perfluorocarbon is lower than that of water, rendering the hydrosomes trappable by a conventional optical tweezer. Using the injector and optical manipulation we demonstrate fusion of two hydrosomes, one containing a calcium-sensitive dye and the other containing calcium chloride, and we measure the mixing times to be on the order of 10 millisecond or less. The monodispersity, repeatability, small size, and fast mixing afforded by this system offer unprecedented opportunities for nanochemistry and single molecule or single nanoparticle studies.

139-Pos Board B18

Simultaneous Measurement Of Ionic Current And Fluorescence From Single Protein Pores

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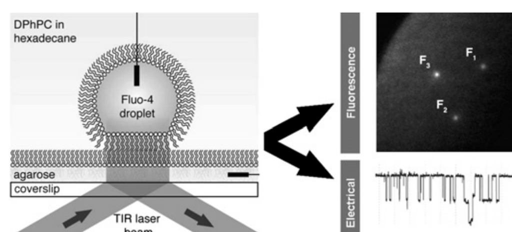
The ability to simultaneously monitor both the ionic current and fluorescence from membrane proteins has the potential to link structural and functional changes in a protein. However, demonstrating the synchronised detection of current and fluorescence at the single molecule level remains a serious challenge. We present a new method for simultaneously measuring single-channel electrical currents and fluorescence from ion channels by using an in vitro water-in-oil droplet bilayer system. We demonstrate fluorescence and electrical detection of stochastic blocking by cyclodextrin in multiple staphylococcal alpha-hemolysin pores. The combined fluorescence signal from individual pores exhibits the same sequence of blocking events as the total current recording, providing unambiguous correlated fluorescence and electrical signals for every protein pore in a bilayer.



140-Pos Board B19

Laser Induced Popcorn-like Conformational Transition of Nano-diamond as a Nanoknife

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Nanodiamond (ND) is surrounded by layers of graphite on its surface. This unique structure feature creates unusual fluorescence spectra, which can be used as an indicator to monitor its surface modification. Meanwhile, the impurity, nitroso (C-N=O) inside the ND can be photolyzed by two-photon absorption, releasing NO to facilitate the formation of a sp^3 diamond structure in the core of ND and transforming it into a sp^2 graphite structure. Such a conformational transition enlarges the size of ND from 8 nm into 90 nm, resulting in a popcorn-like structure. This transition reaction may be useful as nano-knives in biomedical application.

141-Pos Board B20

High throughput Single-Molecule Spectroscopy Using Nanoporous Membranes

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We describe a novel approach for optically detecting DNA translocation events through an array of solid-state nanopores that potentially allows for ultra high-throughput, parallel detection at the single-molecule level. The approach functions by electrokinetically driving DNA strands through sub micrometer-sized holes on an aluminum/silicon nitride membrane. During the translocation process, the molecules are confined to the walls of the nanofluidic channels, allowing 100% detection efficiency. Importantly, the opaque aluminum layer acts as an optical barrier between the illuminated region and the analyte reservoir. In these conditions, high-contrast imaging of single-molecule events can be performed. To demonstrate the efficiency of the approach, a 10 pM fluorescently labeled lambda DNA solution was used as a model system to detect simultaneous translocation events using electron multiplying CCD imaging. Single-pore translocation events are also successfully detected using single-point confocal spectroscopy.

142-Pos Board B21

Using Nanomaterials to Probe Rotation of Individual Cell Surface Receptors

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Rotation of membrane proteins is a sensitive measure of their aggregation state and their interactions with other membrane species. We have explored the use of nanomaterials, including both gold nanorods (GNR) and asymmetric quantum dots (QD), as non-bleaching imaging probes of the rotation of individual cell surface proteins. GNR exhibit resonant light scattering between 700-800 nm which is highly polarized along the rod axis. Orthogonally-polarized, dark-field images of GNR immobilized on glass contain polarized spots suggesting randomly-oriented individual rods. However, imaging individual antibody-conjugated GNR on cell surfaces has been problematic. QD can also provide orientation-dependent optical signals: one commercial product, rod-like in shape, exhibits an initial fluorescence anisotropy >0.2. These QD, conjugated to anti-insulin receptor β-chain antibody, are easily visualized bound to 2H3 cell insulin receptors (IR) as well as immobilized on glass. Blinking of spots in orthogonally-polarized fluorescence images demonstrates imaging of individual QD. The time-autocorrelation function for fluorescence anisotropy from individual cell-bound QD exhibits a slow component decaying on the 100-200 ms timescale. Whether this represents intrinsically-hindered rotation of individual IR or results from crosslinking of multiple receptors by single QD remains to be determined. Supported in part by NIH grant RR023156 and NSF grant CHE-0628260.

143-Pos Board B22

Manipulating The Environment Of a Single Lipase Molecule

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Lipase are interfacial enzymes with attractive applications. Their activity is greatly enhanced in the presence of a hydrophobic surface, a process called interfacial activation. However, the kinetics of this behavior is not yet fully understood. We measured this kinetics of a lipase from Thermomyces Lanuginose at single enzyme level. We utilized single vesicle arrays as a novel biocompatible scaffold to immobilize enzymes and as an interface to study the effect of the enzyme's binding to the membrane on the observed activity. We used organic polymers to vary the accessibility of the enzyme molecule to the bilayer. By this kind of direct control of the microenviroment of the enzyme, we quantified the activity of enzyme at different degrees of accessibility to the bilayer. Therefore, we gained a clear insight of interfacial activation of lipase and we are currently using this platform to quantify the influence of additional parameters, such as size of vesicles and lipid composition, on enzyme's catalytic behavior.

144-Pos Board B23

Single Molecule Studies of the Interactions between β-Amyloid 1-40 and Supported Planar Lipid Membrane

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Recent evidence supports the hypothesis that the early oligomers formed by the β-Amyloid peptide are cytotoxic and may feature in Alzheimer's disease (AD). While the mechanism of this cytotoxicity remains unclear, interactions of these oligomers with neuronal membrane are believed to be involved. Identifying the cytotoxic species is challenging because the β-Amyloid oligomers are extremely heterogeneous, metastable, and form at very low physiological concentrations (nM). In our study, we use single molecule spectroscopy (SMS) to study the interactions between β-Amyloid 1-40 and supported planar lipid membranes. The evolution of β-Amyloid species on lipid membranes were monitored for up to a few days. The results indicate that the interactions between β-Amyloid 1-40 and planar lipid membranes follow three stages. First, a very small fraction of β-Amyloid peptide binds to the membranes with high affinity (Kd $<$ 470pM), covering the membrane surface uniformly and also diffusing within the lipid molecules. In the second stage, β-Amyloid peptides assemble to form oligomers in the membrane. We observed at least two different pathways of oligomer formation, depending on the aqueous β-Amyloid peptide concentration. In the final stage, after prolonged incubation with the lipid membranes, β-Amyloid peptides start forming mesh-like deposits. With the high sensitivity and spatial and temporal resolution of single molecule spectroscopy, we successfully traced the interactions between β-Amyloid 1-40 peptide and planar lipid membranes at the molecular level. Our results are also in agreement with the molecular model of pore-forming peptides suggested by H.W. Huang et al. (1).

1. Huang, H.W., F.Y. Chen, and M.T. Lee. 2004. Molecular Mechanism of Peptide-Induced Pores in Membranes. Physical Review Letters. 92:198304.