rotating motion of an individual nanocrystal, which is linked to specific sites in single protein molecules, using a time-resolved Laue diffraction technique. This method needs a very strong X-ray source, such as the SPring-8, so we began to develop a compact instrument for monitoring the rotation of the single protein molecules, using the electron beam instead of the X-ray.
Instead of the Laue diffraction using white X-ray, the Electron Back-Scattered Diffraction Pattern (EBSP) is adopted to monitor the crystal orientation of the nano-crystals linked to the single protein molecules. For this purpose, it is necessary to realize (1) wet cell with very thin sealing film, (2) EBSP system with high sensitivity, (3) damage-less electron irradiation technique and (4) perfect gold nano-crystals.
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1989-Plat
Calibration of Holographic Optical Tweezers for Force Measurements on Biomaterials
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Optical tweezers have been widely applied in the field of single-molecule biophysics, as the picoNewton forces that can be exerted and measured with this noninvasive technique lie in the force range of many biomolecular properties and events. By using trapped micrometer-sized particles as handles, the force-extension relations of macromolecules such as DNA and proteins have been probed. When probing more complex systems, however, such as cells or protein networks, the 3D character of these materials requires more flexibility in manipulating particles. With holographic optical tweezers, multiple optical traps can be manipulated independently in three dimensions in real time, adding this necessary flexibility to the interactive control over multiple particles. Thus far, however, holographic tweezers have not been an accepted tool in the biophysics community, in large part due to lack of evidence as to how exerted forces vary as the positions of holographic traps are changed.
To perform quantitative force measurements, parameters such as trap stiffness and its position dependence, range of trap steering, and minimum step size are of key importance. Here, we systematically characterize the stiffness of traps within our holographic tweezers setup, in which high-speed (>kHz) camera imaging is used for particle position detection. We create multiple traps and steer one or more over small and large distances, and find that over a range of ~25 μm the trap stiffness does not change significantly. Also, we determine the efficiency with which the laser power is directed towards intended traps. In addition, we control and detect trap displacements to ~1 nm, comparable to the position detection limit of our system. Our results suggest that after full characterization, holographic optical tweezers can be successfully employed in quantitative experiments on biomaterials, e.g., probing elastomeric properties of structural protein networks.

1990-Plat
Measuring the Molecular Scale Dynamics of Protein Receptor Endocytic Trafficking in Neural Cells using Quantum Dot Bioconjguate Probes
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Protein trafficking is critical in neurons since neurons must orchestrate the movement of a plethora of discrete intracellular signaling proteins from the cell body to the ends of their axons, over distances that may span up to several meters. A problem in studying protein trafficking has been a lack of tools to visualize the movement of discrete proteins inside live neurons, in real time. An integrated understanding of endocytic trafficking at the level of single or small numbers of receptor complexes inside live cells is currently hampered by technical limitations. Here, we develop and apply quantum dot QD bioconjugates for imaging discrete receptor endocytic events inside live neural cells. QD probes can bind with specific cognate receptors consequently cell signaling cascades to regulate neural sprouting. Furthermore, QD-receptor complexes are internalized by cells dynamically traffic discrete receptor bound QDs on their membrane surface as well as along vast distances along intracellular microtubule tracks of neural processes. Using single particle tracking and immuno-colocalization, we illustrate and validate the use of QD-receptor complexes for imaging receptor trafficking at synchronized time points after QD-receptor binding and internalization (t= 15-150 minutes). The unique value of these probes is illustrated by new dynamic observations: 1) that endocytosis proceeds at strikingly regulated fashion, and 2) that diffusive and active forms of transport inside cells are rapid and efficient. QDs are powerful intracellular probes that can provide investigators with new capabilities and fresh insight for studying endocytic receptor signaling events, in real time, and at the resolution of single or small numbers of receptors in live cells.

Platform AN: Actin & Actin-binding Proteins

1991-Plat
Depletion of F-actin Near Surfaces
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Proximity to membranes is required of actin networks for many key cell functions, including mechanics and motility. However, F-actin rigidity should hinder a filament's approach to surfaces. Using confocal microscopy, we monitor the distribution of fluorescent actin near non-adherent glass surfaces. Initially uniform, monomers polymerize to create a depletion zone where F-actin is absent at the surface but increases monotonically with distance from the surface. At its largest, depletion effects can extend >35 μm, comparable to mass-weighted filament lengths. Increasing the rigidity of actin filaments with phalloidin increases the extent of depletion, whereas shortening filaments using capping protein reduces it proportionally. In addition, depletion kinetics are faster with higher actin concentrations, consistent with faster polymerization and faster Brownian-ratchet-driven motion. Conversely, the extent of depletion decreases with actin concentration, suggesting that entropy is the thermodynamic driving force. Quantitatively, depletion kinetics and extent match existing actin kinetics, rigidity and lengths. However, explaining depletion profiles and concentration-dependence (power-law of -1) requires modifying the rigid rod model. Dynamically crosslinked and dendritic (ARP2/3) networks either slow or enhance the extent of depletion, respectively. In cells, surface depletion should slow membrane-associated F-actin reactions another ~10-fold beyond hydrodynamic considerations, and to favor membrane invaginations by decreased surface tension. Similar depletion principles underlie the thermodynamics of all surface-associated reactions with mechanical structures, ranging from DNA to filaments to networks. For various functions, cells must actively resist the thermodynamics of depletion.

1992-Plat
Interactions of WASp Nucleation Promoting Factors with Fission Yeast Arp2/3 Complex
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Arp2/3 complex mediates the formation of actin filament branches during endocytosis and at the leading edge of motile cells. The minimum requirements to reconstruct Arp2/3 complex mediated nucleation in vitro are actin monomers, a nulceation promoting factor (NPF), mother actin filaments and Arp2/3 complex. Although several reaction parameters have been measured, the pathway of branch formation from these reactants is still ambiguous owing to missing parameters. We use the CA motifs from the C-terminus of the fission yeast NPF, Wsp1p, to investigate the effect of actin filaments on the interaction between Arp2/3 complex and VCA. Actin filaments increase the affinity of CA for Arp2/3 complex 6-fold (Kd=300nM without filaments and 50nM with filaments). Equilibrium binding experiments and isothermal titration calorimetry both indicate that Arp2/3 complex binds two CAs with different affinities. These results show that the mechanism of Arp2/3 complex mediated actin nucleation involves CA binding to two different sites.

1993-Plat
Nanotether Extrusion to probe Membrane-Cytoskeleton Interaction in Model Systems
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We have recently introduced a method allowing the reconstitution of a dynamic actin cortex inside a liposome (Pontani et al., Biophysical Journal, in press). Liposomes encapsulating the cellular machinery required for actin polymerization are prepared with the inverse emulsion technique and actin polymerization is triggered at the liposome internal membrane. Those liposomes can thus model the cellular cortex. To extrude membrane tethers from liposomes, we use a novel experimental setup combining micropipette aspiration and optical tweezers in a confocal microscope. The variation of the tether extrusion force at various membrane tension allow the measurement of the adhesion energy between the membrane and the cytoskeleton. We can also measure the elastic properties of the actin cortex by monitoring its deformation as a function of the aspiration pressure in the micropipette. This mechanical characterization of liposomes encapsulating an actin cortex opens the way to their further use as model systems to study cellular plasma membrane.