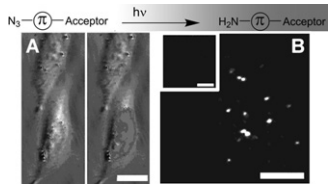
1052-Plat
Intracellular Delivery and Fate of Peptide-Capped Gold Nanoparticles
Yann Cesbron[1], Violaine Sée[1], Paul Free[1], Paula Nativo[1], Umbreen Shaheen[1], Daniel J. Rigden[1], David G. Spiller[1], David G. Fernig[1], Michael R.H. White[1], Ian A. Prior[1], Mathias Brust[1], Brahim Lounis[2], Raphaël Lévy[1].
[1]University of Liverpool, Liverpool, United Kingdom, [2]Université Bordeaux 1/CNRS, Talence, France.
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
Samuel J. Lord[1], Nicholas R. Conley[1], Hsiao-lu D. Lee[1], Marissa K. Lee[1], Na Liu[2], Reichel Samuel[2], Robert J. Twieg[2], W.E. Moerner[1].
[1]Stanford University, Stanford, CA, USA, [2]Kent State University, Kent, OH, USA.
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
James H. Werner[1], Nathan P. Wells[1], Guillaume A. Lessard[1], Mary E. Phipps[1], Patrick J. Cutler[2], Diane S. Lidke[2], Bridget S. Wilson[2].
[1]Los Alamos National Laboratory, Los Alamos, NM, USA, [2]University of New Mexico, Albuquerque, NM, USA.
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
Ahmet Yildiz[1], Shengmin Shih[1], Fatih Kocabas[2].
[1]University of California Berkeley, Berkeley, CA, USA, [2]University of Texas Southwestern Medical School, Dallas, TX, USA.
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
Patrick Moyer, **Ryan Hefti**.
University of North Carolina at Charlotte, Charlotte, NC, USA.
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
Xavier Michalet, Fabien Pinaud, Shimon Weiss.
UCLA, Los Angeles, CA, USA.

Single quantum dots are increasingly used for single-molecule imaging, thanks to their brightness, functionalization versatility and relatively small size. So far, however, experimentalists have limited themselves to standard analysis methods developed for single-dye imaging, which are notoriously short-lived, and require statistical analysis of many trajectories. We show that there is much more information to be extracted from long single quantum dot trajectories than afforded by these standard methods. In particular, we show by simulations and using real data obtained in live cells that it is possible to identify the presence of several diffusion modes within a single trajectory and characterize these different diffusion modes quantitatively. Our approach is based on the probability distribution of square displacements measured for different time lags. We use both simulated data and live cell single quantum dot tracking data to illustrate the capability of our method and compare it to other commonly used techniques. This method was recently used to demonstrate the existence of two diffusion regimes for GPI-anchored proteins in the membrane of HeLa cells, identified respectively with diffusion in and out of raft domains (1).
Reference:
1. Pinaud et al., Traffic 10 (2009) 691.

1058-Plat
Far Field Fluorescence Super Resolution Imaging of Molecular Scale Biological Structures
Alexandros Pertsinidis[1], Yunxiang Zhang[1], Steven Chu[1,2].
[1]UC Berkeley, Berkeley, CA, USA, [2]US Department of Energy, Washington DC, DC, USA.
We have recently demonstrated measurements of relative distances between individual, spectrally distinct fluorescent probes that are attached on a biological molecule with precision and accuracy <1nm. Here we extend this technique to measure relative distances between two (or more) fluorophores of the same spectral characteristics.
We show that the improved resolution afforded by our microscope imaging system allows characterizing biological structures at the molecular scale. In one application we applied our technique to image individual dimers of Endothelial cadherin (E-cadherin) molecules. At 1mM Ca++ we find the majority of the dimers in an extended configuration, consistent with the crystal structure of the full C-cadherin ectodomain. Interestingly, we observe a separate population in a less extended configuration, possibly related to flexibility of the E-cadherin binding interface. As a function of the free Ca++ concentration we observe a continuous, cooperative transition of the extension of the molecules from the rigid extended configuration to a collapsed state. The apparent Kd=70uM and degree of cooperativity ~1.5 from our measurements are consistent with various previous indirect investigations (e.g. intrinsic fluorescence, proteolytic sensitivity, circular dichroism, NMR etc).
These results clearly demonstrate the power of our approach to image individual molecular scale structures and detect conformational changes at the nanometer scale, establishing it as a unique Structural Biology tool, that can operating in ambient, physiological conditions and is based exclusively on optical far-field fluorescence imaging.

Platform O: Membrane Structure I

1059-Plat
Molecular Organization of Cholesterol in Phospholipids
Andrey Ivankin[1], Ivan Kuzmenko[2], **David Gidalevitz**[1].
[1]Illinois Institute of Technology, Chicago, IL, USA, [2]APS, Argonne National Laboratory, Argonne, IL, USA.
Cholesterol-enriched domains in cell membranes are involved in a wide variety of cellular activities including protein sorting, signal transduction, and host-pathogen interactions. Several models describing the structural organization of cholesterol-phospholipid membranes have been proposed, but consensus on cholesterol-lipid organization within the cell membrane has not yet been reached. For instance, the "superlattice" model suggests regular distribution of cholesterol in a hexagonal lipid matrix, whilst the condensed complex model states that cholesterol and lipids form complexes only at a particular stoichiometry. Moreover, current models constrain cholesterol placement to exclusively being within the lipid acyl chains regardless of its mole fraction (χ_{CHOL}). Here we present a systematic structural study of cholesterol-dipalmitoylphosphatidylcholine (DPPC) mixed monolayers at the air-liquid interface by surface pressure-molecular area Langmuir isotherms, epifluorescence microscopy, X-ray reflectivity (XR) and grazing incident-angle X-ray diffraction (GIXD) using synchrotron radiation. The electron density profile across the monolayer, derived from XR data, demonstrates that the vertical position of cholesterol relative to phospholipids strongly depends on χ_{CHOL}. At a range of sterol concentrations, cholesterol and DPPC form new alloy-like mixed domains of short-range order and stoichiometry defined by the overall molecular composition of the film. Since these data cannot be explained within the existing models, we propose a new model of cholesterol-lipid organization in mixed monolayers that is consistent with both the "condensing" effect of cholesterol and a sharp increase in its chemical activity at $\chi_{CHOL}>0.4$. Our data above all does not support the theory that membrane domains exist as independent ordered lipid/cholesterol entities ready to accommodate protein molecules.

1060-Plat
In-situ Measurement of Cholesterol Transport in Model-Membrane Systems Studied by Time Resolve Small Angle Neutron Scattering and Comparison with MD Simulation
Sumit Garg[1], Lionel Porcar[2], Paul Butler[3], Francisco Castro-Roman[4], Ursula Perez-Salas[5].
[1]Argonne National Lab, Argonne, IL, USA, [2]Institut Laue-Langevin, Grenoble, France, [3]National Institute of Standards and Technology, Gaithersburg, MD, USA, [4]Cinvestav - IPN, Mexico City, Mexico, [5]University of Illinois at Chicago, Chicago, IL, USA.
Cholesterol is essential for a myriad of biological functions, but its excess is toxic. Cholesterol levels are maintained by various cholesterol metabolic pathways which depend critically on its intracellular transport and homeostasis. Any disorder in intracellular cholesterol distribution will lead to diseases, from neurodegenerative, such as Niemann Pick TYPE-C and Alzheimer, to cholelithiasis and atherosclerosis. Unfortunately, the understanding of intracellular transport of cholesterol has lagged behind other aspect of cholesterol metabolism due to limitations in the techniques used to date. These limitations have resulted in reported cholesterol transport rates showing huge inconsistencies. This is due to the fact that these measurements are not done in-situ, but rather require biochemical isolation of cholesterol-donor and cholesterol-acceptor vesicles. Another important limitation has been the need for a biochemical tag on cholesterol which has a significant effect on the resulting rates of transfer. This work presents in-situ Time-Resolved Small Angle Neutron Scattering (in-situ TR-SANS)studies of the Intra- and Inter-membrane cholesterol exchange rates in POPC model vesicles. This technique does not require any biochemical tag and transport of cholesterol from donor to acceptor vesicles can be measured continuously by following the changes in the scattering intensity. Interestingly our approach finds that trans-membrane flipping rates of cholesterol are much slower without any foreign particles in contrast to high flipping rates reported in literature. Molecular dynamics simulations have also been performed to investigate the energetic and kinetic behavior of cholesterol. We found that simulation results are in agreement with our SANS results, providing a more detailed thermodynamic description at the molecular level. Such a synergistic approach combining TR-SANS and MD simulation will provide new insight into the ongoing efforts of understanding cholesterol traffic and related disorders.

1061-Plat
Sterol Transfer from Vesicles to MBCD is Governed by the Extent of Sterol Superlattice
Berenice Venegas, Parkson L.-G. Chong.
Temple University School of Medicine, Philadelphia, PA, USA.
Cholesterol transfer regulates the intracellular distribution and the metabolism of cholesterol, thus having a direct impact on cholesterol homeostasis in cells. This work investigates the effect of lipid lateral organization on sterol transfer from liposomes to methyl-B-cyclodextrin (MBCD), a water-soluble macrocyclic compound able to deplete sterols from membranes. Large unilamellar vesicles (LUVs) composed of POPC, dehydroergosterol (DHE) and Dansyl-PE were examined. DHE content was varied in steps of 0.4 mol% in a range of concentrations covering the theoretically predicted critical mole fractions (C_r,e.g., 20.0, 22.2, 25.0, 33.3, 40.0 and 50.0 mol%) for maximal sterol superlattice formation. The molar ratio of DHE to Dansyl-PE was kept constant (15:1) in all samples. The rate of sterol transfer was monitored based on the fluorescence resonance energy transfer (FRET) between DHE (donor) and Dansyl-PE (acceptor). When DHE is transferred from LUVs to MBCD, FRET efficiency is decreased and, consequently, the fluorescence intensity of Dansyl-PE is decreased over time. The initial rate of the DHE transfer was found to vary with DHE content in the original membrane in a biphasic manner, reaching a local maximum at all C_r examined. This result demonstrates that the rate of DHE transfer from LUVs to MBCD is governed by the extent of sterol superlattice in the liposomal membrane, a conclusion different from that given in a previous study[1]. Both studies showed the same general trend, i.e., that sterol chemical potential increases with increasing sterol content. The difference lies in the fine details of how sterol transfer rate varies with sterol content in the immediate vicinity of C_r (AHA, DOD and PDOH).
[1] Ali *et al.* (2007) PNAS 104:5372-7.