1052-Plat
Intracellular Delivery and Fate of Peptide-Capped Gold Nanoparticles
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Gold nanoparticles (NPs) have extraordinary optical properties that make them very attractive single molecule labels. Although understanding their dynamic interactions with biomolecules, living cells and organisms is a prerequisite for their use as *in situ* sensors or actuators. While recent research has provided indications on the effect of size, shape, and surface properties of NPs on their internalization by living cells, the biochemical fate of NPs after internalization has been essentially unknown. Here we show that peptide-capped gold NPs enter mammalian cells by endocytosis. We demonstrate that the peptide layer is subsequently degraded within the endosomal compartments through peptide cleavage by the ubiquitous endosomal protease cathepsin L. Preservation of the peptide layer integrity and cytosolic delivery of NPs can be achieved by a combination of cathepsin inhibition and endosome disruption. This is demonstrated using a combination of distance-dependant fluorescence unquenching and photothermal heterodyne imaging. These results prove the potential of peptide-capped gold NPs as cellular biosensors. Current efforts focus on in-vivo labeling of NPs, nanoparticle-based real-time sensing of enzyme activity in living cells, and the development of photothermal microscopy for single nanoparticle imaging in living cells.

1053-Plat
Photoactivatable Azido Push-Pull Fluorophores for Single-Molecule Imaging in and out of Cells
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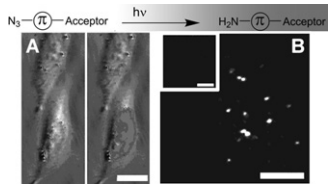
We have designed a series of photoactivatable push-pull fluorophores, single molecules of which can be imaged in living cells. Photoactivatable probes are needed for super-resolution imaging schemes that require active control of single-molecule emission. Dark azido push-pull chromophores have the ability to be photoactivated to produce bright fluorescent labels. Activation of an azide functionality in the fluorogens induces a photochemical conversion to an amine, thus restoring fluorescence. Moreover, photoactivated push-pull dyes can insert into bonds of nearby biomolecules, simultaneously forming a covalent bond and becoming fluorescent (fluorogenic photoaffinity labeling). We demonstrate that the azide-to-amine photoactivation process is generally applicable to a variety of push-pull chromophores, and we characterize the photophysical parameters including photoconversion quantum yield, photostability, and turn-on ratio. Azido push-pull fluorogens provide a new class of photoactivatable single-molecule probes for fluorescent labeling and super-resolution microscopy.
(top) Photoconversion of an azido push-pull fluorogen produces a fluorescent amino molecule. (A) Living cells incubated with an azido fluorogen before and after photoactivation. Scalebar approximately 15 um. (B) Image of single molecules in a polymer film immediately after photoactivation. Inset is the frame immediately before activating. Scalebars approximately 2 um.



1054-Plat
Confocal, 3D Tracking of Single Quantum Dots: Following Receptor Traffic and Membrane Topology
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We have constructed a new confocal fluorescence-microscope that uses active feed-back and a unique spatial filter geometry to follow individual fluorescent quantum dots as they diffuse throughout 3 dimensional space at rates faster than most intracellular transport processes (~microns/second) {Lessard et al. Appl. Phys. Lett., 91, 2007; Wells et al. Anal. Chem., 80, 2008}. This system can follow individual molecular motion over an extended X, Y, and Z range (tens of microns), enabling one to study the transport of individual fluorescently labeled biomolecules (proteins, DNA, or RNA) performing their functions inside living cells. Our preliminary investigations in this area are focused on the spatial dynamics of the IgE receptor Fc{epsilon}RI on rat mast cells, an important signaling molecule for the allergic response. We find the types of motion of this receptor on the surface are highly heterogeneous, with substantial and measurable excursions in all three spatial dimensions (X, Y, and Z). The types of motion seen are consistent with prior studies of two dimensional membrane diffusion of this receptor {Andrews et al., Nat. Cell Biol., 10, 2008}. In contrast to CCD camera based approaches to single particle tracking, the use of single element detectors enables one to record the arrival time of individual photons with ~100 picoseconds resolution, enabling time-resolved spectroscopy to be performed on the molecules being tracked. We have used this added temporal information to measure changes in the emission lifetime as a function of position and positively identify single quantum dots via photon-pair correlations (photon anti-bunching). Since the timing of individual photons are recorded with 100 picoseconds resolution and 3D trajectories are recorded for periods up to minutes, this system bridges the enormous time-scale difference between fast biomolecular conformational fluctuations and cellular signaling processes.

1055-Plat
High Precision Tracking of Intracellular Transport with Fluorescent Nanoparticles
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To grow and maintain eukaryotic cilia and flagella, axonemal precursors and membrane proteins are moved continuously along the unipolar array of outer microtubules by intraflagellar transport (IFT). To understand the role of dynein and kinesin motors in this process, we have used paralyzed flagella mutants of Chlamydomonas as a model organism. Flagella was rigidly immobilized to a glass coverslip and fluorescent nanoparticles were attached to transmembrane component of moving cargoes. Fluorescent signals of nanoparticles were tracked by ultrahigh spatial (~1 nm error) and temporal (600 microsecond) resolution. Cargoes were moved by 8 nm steps towards both directions, in agreement with kinesin and dynein step sizes. Movement was highly directional (no backward steps) which is different from other bidirectional transport systems, i.e. axonal transport in neurons. The bead usually changes the direction of movement at the tip of the flagellum and change in direction in the middle of the flagella was less common. Remarkably, just before the change in direction we observed high fluctuations in position for ~50 msec. The cargo then abruptly starts moving on the opposite direction without showing any forward-backward stepping. The results imply that the motors responsible of the transport can be switched on and off quickly in turnaround zones by the cell so that they do not compete against each other to determine the direction of cargo transport.

1056-Plat
Intracellular Myosin Motor Protein Motion Using Laser Scanning Confocal Microscopy
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We focus on live cell single molecule imaging and tracking with particular focus on measuring single myosin V steps along actin filaments within live cells. The goal of the work is to acquire information that will offer new insights into the mechanism of these motors' processivity. Conventionally, single molecule Myosin studies have been accomplished through total internal reflection fluorescence microscopy (TIRF).[1,2] However, TIRF methods have very limited applicability to live cell *in vivo* studies. As such, we apply confocal laser scanning microcopy (CLSM) to imaging and tracking of Myosin V. CLSM also offers the capability to provide full 3-dimensional tracking of biomolecular motors. Our experiments have confirmed step size results from previous TIRF experiments. In terms of instrumentation, we overcome the nominally slow scanning speed of CLSM by the use of a fiber scanning technique that allows us to scan with image acquisition times of less than 100 ms. We make use of home-made streptavidin-functionalized quantum dots in conjunction with this tracking technique to increase the quantum efficiency and stability of the fluorophores. The QD fabrication and sample preparation techniques will also be presented.
1. A. Yildiz, J. N. Forkey, S. A. McKinney, T. Ha, Y. E. Goldman, and P. R. Selvin, "Myosin V walks hand-over-hand: Single fluorophore imaging with 1.5-nm localization," Science **300** (5628), 2061-2065 (2003).
2. T. Sakamoto, A. Yildez, P. R. Selvin, and J. R. Sellers, "Step-size is determined by neck length in myosin V," Biochemistry **44** (49), 16203-16210 (2005).

1057-Plat
Single Quantum Dot Trajectory Analysis: Beyond the Single Diffusion Mode Model
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