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Recent evidence suggests that soluble amyloid-β (Aβ) oligomers as small as dimers may be linked to the progression of Alzheimer's disease. We have used single-pair FRET measurements to investigate heterogeneity in surface-tethered dimers of Aβ40, probing for preferred structures. Dimers are prepared by combining monomers singly labeled with donor and acceptor dyes; dimers prepared in solution (prior to surface-tethering) and on the functionalized surface have been examined. Donor and acceptor fluorescence are separated onto two detectors, such that co-localized spots in two-color images are indicative of at least two associated peptides. Dimers are verified based on the observation of single-step photobleaching in each detection channel; larger oligomers are excluded from analysis. By measuring donor and acceptor fluorescence as a function of time, we have determined time-dependent FRET efficiencies for dozens of individual dimers, permitting insight into inter-dye distances and dimer structures. These results are further complemented by comparison to published structures of simulated Aβ40 dimers. Together, experiment and simulation may reveal a subset of preferred structures for Aβ40 dimers.

3048-Pos
High Density Single Particle Tracking Using Bayesian Multi-Fluorophore Fitting through Vertices
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Single particle tracking (SPT) has become a common technique for studying protein and lipid behavior in cell membranes. Using photo-stable fluorescent probes such as quantum dots, SPT is now being applied using multi-color strategies in order to look at nano-scale protein-protein interactions. However, the sparse labeling of each spectral species needed to limit the occurrence of fluorophores overlapping within the diffraction limit reduces the probability of overlap from opposing spectral species and the frequency of observed interactions.
We present a single particle tracking method that allows a higher density of labels by performing a Bayesian multi-fluorophore fit to particles that are spaced closer than the diffraction limit. We call a single event of overlapping trajectories a 'vertex.' We use knowledge of the particles' intensities, diffusion constants positions before and after the vertex, and possibly blinking rates to improve the multi-fluorophore fits through the vertex. We examine the accuracy of the trajectories and fits as a function of label density. Furthermore, we show that under a range of frame and blinking rates, fluorescence intermittency from blinking probes can improve the accuracy of multi-fluorophore fits. We show that single particle and Bayesian multi-particle fits can be performed at more than 10^5 fits per second using an iterative fitting method implemented on GPU architecture.

3049-Pos
Dynamical Observations of Brownian Motions on Single Protein Molecules Using Diffracted Electron Tracking (DET)
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In order to improve both monitoring super-precisions of conformational changes and stability of the dynamical signal intensity from single molecular units under in vitro physiological conditions, we have proposed new single molecular techniques using shorten wavelength, for example, X-rays, electrons, and neutron. Diffracted X-Ray tracking (DXT) has been developed for obtaining the information about the dynamics of single molecules. This method can observe the rotating motion of an individual nanocrystal, which is linked to specific sites in single protein molecules, using a time-resolved Laue diffraction technique. However, this method needs a very strong X-ray source, so we began to develop a compact instrument for monitoring the motions of the single protein molecules, using the electron beam instead of the X-ray. In this work, we demonstrated three-dimensional tracking of single nanocrystals labeled with individual single molecular units using Scanning Electron Microscope (SEM). We called Diffracted Electron Tracking (DET). Instead of the Laue diffraction using white X-ray, the Electron Back-Scattered Diffraction Pattern (EBSP) in SEM is adopted to monitor the crystal orientation of the nanocrystals linked to the single protein molecules.
We used SEM (JSM-7000F TYPE A, JEOL) to monitor EBSP from the labeled gold nanocrystals (Diameter size= 30-60nm) in the thin aqueous solution (thickness is about 100nm). We observed dynamical EBSP during 3s in each integrated time of 30ms. We determined three Euler angles from EBSP mapping of the observed gold nanocrystals. Thus, we observed three-dimensional Brownian motions of the labeled gold nanocrystal. Additionally, we checked the relationship between the sizes of the labeled nanocrystals and Brownian motions from dynamical EBSP data.

3050-Pos
Single-Image Measurements of Monochromatic Subdiffraction Dimolecular Separations
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One of the current challenges in single-molecule imaging is the dynamic separation measurement between two subdiffraction-separated identical fluorophores. The combined intensity profile of two subdiffraction separated fluorophores can be approximated by a 2D Gaussian function, and we have developed a simple method to measures the dimolecular separation using this combined Gaussian intensity profile. By measuring the standard deviation (SD) of the convolved Gaussian image we show that we can (1) differentiate dimers from monomers and (2) measure the dimolecular separation with a known precision depending on the number of detected photons, all using a single image of milliseconds exposure time. We have constructed diagrams showing (a) the number of photons required to differentiate dimers from monomers, (b) dimer SD vs. separation, and (c) the precision in the measured dimer separation. This study demonstrates a simple method that allows dynamic measurements of monochromatic subdiffraction dimolecular separations. For example, a single image of 10,000 collected photons in 100 ms can determine the dimolecular separation down to 70 nm with a precision of 20 nm.

3051-Pos
Single Molecule Studies of Protein Surface Adsorption Kinetics
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The fact that most protein-based biotechnological and medical procedures involve protein-surface interactions demands that the protein-surface adsorption process be studied in greater detail to prevent undesirable nonspecific adsorption of proteins to surfaces. To solve this problem, the mechanisms responsible for protein adsorption to surfaces must be identified and quantified. Unlike conventional bulk measurements where adsorbed proteins of one kind cannot be differentiated from another, single-molecule imaging studies can identify the adsorption mechanisms using real-time imaging of the adsorption process. Here we report on Total Internal Reflection Fluorescence (TIRF) Microscopy imaging of single Streptavidin-Cy3 molecules interacting with hydrophobic fused-silica surfaces. The results reveal the different mechanisms responsible for protein-surface adsorption, and their kinetics. We have observed reversible and irreversible adsorptions due to the intrinsic interaction of proteins with surfaces at the water-surface interface, and irreversible adsorptions due to the protein deposition process at the air-surface interface. We will discuss the extent of contribution to total surface-protein adsorption for each adsorption mechanism.

3052-Pos
De-Noising Single Molecule FRET Trajectories using Wavelent and Bayesian Techniques
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Extracting quantitative information from low signal-to-noise single molecule FRET trajectories remains a significant challenge. In particular, biological systems that derive their function from shallowly defined or continua of states are not amenable to recent Markovian analysis algorithms. A method to de-noise single-molecule fluorescence resonance energy (smFRET) trajectories using wavelet detail thresholding and Bayesian inference is presented. Bayesian methods are developed to identify fluorophore photoblinks in the time trajectories. Simulated data are used to quantify the improvement in static and dynamic data analysis. Application of the method to experimental smFRET data shows that it distinguishes photoblinks from large shifts in smFRET efficiency while maintaining the important advantage of an unbiased approach. Known sources of experimental noise are examined and quantified as a means to remove their contributions via soft thresholding of wavelet coefficients. A wavelet decomposition algorithm is described, and thresholds are produced through the knowledge of noise parameters in the discrete-time photon signals. Reconstruction of the signals from thresholded coefficients produces signals that contain noise arising only from unquantifiable parameters. The method is applied to simulated and observed smFRET data, and it is found that the denoised data retain their underlying dynamical properties, but with increased resolution.