1695-Plat
Deconstructing PIFE: A Spectroscopic Investigation of the "Protein Induced Fluorescence Enhancement" Phenomenon in Cy3
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PIFE, an acronym that stands for "Protein Induced Fluorescence Enhancement", is a term that has been coined to describe the enhancement of fluorescence intensity that the dye Cy3 experiences in the proximity of a protein. The approach has been used to study dynamic aspects of a large number of DNA- and RNA-protein interactions at the single molecule level. We and others have hypothesized that the phenomenon results from a restriction in the photoisomerization deactivation pathway of the dye. Here, we present the results of a detailed spectroscopic study that aims to characterize PIFE at the molecular level. We used time-resolved fluorescence, fluorescence anisotropy and transient spectroscopy to fully characterize the deactivation pathways of the dye on DNA when next to a protein. Our results allowed us to confirm our hypothesis that the enhancement in fluorescence correlates with a decreased yield of photoisomer.

1696-Plat
Fabrication and Surface Functionalization of Highly Birefringent Rutile Particles for Trapping in an Optical Torque Wrench
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The optical torque wrench (OTW) allows the direct application and measurement of torque on biomolecules, such as DNA or DNA-protein complexes, or rotary motors like the F0F1-ATP-synthase or the bacterial flagellar motor. The applicable torque of the OTW is a function of the size and birefringence of the particle. Quartz has proven a convenient material, but its quite low birefringence limits full investigation of torque-speed relationships of diverse biological systems. In contrast, rutile exhibits a much higher birefringence - exceeding that of quartz by a factor of 30 - but its utilization has been infrequent because of the difficulties in optical trapping and fabrication.
To enhance the applicability of the OTW, we have improved both the design and fabrication of cylindrical rutile particles. We have employed finite element method calculations to determine the optimal dimension of stably trappable rutile cylinders. To obtain rutile cylinders with the optimal dimensions, we developed a protocol for full control of size and sidewall angle. In our fabrication protocol, a chromium etch mask provides increased resistance to dry etching and allows the fabrication of structures with both high aspect ratio and anisotropy. Also, the sidewall angle of cylinders can be readily tuned by adjusting a single process parameter, namely the oxygen flow rate during dry etching. The fabricated cylinders were characterized in the OTW setup to reveal their linear and angular trapping properties. The fabrication process is compatible with common chemical functionalization procedures and permits covalent biomolecule attachment. To enhance biomolecule coverage, we used ethanolamine and poly(ethylene glycol) as biomolecular crosslinkers to obtain homogenous and dense coatings. Our recent results, in which we use functionalized, trapped rutile cylinders to study single biomolecules and motor proteins, will be presented.

1697-Plat
Electron Paramagnetic Resonance from a Single Biomolecule
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Electron paramagnetic resonance (EPR) is an established and powerful tool for studying atomic-scale biomolecular structure and dynamics. Yet it requires a homogenous sample size of ~10^15 spin labeled biomolecules. Single molecule measurements provide improved insights into heterogeneous behaviors that can be masked by ensemble measurements and are often essential for illuminating the molecular mechanisms behind the function of a biomolecule. We report EPR measurements of single labeled biomolecule demonstrating the merging of these two powerful techniques. We selectively labelled individual double-stranded DNA molecules with nanodiamonds containing nitrogen-vacancy (NV) centers, and optically detected the paramagnetic resonance of the single NV nanodiamond probe. Analysis of the spectrum reveals that the characteristic time scale for reorientation of the labeled molecule relative to the applied magnetic field is slow compared to the inverse of the EPR linewidth. This demonstration of EPR spectroscopic determination of the dynamics of an individual labeled biomolecular construct provides the foundation for single molecule magnetic resonance studies of complex biomolecular systems.

1698-Plat
Dynamics of Polymeric Protein Assemblies in Live Cells Revealed by Fluorescence Polarization Imaging of Single Molecules
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Fluorescence single molecule imaging is a powerful means of investigating the distribution and dynamics of individual biological molecules and their assemblies. Molecular orientation underpins the biophysical properties of the biopolymers (e.g., DNA, cytoskeleton, extracellular matrix and misfolded protein oligomers). Therefore, methods that reveal the molecular orientation have yielded new insights in the structure and function of biomolecular assemblies. However, current methods are limited to measurement of orientation of single fluorophores and have not permitted robust imaging of orientation of many single molecules in parallel. We have developed a new fluorescence polarization microscope that instantaneously and efficiently sorts the emitted fluorescence along four polarization orientations (separated by 45 degrees) to provide instantaneous imaging of position and orientation of single fluorescent molecules and their assemblies. With aid of computational algorithms, the microscope provides diffraction limited spatial resolution, 10fps temporal resolution, and 10 degree of angular resolution in living cells.
Our technique has enabled analysis of molecular position and orientation in vitro and in living cells. We found that phalloidin-Alexa Fluor 488 reports local orientation of sparsely labeled actin filaments. Taking advantage of this signal, we have measured changes in the orientation of local actin filaments as they undergo retrograde flow at the leading edge of migrating human keratinocytes. We also used our system to study organization of septins, a highly conserved cytoskeleton critical for cytokinesis and intracellular compartmentalization. We found that individual septin molecules labeled with constrained GFP attach to the cell cortex with consistent orientation. The consistent alignment of single septin-GFP suggested the presence of highly ordered scaffold.
Our single molecule fluorescence orientation imaging technique is also promising to explore conformational changes of single molecules or mechanisms of protein assembly in living cells.

1699-Plat
Single-Molecule-Sensitive FRET in Freely-Diffusing Attoliter Droplets
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Fluorescence resonance energy transfer (FRET) from RNA confined in freely-diffusing attoliter aqueous droplets shows dramatic differences from that of RNA in solution. First, the use of droplet confinement is shown to substantially increase the signal-to-noise ratio of single-molecule sensitive measurements. However, a distinct shift in FRET is also observed. These differences can be attributed to a modified pH in the confining environment, which is a result of the well-documented autolysis of water and accumulation of hydroxide ions near the water interface. This outcome has implications for the use of droplets for protein crystallization, nanoparticle synthesis, biomicrofluidics, and analytical chemistry, where careful attention to the use of appropriate buffers or surfactants to control pH in the confined phase will be required. This work was funded by NSF MCB-0920139 and NSF DBI-1152386.

Platform: Skeletal Muscle Mechanics, Structure, and Regulation

1700-Plat
Activation and Relaxation Kinetics in Skeletal and Cardiac Muscles
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A large amount of data is available on the Ca^{2+} regulation of the thin filament of skeletal and cardiac muscle, but some general concepts are still under debate. To quantitatively describe muscle contraction and relaxation in the 3D multi-sarcomere geometry we have implemented in computational platform MUSICO: (i) a nine state actomyosin ATPase cycle, (ii) extensibility of thick