complex biological problems that simply can not be addressed through traditional biochemical approaches. The primary advantages of our approaches are that we can actually see what proteins are bound to DNA, where they are bound, how they move, and how they influence other components of the system - all in real-time, at the level of a single reaction. Our research program is focused on studying the regulation and activity of proteins that are involved in repairing damaged chromosomes. We are particularly interested in determining the physical basis for the mechanisms that proteins use to survey DNA molecules for damage and initiate repair processes, and how these initial steps are coordinated with downstream events that lead to completion of repair. As part of our work, we are also actively pursuing the development of novel experimental tools that can be used to facilitate the study of single biochemical reactions. In particular, we are applying techniques derived from nanotechnology to our biological research, and using nano- and micro-scale engineering to facilitate the development of new, robust experimental platforms that enable ''high throughput'' single molecule imaging.

962-Pos

Watching Conformational and Photo-Dynamics of Single Fluorescent Proteins in Solution

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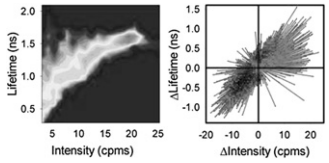
Observation of dynamics of single biomolecules over a prolonged time period without significantly altering the biomolecule via immobilization is a difficult challenge. This result is achieved with the Anti-Brownian ELectrokinetic (ABEL) Trap, which allows extended investigation of solution-phase biomolecules without immobilization via real-time electrokinetic feedback. We apply the ABEL trap to study an important photosynthetic antenna protein, Allophycocyanin (APC). Single molecules of solutionphase APC can often be studied for more than one second. We observe a complex relationship between fluorescence intensity and lifetime that cannot be explained by simple static kinetic models. Light-induced conformational changes are shown to occur. Further, evidence is obtained for fluctuations in the spontaneous emission lifetime, which is typically assumed to be constant. Our observations provide a new window into the dynamics of fluorescent proteins and are relevant for interpretation of in vivo singlemolecule imaging experiments, bacterial photosynthetic regulation, and biomaterials for solar energy harvesting.



963-Pos

What Can We Learn From Single-Molecule Diffusion

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There is increasing interest in a detailed understanding of the structure and dynamics of the cellular plasma membrane, primarily based on recognizing its essential role for controlling cellular signaling processes. Various pictures emerged, which ascribe the plasma membrane a high degree of organization at very short length scales of tens of nanometers. We employed single molecule fluorescence microscopy to study diffusion of CD59, a GPI-anchored protein, in the plasma membrane of living T24 cells at sub-wavelength resolution, both on the cell body and on tunneling nanotubules connecting cells. By separating longitudinal and transversal mobility, we found isotropic diffusion behavior on the surface of tunneling nanotubules, rendering direct influences of the membrane skeleton unlikely.

In both studies we analyzed the mean square displacement as a function of the time-lag and the distribution of displacement steps. However, a closed analytical theory for these analysis is only available for the simplest models. To address a suspected diffusion process we reasoned that a full analytical description may not be required; it may well be sufficient to compare the experimental data with Monte Carlo simulations of the process. We demonstrated the working principle for the analysis of free diffusion, hop diffusion and transient binding of the tracer molecule to slowly moving receptors.

In the recent years increasing evidence was reported for an inherent heterogeneity of cell populations. Our reasoning was that mobility probes nanometer-sized properties of the moving protein and its local environment. Automated and tailored data analysis routines allowed for the analysis of the required large data sets: ~200.000 trajectories obtained on ~350 cells were analyzed in total. We found up to five-fold higher variability of the diffusion constant between cells compared to the uncertainty for the determination of the diffusion constant on a single cell.

964-Pos

Electrostatic Switching of Polysaccharide Conformation Probed at the Single Molecule Level

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Polysaccharides play a key mechanical role in maintaining cell integrity and in cell-cell recognition. Single molecule AFM stretching measurements have revealed that upon loading, the backbone of polysaccharide molecules change their conformation and these conformational changes depend on the linkages between the sugar rings. It has been proposed that these force-induced conformational transitions may play an important role in biological systems. However a mechanism to switch on/off these conformational transitions and control the nanomechanical properties of carbohydrates has not yet been shown.

Here we demonstrate an electrostatic switch that can be used to toggle the force dependent conformational transition in acidic polysaccharides. Using single molecule AFM force spectroscopy we show that the tension dependent conformation of the polysaccharide molecules can be controlled by varying the backbone charge density and solution electrostatics.

965-Pos

An Optical Conveyor for Molecules

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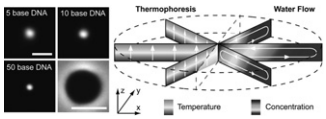
We optically trap molecules in free solution, which allows to accumulate 5-base DNA to a hundredfold excess within seconds [1]. The concentration of the trapped DNA scales exponentially with length, reaching trapping potential depths of 14kT for 50 bases. This novel way to trap molecules could be used to enhance diffusion-limited surface reactions, redirect cellular signaling, observe individual biomolecules over a prolonged time or separate small molecules in solution by their diffusion constant.

The mechanism is based on the microscale analog of a conveyor belt: a bidirectional flow, driven optically by the recently shown thermo-viscous fluid pump [2,3], is combined with a perpendicular thermophoretic molecule drift. Arranged in a toroidal geometry, no microfluidics, electrodes or surface modifications are required. As a result, the trap can be dynamically relocated.

[1] Weinert and Braun, Nano Letters, accepted
[2] Weinert, Kraus, Franosch and Braun, PRL 100, 164501 (2008)
[3] Weinert and Braun, JAP 104, 104701 (2008)



966-Pos

Recovering Absolute Fret Efficiency from Single Molecules: Comparing Methods of Gamma Correction

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Fluorescence resonance energy transfer is widely thought of as a ''spectroscopic ruler.'' Because biological processes and cellular assemblies occur on the nanometer scale, FRET is a popular tool for structural biology. In contrast to ensemble solution FRET measurements which record the entire emission spectrum, microscopy- based FRET experiments separate donor and acceptor intensity by passing the emission through a series of optical elements. Observed FRET efficiency, determined from the uncorrected donor and acceptor intensities, has been called a relative proximity ratio, which is internally consistent *only* if the photophysical properties and instrument remain unchanged. However, it is desirable to measure absolute distances using FRET, which requires that FRET efficiency be corrected for both instrument response and fluorophore properties. Thus, ''gamma'' correction adjusts for differences between the donor and acceptor dyes in their probability of photon emission upon excitation and the probability that emitted photons will be detected. Methods of gamma correction vary depending on the single molecule methodology. To test different methods for correcting FRET efficiency, we recorded smFRET distributions for protein and DNA on different instruments and with different filter sets which altered the observed FRET efficiency. Knowledge of filter set transmission allows for comparison of results between groups using different instruments. Applying empirically-derived corrections for instrument response and quantum yield was only slightly better than corrections based solely on filter set transmission data. We found that gamma correction based on single molecule photobleaching was the most effective particularly when gamma was determined for each sample or even each molecule. Variations in focus of the two colors and sub-pixel errors

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

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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

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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

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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

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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 $\pm 120^\circ$ 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 $+240^\circ$ and $+320^\circ$ 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 $\Delta E = 5.5\ k_BT$/rad, which was almost 55% of the net rotary torque of F_1.

971-Pos

Position Dependent Site-Exposure Nucleosome Dynamics by FRET-FCS

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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

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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)