1695-Plat
Decohering 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 photosensitization 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

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 Bimolecule

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A large amount of data is available on the Ca2+ 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-sarcome geometry we have implemented in computational platform MUSICO: (i) a nine state actomyosin ATPase cycle, (ii) extensibility of thick