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)