in image mapping affect both FRET and gamma. As such, per molecule correction affects distribution width because FRET outliers may also have anomalous gamma values.

967-Pos
Diffusion of Membrane Proteins in Living Bacteria: Quantifying Complex Dynamics from Single-Molecule Tracking Experiments

Siet M.J.L. van den Wildenberg, Yves J.M. Bollen, **Erwin J.G. Peterman**.
VU University, Amsterdam, Netherlands.

Understanding the dynamics of trans-membrane proteins diffusing through membranes is essential for full comprehension of their function en mechanism. Over the last years it has become possible to study this diffusion process very directly in living bacteria using single-molecule fluorescence microscopy. In our labs, we are interested in unraveling the mechanism of the *E. coli* TAT protein-translocation machinery, which consists of dynamic complexes consisting of three different trans-membrane proteins, TatA, TatB, and TatC. To this end, we have created GFP-fusions of TatA and studied their behavior under different conditions, including excess of substrate and without proton motive force. We noticed that the complex diffusion behavior changed dramatically under different conditions. In this contribution we discuss our approach to quantify these changes. From our data, we calculate cumulative distribution functions (CDF), describing the probability of finding the particle inside a circular region after a given time lag. This approach is superior in the more regularly used mean-squared displacement approach, since it allows quantification of multiple diffusion components. Using this CDF approach we find complex behavior, which we show, using Monte-Carlo simulations, arises from the projection of the 3-dimensional shape of the bacterium on a plane and from the presence of at least two species diffusing with substantially different diffusion constants. We discuss our approach and effects of localization inaccuracy, shape of the bacterium and the presence of a non-moving fraction. Our results allow us to unravel the diffusion behavior of the Tat-complexes and shed light on their working mechanism.

968-Pos
Quantifying Sources of Low-Frequency Drift During Single-Molecule Experiments

Fabian Czerwinski[1,2], Ulrich F. Keyser[2], Lene B. Oddershede[1].
[1]University of Copenhagen, Copenhagen, Denmark, [2]University of Cambridge, Cambridge, United Kingdom.

Single-molecule techniques have evolved to the point where quantitative force measurements on biological systems can be performed down into the femtonewton range. As resolution is constantly improving, the pinpointing and elimination of noise sources become increasingly important. Complementary to Fourier analysis, Allan-variance analysis is ideally suited for this task; adjacent time series are recorded and the variations between observation intervals are calculated. Here, we provide a comprehensive toolbox consisting of acquisition and analysis software as well as fitting scripts to directly extract parameters of noise and low-frequency drift sources [1].
Furthermore, the validity and robustness of Allan-variance analysis is demonstrated in data obtained from various optical-tweezers setups wherein laboratory-specific noise sources are detected. This allows for a quantitative discrimination as well of common detection systems as of different calibration methods. In addition, we demonstrate how our toolbox can be applied during single-molecule experiments. Here, we determine the optimal calibration interval for any setup, suitable settings for variance and update rates in force-feedback loops, and variations due to the geometrical constraints of the sample chamber.
As outlook, we present data from other single-molecule techniques such as solid-state nanopores and magnetic tweezers. These emphasize the fact that Allan-variance analysis can be used as a standard tool enabling precise quantification of noise and drift effects.

[1] F. Czerwinski, A.C. Richardson, and L.B. Oddershede, ''Quantifying Noise in Optical Tweezers by Allan Variance,'' Opt. Express 17, 13255-13269 (2009)

969-Pos
Single Quantum Dot Imaging with 2-Photon Excitation Under Ambient Conditions

Ruobing Zhang, Eli Rothenberg, Paul R. Selvin.
University of Illinois at Urbana-Champaign, Urbana, IL, USA.

Quantum dots (q-dots) are bright and long-lasting fluorescence probes that are widely used in *in vitro* and *in vivo* experiments. Here, we report 2-photon (2P) widefield excitation of single q-dots in aqueous solutions which allows fast real time imaging. We show this on a Myosin V, labeled on one head with a 655 nm qdot. With 50 msec integration time, 2 μM ATP, 840 nm excitation, we achieve 1.0 nm spatial accuracy and can watch the motor move over 20 steps. This compares well with 1-photon excitation at 532 nm. We find that adding DTT or BME to the imaging buffer essentially eliminates blinking. We also demonstrate 2P microscopy of individual q-dots by scanning with a diffraction-limited spot, allowing z-resolution depth discrimination. In this optical arrangement, we can use an EMCCD, instead of the usual PMT, as the detector, which considerable simplifies matters. In this arrangement, we show 3D imaging of q-dot-labeled *E.coli* LamB receptors. Our technique opens great possibilities for fast live cell and tissue imaging.

970-Pos
The Role of Pi-Release as the Main Torque Generating Step of F_1-Atpase

Rikiya Watanabe, Hiroshi Ueno, Ryota Iino, Hiroyuki Noji.
Institute of scientific and industrial research, Osaka university, Ibaraki, Japan.

F_1-ATPase ($\alpha_3\beta_3\gamma$) is a rotary motor protein, which couples ATP hydrolysis to the rotary motion. Extensive studies on F_1-ATPase revealed that each of three β-subunits, which has the catalytic site, follows the same reaction pathway of ATP hydrolysis, but they are always in a reaction phase differing by $\pm 120°$ from each other. When we focus on one β-subunit, the β binds ATP at a particular binding angle. After the γ rotates 200°, the β cleavages the bound ATP into ADP and Pi. The produced ADP and Pi are released from the β after further 40°and 120° rotation, at +240° and +320° from the ATP-binding angle, respectively. In this study, we observed the rotating F_1 and measured the equilibrium of ATP cleavage and synthesis at the single molecule level. As F_1 released the produced Pi, the equilibrium was shifted to ATP cleavage; therefore, from the time course of the probability of ATP cleavage, we determined the rate of Pi-release at the angles for ATP cleavage and ADP release as $0.021 s^{-1}$and $0.94 s^{-1}$, respectively. We also determined the rate at the proper angle for Pi-release as $2{,}600 s^{-1}$ by using the fast-framing camera with 18,000 fps. From these results, we found that the rate of Pi release strongly depended on the rotary angle, and the dependence of activation energy on the rotary angle was determined to be $\Delta E = 5.5\ k_B T/\mathrm{rad}$, which was almost 55% of the net rotary torque of F_1.

971-Pos
Position Dependent Site-Exposure Nucleosome Dynamics by FRET-FCS

Kaushik Gurunathan, Marcia Levitus.
Arizona State University, Tempe, AZ, USA.

Nucleosomes are the fundamental repeating unit of eukaryotic chromatin. Often large protein complexes encounter a hurdle when their target DNA sites are sterically occluded inside these nucleosomes. One of the models by which DNA sites are exposed is by spontaneous unwrapping and rewrapping of DNA stretches and hence it is called Site-Exposure model. Here, in collaboration with Widom lab, we use a FRET-FCS method to study the dynamic rates of unwrapping and rewrapping at sites inside the nucleosomes. Our FRET system consists of labeling the DNA with a FRET donor (Cy3) at positions along the length of the DNA, starting from one end all the way to the center of the dyad axis, and labeling a histone protein with a FRET acceptor (Cy5). Using FCS measurements, we measured the relaxation time of this dynamic process which is dominated by the re-wrapping rate of the nucleosomes. Our results show that although the re-wrapping rate decreases with greater lengths unwrapped, it is not as dramatic as one would expect.

972-Pos
The Intra Dynamics of Group II Chaperonin Detected by Diffracted X-Ray Tracking Method

Hiroshi Sekiguchi[1], Ayumi Nakagawa[2], Taro Kanzaki[2], Masafumi Yohda[2], Yuji C. Sasaki[1].
[1]The University of Tokyo, Kashiwa city, Japan, [2]Tokyo University of Agriculture and Technology, Koganei city, Japan.

Besides the static structural information of a protein, it is crucial for revealing mechanism of protein's function that to pursue the intra-dynamics information of the objective protein in millisecond and atomic scale.
We had proposed the Diffracted X-ray Tracking (DXT) for detecting subtle intra-movement of the target protein, and applied this method for some proteins, such as bactriorhodopsin [1], antibody [2] and KcsA channel [3]. In DXT, the dynamics of a single protein can be monitored through trajectory of the Laue spots from the nano crystal which was labeled on the objective proteins immobilized on the substrate surface.
In this study, we applied the DXT method for monitoring ATP driven conformational change of archaeal group II chaperonin, known as the protein machinery that interacts with misfolded proteins, confine them in its cavity by closure of the built-in lid, and assists them to re-fold correctly in the cavity [4].
Optimizing the experimental condition of DXT method, such as immobilization of proteins and prepation of gold nanocrystals, the dynamics of open and closure of the chaperonin's built-in lid will be discussed.

[1] Y. Okumura et al., Phys. Rev. E, 70:021917-1-7 (2004)
[2] T. Sagawa et al., Biochem. Biophy. Res. Commun. 335:770-775 (2007)

[3] H. Shimizu et al., Cell 132:67-78 (2008)
[4] T. Kanzaki et al., J. Biol. Chem. 283:34773-34784

973-Pos
Optimal Estimation of the Diffusion Coefficient from Noisy Time-Series Measurements
Christian L. Vestergaard[1], Paul Blainey[2], Xiaoliang Sunney Xie[3], Henrik Flyvbjerg[1].
[1]Technical University of Denmark, Kgs. Lyngby, Denmark, [2]Stanford University, Stanford, CA, USA, [3]Harvard University, Cambridge, MA, USA.
Single-molecule time-lapse measurements of diffusing proteins often contain considerable localization error. The standard method for estimating the diffusion coefficient is based on the mean square displacements. This method is highly inefficient, since it ignores the high correlations inherent to these. A Generalized least squares method, which takes into account these correlations, is presented and it is shown that it attains the maximum precision possible according to information theory. The method is demonstrated on data from high-speed time-lapse photography of the hOgg1 repair protein diffusing on DNA.

974-Pos
Adaptive Platform for Highly Parallel Low-Noise Recordings of Single Membrane Proteins
Gerhard Baaken[1,2], Srujan Kumar Dondapati[1], Juergen Ruehe[2], Jan C. Behrends[1].
[1]Laboratory for Electrophysiology and Biotechnology, Department of Physiology, University of Freiburg, Freiburg, Germany, [2]Laboratory for Chemistry and Physics of Interfaces, Department of Microsystems Engineering (IMTEK), University of Freiburg, Freiburg, Germany.
Highly parallel, low noise electrophysiological recordings of single ion channels are of interest both for basic research and drug development. Here, a microsystems approach is presented (see Fig.1A) which greatly simplifies the recording configuration and optimizes the electrical parameters governing noise. The lipid bilayer is formed on a picoliter cavity generated within a photochemical resist acting as a dielectric (see Fig. 1B). On the bottom of each cavity a microelectrode is placed.
Using standard photolithographical techniques this design allows for many such setups on one chip, and is therefore in principle well suited for highly parallel single channel recordings. Parallel recordings (16-electrode multiarray) of currents mediated by alamethicin are shown (see Fig.1C), illustrating the potential of this novel approach towards high-throughput measurements of single membrane proteins. Furthermore, the flexibility of this approach is outlined by the reconstitution of α-hemolysin in the lipid bilayer. The capability for molecular analysis is demonstrated by the detection of oligomers diffusing through the bacterial pores.

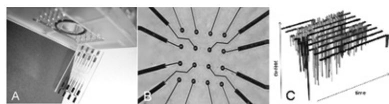
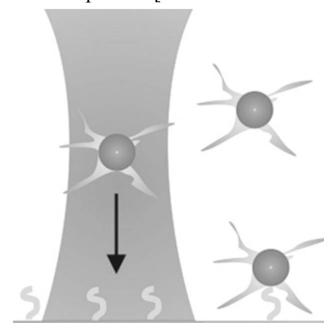


Fig. 1. *Patch16Chip* ***(B)*** *Connecting lines and cavities over the circular shaped electrodes.* ***(C)*** *Current traces of single alamethicin-channels, detected in parallel at 9 different cavities.*

975-Pos
A New Closed Cell, Horizontal Magnetic Tweezer
Christopher P. McAndrew.
The Catholic University of America, Washington, DC, USA.
We report on the development of a magnetic force transducer or tweezer that can apply piconewton forces on single DNA molecules in the focus or horizontal plane. Since changes in the DNA's end-to-end extension are coplanar with the pulling force there is no requirement to continually refocus the tethered beads thus considerably simplifying single molecule micromanipulation experiments in our setup. The DNA constructs (γ-DNA end-labeled with a 3μm polystyrene bead and a 2.8μm magnetic sphere) and buffer are introduced into a 200μL to 500 μL closed cell created by using two glass slides separated by 1mm spacers and a thin viscoelastic perimeter wall. This closed cell configuration isolates our sample and produces low-noise force and extension measurements. Breaching the viscoelastic barrier are five pipettes: a 1-2μm inner diameter suction pipette used for capturing the polystyrene bead, a magnet-tipped pipette used to pull the magnetic sphere, a 0.5mm-inner-diameter injection pipette used to introduce proteins of interest, and two 0.5mm-inner-diameter pipettes used to maintain flow. The suction micropipette and the injection pipette are rigidly coupled and positioned by a manual three axis manipulator that can produce continuous displacements of 15mm, 25mm and 25mm in the x-, y-, and z-axis respectively. The magnet-tipped pipette is controlled by a motorized two axis micromanipulator capable of continuously spanning the full width of the cell. A motorized micromanipulator with a defined 157nm step size maneuvers the cell over a 32X/0.40NA objective and between the two mechanical manipulators. Initial tests show the capability of the device to easily and repeatedly find, capture, and manipulate end-labeled DNA constructs.

976-Pos
Optical Trapping and Two-Photon Exitations of Quantum Dots
Liselotte Jauffred, Andrew C. Richardson, Lene B. Oddershede.
Niels Bohr Institute, Copenhagen, Denmark.
Individual colloidal quantum dots can be optically trapped and manipulated by a single infrared laserbeam operated a low laser powers [Jauffred et al. Nano letters 2008 (**10**)] and the spring constant of the force, exerted by the harmonic optical trap on a quantum dot, have been found to be of the order of 10^{-4} pN/nm. We measured the optical trapping strength of individual colloidal quantum dots with different emission wavelenghts (from 525 nm to 800 nm) and different physical sizes, with the result that these diverse quantum dots have identical trapping capabilities.



Furthermore, we show that the trapping laserlight can also act as a source for two-photon excitation of the trapped quantum dots, thus eliminating the demand for an excitation light source in addition to the trapping laser beam.

977-Pos
Optical Torque Wrench for Single Molecule Studies
Francesco Pedaci, Sven Klijnhout, Maarten van Oene, Jacob W.J. Kerssemakers, Nynke H. Dekker.
TU Delft, Delft, Netherlands.
At the molecular level, the torque applied to biopolymers plays a central role in many processes involving their conformational changes and interactions with proteins. We will study the torque-sensitivity of individual nucleic acid molecules and their interactions with proteins using a novel optical tweezers configuration termed the optical torque wrench [1].
In standard single-molecule techniques, torque cannot be simultaneously controlled and detected, in contrast with the case of the applied force. With this new technique we will be able to control both parameters in real-time in single molecules of DNA or RNA, with high temporal (250 kHz) and spatial (nm) resolution typical of optical tweezers. Here we present a first characterization of our instrument.
This will allow us to acquire fundamental insight into the torque-sensitivity and dynamics of nucleic acids, DNA packaging, polymerase activity in DNA replication or transcription, and related biological processes.
[1] A. La Porta and M.D.Wang, Phys. Rev. Lett. 92, 190801, 2004.

Atomic Force Spectroscopy

978-Pos
Fishing on Living Cells with AFM: Novel Method to Study Topology and Dynamics of Cotransporter SGLT1 Protein
Theeraporn Puntheeranurak[1], Rolf K.H. Kinne[2], Peter Hinterdorfer[3].
[1]Faculty of Science, Mahidol University, Bangkok, Thailand, [2]Max Planck Institute of Molecular Physiology, Dortmund, Germany, [3]Institute for Biophysics, Johannes Kepler University of Linz, Linz, Austria.
In the apical membrane of epithelial cells from the small intestine and the kidney, the high-affinity Na^+/D-glucose cotransporter SGLT1 plays a crucial role in intestinal glucose absorption and in renal glucose reabsorption. Here the over-expression of rabbit SGLT1 in rbSGLT1-transfected Chinese hamster ovary (CHO) cells was first characterized using the immuno-staining method on non-permeabilized cells. The functionality of the SGLT1 was verified by biochemical approaches. Atomic force microscopy (AFM) was employed to probe initial substrate-carrier interactions, topology, and function of SGLT1 in living cells on the single-molecule level. Specific recognition events in force distance cycles were detected using epitope specific antibodies or thio-glucoside bound to the AFM tip. Upon addition of D-glucose or the specific inhibitor phlorizin, binding of antibody primed AFM tips drastically decreased suggesting recognition sites for D-glucose in the extracellular loop 8-9 and for phlorizin in the extracellular loop 13-14. The binding probability of the thio-D-Glucose tip was reduced by various sugars in a potency sequence that differed markedly from transport studies. We therefore propose that the first of several selectivity filters of SGLT1 is formed by the two extracellular loops 8-9 and