John J. Skehel, and Claudia Veigel — National Institute for Medical Research, London, UK

The Influenza virus is an enveloped virus from the Orthomyxovirus family. The protein-rich membrane of the viral particle needs to persist in the often hostile extracellular environment when the virus transfers from host to host, but, to allow infection, it also needs to permit membrane fusion within the acidic compartments of the target cell. To investigate how the virus negotiates these apparently conflicting demands on its rigidity, we developed methods to image this relatively large virus (˜100 nm diameter) using an atomic force microscope, and to probe its mechanical properties under conditions mimicking the different stages of the viral life-cycle. We compared the complex response of the viral envelope with the behaviour of simplified model systems to understand the contribution of the various parts of the viral structure to its mechanical properties. In addition we investigated how the acid-induced conformational change of the Influenza Hemagglutinin protein (involved in membrane fusion) disturbs lipid membranes and we set out to identify the responsible parts of the protein (the fusion peptide or the transmembrane region).

BP 15.10 Wed 16:30 C 243

TIRFM evanescent field calibration using tilted microtubules — •Chris Gell, Michael Berndt, and Stefan Diez — MPI-CBG, Dresden, Germany

Total internal reflection fluorescence microscopy (TIRFM) has become a powerful tool to study the dynamics of subcellular structures and single molecules near substrate surfaces. However, the penetration depth of the evanescent field , i.e. the distance at which the excitation intensity has exponentially decayed to 1/e, is often left undetermined. This presents a limit on the spatial information about the imaged structures. Moreover, in multi-color TIRFM applications, e.g. to perform colocalization studies, it is crucial to ensure equal penetration depths for the different excitation wavelengths. Here, we present a novel method to quantitatively characterise the illumination in TIRFM using tilted, fluorescently labelled, microtubules. Importantly, the use of in vitro reconstituted microtubules as nanoscale rulers results in a minimal perturbation of the evanescent field. Excitation light scattering is essentially eliminated and the refractive index of the sample environment is virtually unchanged. Our method has the potential to provide a generic tool for in-situ calibration of the evanescent field.

BP 15.11 Wed 16:45 C 243

C-Ring conformational rotation of a single F0F1- ATP synthase motor using alternating laser excitation — •Stefan Ernst[1], Monika Düser[1], Nawid Zarrabi[1], Rolf Reuter[1], Stanley D. Dunn[2], Gary D. Glick[3], and Michael Börsch[1] — [1]3rd Institute of Physics, University of Stuttgart, Pfaffenwaldring 57, 70550 Stuttgart, Germany — [2]Department of Biochemistry, University of Western Ontario, London, Ontario, Canada N6A 5C1 — [3]Department of Chemistry, University of Michigan, Ann Arbor, MI, USA 48109-1001

Formation of ATP from ADP and Phosphate (ATP synthese) is of great importance for any living cell. This chemical reaction is catalyzed by the enzyme F0F1- ATP synthase. By hydrolyzing ATP the enzyme can also work as an proton pump. Catalysis driven by a stepwise internal rotation of subunits of the lipid membrane embedded enzyme. To detect these substeps the single molecule fluorescence resonance energy trasfer (FRET) approach was used. We labeled one rotary c subunit with the FRET acceptor dye and the static a subunit with the FRET donor.

It was possible to determine the stepsize of rotation and dwell times. It was also possible to study the influence of different bacterial drugs, i.e. inhibitors of F0F1- ATP synthase like AMP-PNP and Aurovertin.

The different substep movements were identified with Hidden Markov Models (HMM). Duty cycle optimized alternating laser excitation provides an acceptor test to improve the accurancy of the single molecile FRET analysis.

BP 15.12 Wed 17:00 C 243

Data Analysis with Hidden Markov Models on a single Kdp-ATPase — •Nawid Zarrabi[1], Michael Börsch[1], Thomas Heitkamp[2], and Jörg Greie[2] — [1]3. Physikalisches Institut, Pfaffenwaldring 57, Universität Stuttgart, 70569 Stuttgart — [2]Universität Osnabrück, Fachbereich Biologie/Chemie, Arbeitsgruppe Mikrobiologie, Barbarastraße 11, 49069 Osnabrück

The membrane-embedded KdpFABC complex belongs to the group of P-type ATPases which transports potassium across a lipid bilayer using ATP hydrolysis. This enzyme contains a central catalytic subunit which mediates ion transport and ATP-hydrolysis.

We measured the stepwise conformational changes of this protein using confocal single-molecule fluorescence resonance energy transfer (FRET) and analyzed this data with Hidden Markov Models (HMM).

To prove the capability of the HMM approach we generated single molecule data of freely diffusing enzymes in liposomes by a Monte-Carlo-simulation. Thereby we included the intensity fluctuations due to Brownian motion. The conformational states of the ATPases were described by a Markov process with predefined rates for the transitions of the reaction cycle.

The aim of the data analysis method was to investigate the reaction cycle of the KdpFABC-complex and, furthermore, to elucidate the effectiveness of different inhibitors of the ion transport mechanisms.