

investigation. Single molecular TIRF microscopy was used to measure the translational diffusional coefficient of Alexa488 labeled monomeric PLB reconstituted into a supported lipid bilayer. The diffusional coefficient of monomeric PLB is $0.7 \mu\text{m}^2/\text{s}$, which is consistent with its molecular weight. Time-resolved phosphorescence anisotropy of erythrosin iodoacetamide (ErIA) labeled SERCA in cardiac sarcoplasmic reticulum (SR) was measured with and without phosphorylation of PLB in presence of high and low Ca concentrations. Phosphorylation of PLB decreased the final anisotropy of ErIA labeled SERCA at low Ca, indicating decreased SERCA self-association. This supports the proposal that PLB inhibits SERCA by inducing SERCA-SERCA association, which is relieved by phosphorylation.

231-Pos Board B110

Single molecule measurements of ATP-myosin V and ADP-myosin V

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We investigate the conformations of myosin V bound to ATP and to ADP via single molecule FRET measurements. The myosin V is labeled with FIAsh in the upper 50kDa domain, and the bound nucleotides are labeled with Rhodamine 101. We have carried out two types of single molecule FRET measurements on this complex: 1) we record the transit of single molecules diffusing through the focal region of a probe laser (473 nm); 2) we record the time trajectory of each molecule while it is encapsulated within an optically trapped femtoliter aqueous nanodroplet (hydrosome). In the latter measurements, an infrared (1064 nm) optical trap holds a single hydrosome within the focal region of the probe (473 nm) beam, which fluorescently excites the single molecule contained within the hydrosome. Our preliminary results to date indicate that our single molecule FRET measurements are consistent with each other and with previous ensemble measurements.

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Fluorescence Labeling And Purification Of Cellulases For Single Molecule Spectroscopy

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The development of highly-sensitive detectors for optical microscopy has enabled the detection of individual fluorescent molecules and allowed life scientists to probe dynamic and conformational properties of enzymes. In single molecule spectroscopy (SMS) an essential requirement is the use of bright, fluorescent moieties. In this sense, organic dyes are small molecules that can confer fluorescence capabilities without compromising enzymatic activity. However, tracking of a single molecule labeled with a single fluorescent moiety is limited by bleaching time and the number of photons emitted by molecule per second. Thus, in SMS it is desirable to have enzymes labeled with multiple fluorescent moieties while retaining native activities.

Most organic dye labeling techniques produce mixtures of populations of molecules labeled with different numbers of fluorophores. For SMS this poly-dispersity of labeled molecules can introduce significant variability. In addition, each of these labeled populations can have properties different from the native protein or enzyme, which further complicates the interpretation of results derived from SMS. To address this we have developed methods to label and purify enzymes with a variety of organic dyes from the Alexa-Fluor family. Our approach explored labeling in free solution and solid phase. Purification methods developed to remove unbound dye were optimized for each of these labeling methods. Separation of populations of labeled molecules was performed via FPLC and optimized for each one of the enzymes labeled. Through these methods we have produced highly purified populations of cellulases Cel5A, Cel6B, and Cel9A labeled with known numbers of dyes. These populations have been characterized for their degree of labeling, location of the fluorescent moiety, and catalytic activity as compared with the native enzymes. We demonstrate the advantage of the use of fluorescently tagged cellulases with well-known physico-chemical properties through SMS measurements.

233-Pos Board B112

Metal-Enhanced Fluorescence (MEF)

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In recent years our laboratories have described the favorable interactions and outcomes of both plasmon supporting particles (Ag, Au, Cu, Zn, Ni, Cr) and

substrates with electronically excited states. These favorable effects have included enhanced fluorescence emission from singlet states, S_1 and S_2 , as well as enhanced phosphorescence yields from triplet, T_1 , states (MEP). In addition, we have observed and described plasmon enhanced chemiluminescence intensities (MEC), as well as highly directional emission. As a result of enhanced triplet yields, we have also observed both enhanced singlet oxygen and superoxide anion yields.

These favorable influences on the photophysical properties of close proximity excited states to plasmon supporting substrates/particles has led to wealth of biochemical applications, such as the high sensitivity and ultra fast detection of proteins, DNA and ultra bright and photostable metal-enhanced fluorescence based particles for downstream cellular imaging applications. In addition, there are a lot downstream applications of MEP such as in photodynamic therapy by surface plasmon controlled single oxygen generation. Current thinking, describes Metal-Enhanced Fluorescence as the near-field coupling of electronic excited states to surface plasmons (a surface mirror dipole), the particle subsequently radiating the photophysical characteristics of the coupled excited state in the far-field, remarkably, even vibronic structure. In this paper, we communicate our recent findings for metal-fluorophore interactions and our current thinking and progress towards developing a unified metal-fluorophore description.

234-Pos Board B113

Action-Spectra of Electrochromic Voltage-Sensitive Dyes in an Intact Excitable Tissue

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Voltage-sensitive dyes (VSDs) provide a spatially resolved optical read-out of electrical signals in excitable tissues. Several common fluorescent VSDs display electrochromic shifts of their emission spectra, making them suitable candidates for ratiometric measurements of transmembrane voltages. These advantages of VSDs are tempered by tissue-specific shifts to their fluorescence emission. In addition, the optimal electrochromic dye response occurs in wavelength bands distinct from the dye's maximal resting emission. This "action spectrum" can undergo tissue-specific shifts, as well.

We have developed a technique for *in-situ* measurements of the action-spectra of VSDs in intact excitable tissues. Fluorescence emission spectra of VSDs during action potential depolarization were obtained within a single sweep of a spectrophotometer equipped with a CCD array detector. To resolve the subtle electrochromic shifts in voltage-induced dye emission, fluorescence emission spectra measured right before and during field-induced action potential depolarization were averaged over about one hundred trials. Removing white noise contributions from the spectrometer's CCD detector/amplifier via low-pass filtering in Fourier space, the action spectra of all dyes could be readily determined.

235-Pos Board B114

Plasmonic Electricity: A Digital form of Metal-Enhanced Fluorescence

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Fluorescence technologies are entrenched in the biosciences today. In nearly all aspects of fluorescence spectroscopy light is focused and collected by a detector which converts the photon flux into a digital signal which is then displayed. To boost optical signatures many groups have shown that the close proximity of fluorescent species to fluorophores, significantly amplifies the fluorescence signatures many fold, a technology recently described as Metal-Enhanced Fluorescence by the Geddes labs¹. However, hidden within these close-range near field fluorophore-metal interactions is an induced plasmonic current, directly proportional to the excitation irradiance and the concentration of the fluorophores present in the near-field, $< 20 \text{ nm}$. The current can be read directly, opening up huge opportunities for both the amplification and the *direct detection of fluorescence*, i.e. digital fluorescence, such as in solar energy conversion, digital immunoassays (Figure 1), DNA detection

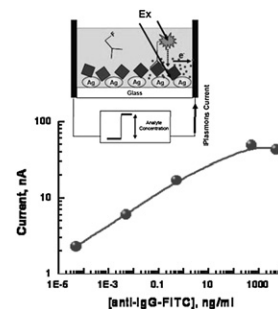


Figure 1. Metal-Enhanced Fluorescence-based digital immunoassays. A model IgG-Anti-IgG assay, demonstrating the direct detection of Fluorescence.