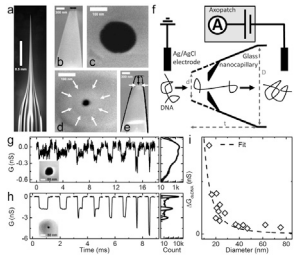
MspA's narrow constriction, we observe currents unique to the bases within the constriction. We find that both, thermal fluctuations and the geometry of MspA, ensure that these currents are governed by ~4 sequential nucleotides. Furthermore, we find that Angstrom-level changes in the position of the DNA within the constriction considerably influence these currents. By varying the voltage we can stretch the DNA and thereby adjust the average position of the DNA within the pore. Our findings improve nanopore sequencing and highlight the power of nanopore technology to probe Angstrom-level distance scales.

3198-Plat
Shrinking Nanocapillaries to Low Noise Nanopores for Single Molecule Detection
Lorenz J. Steinbock, Swati Krishnan, Roman Bulushev, Aleksandra Radenovic.
EPFL, Lausanne, Switzerland.
Glass nanocapillaries (Figure a) can be shrunken under an electron beam of an SEM. Figure b and c illustrate the tip of an unmodified nanocapillary from the side and the top. The tip is approximately 230 nm broad (black arrow) and the orifice measures 190 nm in diameter. After 4 minutes of irradiation the capillary has shrunken in horizontal (white arrow in e) and vertical direction (black arrow in e). The tip is now 180 nm broad and the orifice 25 nm big. The capillary is then placed into a resistive pulse setup with a current amplifier. Scheme in f shows the setup and the effect of the shrinking process. DNA is added to the reservoir in front of the nanocapillary and is translocated through the glass nanopore by applying a potential. This translocation causes conductance decreases as demonstrated in Figure g and h. For smaller diameters bigger decreases in the conductance can be measured. Figure i shows the conductance decrease caused by one DNA molecule as a function of the diameter. A model was developed to simulate this dependence, which shows good agreement with the data (fit in i).



3199-Plat
Novel Quantum Dot Probes for Single-Molecule Biophysics
Sara M. Wichner[1], Mark A. DeWitt[1], Bruce E. Cohen[2], Ahmet Yildiz[1].
[1]University of California, Berkeley, Berkeley, CA, USA, [2]Lawrence Berkeley National Lab, Berkeley, CA, USA.
Quantum dots (QDs) have proved to be invaluable fluorophores across many in vitro microscopy applications because of their greater photostability and brightness than organic dyes and fluorescent proteins. However, commercially available QDs are ~20 nm in diameter due to their bulky passivating layer, a diameter which is prohibitively large for use in small or confined systems. We have developed a novel wrapping and functionalizing procedure of hydrophobic QD core-shells. Our small QDs have similar brightness and water solubility to commercially available QDs and their size (6-8 nm in diameter) is comparable to that of GFP. Development of small QDs enabled us to study the stepping mechanism of kinesin using two different colors at high spatiotemporal resolution. These studies provided further insight into the coordination mechanism between the two kinesin heads during processive motility. We anticipate that our small QDs will allow high signal-to-noise measurements in a diverse range of systems currently inaccessible via bulky hydrophilic quantum dots.

3200-Plat
Split-FP Conjugated Metal Nanoparticle Raman Nanoprobes for Ultra-Sensitive Molecular Detection
Tugba Koker, Fabien Pinaud.
University of Southern California, Los Angeles, CA, USA.
The recent convergence of biotechnology and nanotechnology now provides unique means to develop hybrid nanomaterials for advanced biosensing applications. In particular, the controlled assembly of metal gold (Au) and silver (Ag) nanoparticles using bio-inspired scaffolds is a promising approach for the design of novel Surface Enhanced Raman Spectroscopy (SERS) nanoprobes capable of single molecule detection sensitivity. Here, we propose a new design for colloidal Raman nanoprobes based on split-FP/metal nanoparticle hybrid composites. We use split fluorescence proteins (split-FPs) as both activatable Raman reporters and molecular glue to assemble Au or Ag nanoparticles into photonically-active SERS nanoclusters. We expressed recombinant split-FPs (sGFP, sYFP, sCFP) with metal binding domains and engineered their complementary peptide fragments for oriented and controlled grafting at the surface of Au and Ag nanoparticles. These split-FPs fragments were respectively coated on various size of metal nanoparticles (5-50nm diameter), and were characterized by electrophoresis, HPLC, Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), and immunoblotting. In vitro, complementary metal nanoparticles functionalized with split-FP fragments can self-assemble and form nanoclusters as confirmed by electrophoresis and TEM measurements. To improve the complementation kinetics of split-FP/peptide pairs on nanoparticles, various point mutations were introduced in split-FPs. We further show that SERS signals from split-FPs are specifically detected upon biomolecular interactions of split-FP fragments and that the unique Raman signature of the FP chromophores is easily distinguished over other protein modes when bound to plasmonic nanomaterials. These results demonstrate the potential for highly selective and sensitive SERS detection based on split-FP/metal nanoparticle hybrid probes.

Platform: Membrane Structure

3201-Plat
Superresolution Microscopy Reveals Nanometer-Scale Reorganization of MG53 Associated with Membrane Repair
Mingzhai Sun[1], Jiaqing Huang[2], Kristyn Gumpper[1], Gejing De[1], Matthew Sermersheim[1], Pei-Hui Lin[1], Haichang Li[1], Pu Duann[1], Jianjie Ma[1].
[1]Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, USA, [2]Department of Electrical and Computer Engineering, The Ohio State University, Columbus, OH, USA.
Repair of acute damage to the plasma membrane is an important aspect of normal cellular physiology and disruption of this process can result in pathophysiology in a number of human diseases. MG53, a tripartite motif family protein discovered in our lab, is an essential component of the acute membrane repair machinery. It functions as a sensor of oxidation that occurs at the injured cell membrane sites. Upon membrane damage, intracellular vesicles containing MG53 translocate and accumulate to the injured sites to form membrane repair patches. When cells are at resting states, MG53 distributes both in the cytoplasm and at the cell surface through the phosphatidylserine (PS) binding motif. Through the use of superresolution single-molecule fluorescence microscopy, we showed that MG53 is organized in nanometer-scale clusters of ~25 nm at the surface of resting cells. At the re-sealed membrane injury sites, clusters that are as big as 100 nm are observed with higher local protein density, consistent with our previous biochemical studies that MG53 forms oligomers upon oxidation. We also investigate two mutant forms of MG53 - the Cys242 point mutation (C242A) and the leucine zipper motif mutant (LZ12), both of which show defective membrane repair capability due to the disrupted disulfide bond formation and impaired oligomerization respectively. Both C242A and LZ12 mutants show decreased cluster size and local protein density at the surface of resting cells.

3202-Plat
Investigating the Cell Membrane via Single Particle Tracking, Bayesian Inference and Hydrodynamic Force Application
Maximilian U. Richly[1], Silvan Türkcan[1], Cedric Bouzigues[1], Michel R. Popoff[2], Jean-Baptiste Masson[3], Jean-Marc Allain[4], Antigoni Alexandrou[1].
[1]Laboratoire d'Optique et Biosciences, Ecole Polytechnique, PALAISEAU Cedex, France, [2]Unité Bactéries Anaérobies et Toxines, Institut Pasteur, Paris, France, [3]Physics of Biological Systems, Institut Pasteur, 75015 Paris, France, [4]Laboratoire de Mécanique des Solides, Ecole Polytechnique, 91128 Palaiseau, France.
We investigate the potential felt by membrane receptors inside membrane microdomains via tracking of single receptors labelled with rare-earth doped luminescent nanoparticles and Bayesian inference analysis of the recorded trajectories. We demonstrated that the potential felt by peptidic toxin receptors confined in lipid rafts is well described by a second-order polynomial potential, possibly due to an inhomogeneous lipid and protein distribution [Türkcan et al., Biophys. J. 2012] In contrast, the potential experienced by transferrin receptors in cytoskeleton-delimited microdomains is localized at the border of the confinement domain. Using the inference approach, we extract the hopping energy, i. e. the barrier height, between adjacent microdomains [Türkcan et al., PLoS One 2013]. We also investigate the interaction of lipid rafts with the cytoskeleton via a hydrodynamic drag force applied on the labelling nanoparticle by a liquid flow inside a microchannel [Türkcan et al., Biophys. J., 2013]. We demonstrate that the receptors are displaced, together with their confining raft platforms, over distances comparable to the cell size against elastic barriers. The number and stiffness of the encountered barriers indicate that they are part of the actin cytoskeleton.