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 $\mu m^2/s$, 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

Shira Stav, Ana Jofre.
University of North Carolina at Charlotte, Charlotte, NC, USA.

We investigate the conformations of myosin V bound to ATP and to ADP via single molecule FRET measurements. The myosin V is labeled with FlAsH 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.

232-Pos Board B111

Fluorescence Labeling And Purification Of Cellulases For Single Molecule Spectroscopy

Jose M. Moran-Mirabal, Stephane C. Corgie, Jacob C. Bolewski, Hanna M. Smith, Larry P. Walker.
Cornell University, Ithaca, NY, USA.

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 a 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)

Yongxia Zhang, Kadir Aslan, Chris D. Geddes.
Umbi, Baltimore, MD, USA.

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

Joseph Foley, Martin Muschol.
University of South Florida, Tampa, FL, USA.

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

Anatoliy Dragan, **Chris D. Geddes**.
University of Maryland Biotechnology Institute, Baltimore, MD, USA.

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 labs[1] 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 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

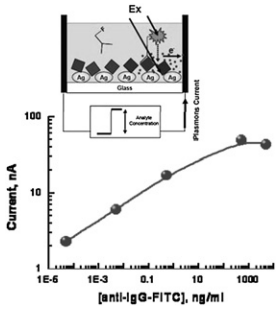

Figure 1. Metal-Enhanced Fluorescence-based digital immunoassays. A model IgG-Anti-IgG assay, demonstrating the direct detection of Fluorescence.

and in fluorescence microscopy. The direct measurement of fluorescence is likely to find profound applications and implications in the biosciences and promises to change both the way we think and use fluorescence spectroscopy today.

1. Metal-Enhanced Fluorescence, edited by Chris D. Geddes, John Wiley and Sons, New Jersey, 2009. - *In Press*.

236-Pos Board B115

Ultrafast Decay of Trp in Biological Macromolecules

Jianhua Xu[1], Olga Tcherkasskaya[1], Angela M. Gronenborn[2], Patrik Callis[3], Dmitri Toptygin[4], Florence K. Gleason[5], Ludwig Brand[4], Jay R. Knutson[1].
[1]National Institutes of Health, Bethesda, MD, USA, [2]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA, [3]Department of Chemistry & Biochemistry, Montana State University, Bozeman, MT, USA, [4]Department of Biology, Johns Hopkins University, Baltimore, MD, USA, [5]Department of Plant Biology, University of Minnesota, St. Paul, MN, USA.

Femtosecond (<300fs fwhm) measurements of fluorescence decay and quenching of Tryptophan (Trp) were performed in a variety of proteins, including GB1, Thioredoxin (both wild type from two species and a human Trx mutant with a single Trp), Cyanovirin and Interleukin-1beta using an upconversion spectrophotofluorometer combined with a time correlated single photon counting apparatus to span the ~200fs to 20ns time scale. Trp is subject both to ultrafast quenching in proteins and spectral energy loss coupled to nearby water dynamics. All fluorescence transients of tryptophan in proteins reveal complex, i.e. multiexponential behavior. In addition to a ''bulk water'' relaxation (~2 ps), a 50 ps fluorescence decay was found in single-Trp thioredoxin which matched the component we had found previously in two-Trp Anabaena and E. coli thioredoxins . In fact, a sub-100ps component is consistently found in all but one of these proteins with positive amplitudes even at longer wavelengths (e.g., 390nm). The exception is GB1, a protein which Toptygin and Brand previously found carried a negative preexponential term near 390nm. Since the lifetime associated with that negative was 65ps, it was just within the edge of TCSPC detection. The more prevalent positive amplitude DAS (decay-associated spectra) we see in the other proteins on these timescales are indicative of ultrafast quenching processes depleting the partly relaxed singlet state. Candidate mechanisms include ET to nearby acceptors and/or collisional quenching. This is similar to our prior observation (J. Am. Chem. Soc., 2006, 128, 1214) that ultrafast quenching, not desorbing water, dominates the time-resolved emission spectra (TRES) of monellin. We will discuss the −/+ data signatures for both water relaxation and fast quenching, including simulations to lay out the circumstances where a fast relaxation accompanied by a direct radiative rate reduction might mask the negative term.

237-Pos Board B116

Rapid Detection of Troponin I from Serum using Microwave-Accelerated Metal-Enhanced Fluorescence

Kadir Aslan, Yongxia Zhang, Chris D. Geddes.
University of Maryland Biotechnology Institute, BALTIMORE, MD, USA.

In a clinical setting, immunoassays for the quantification of cardiac markers are usually run in serum and can take > 15 minutes to process per step, per marker; resulting in a long patient screening process. Some commercially available tests for cardiac markers offer results from whole blood in approximately 15 minutes. However, these systems measure one sample at a time and have high initial and maintenance / supply costs. In this regard, the development of new ultra-fast (< 30 seconds) and sensitive immunoassays for cardiac markers, that can predict an AMI accurately, earlier and more economically, will significantly benefit human health. Our Laboratory recently reported the application of a platform technology, namely ''Microwave-Accelerated Metal Enhanced Fluorescence (MAMEF)'' to a model protein assay in HTS well plates, where low concentrations of a target protein were detected in less than 30 seconds.[1] Here we present our findings on the rapid detection of Troponin I from samples prepared in buffer and serum on HTS well plates using the MAMEF platform technology. In this regard, HTS wells were firstly modified with silver colloids and cardiac marker specific capture antibody. Subsequently, the Troponin I immunoassay was undertaken by the incubation of Troponin I and the detection antibody under microwave irradiation for 30 seconds for each step. A lower detection of < 1 ng/mL for Troponin I in buffer and serum was recorded. In addition, the detection of Troponin I from I-T-C complex in buffer and serum was also achieved with a lower detection limit < 1 ng/mL using MAMEF. Our findings demonstrate that cardiac markers can be determined in < 30 seconds at clinically relevant levels.

1. Aslan, K., Holley, P. & Geddes, C.D. *Journal of Immunological Methods* **312**, 137 (2006).

238-Pos Board B117

Structural and Mechanistic Characterization of the Mannitol Transporter from E. coli using 5-fluorotryptophan as a Spectroscopic Probe

Milena Opacic, Ben H. Hesp, Jaap Broos.
University of Groningen, Groningen, Netherlands.

The mannitol permease (EIImtl) of E. coli is an integral membrane protein responsible for the active transport of mannitol over the cytoplasmic membrane. It is composed of three domains: two cytosolic domains A and B, and transmembrane C domain. The structures of A an B domains were solved by X-ray crystallography and NMR spectroscopy. For the transmembrane C domain a 5Å 2D projection map is available and several topology models. EIImtl is functional as a dimer.

A dozen single Trp mutants of EIImtl were made and 5-fluoroTrp was incorporated in the C domain with ≥ 95% efficiency. Compared to Trp, 5-fluoroTrp shows the advantage that the fluorescence decay kinetics is much more homogeneous. 5-fluoroTrp is also a good energy donor, which makes it suitable for resonance energy transfer (RET) experiments. An analogue of mtl, azi-mtl, was used as an acceptor. Steady state fluorescence spectroscopy was used to characterize the solvent exposure of specific positions within the transmembrane C domain. Time resolved fluorescence spectroscopy was used to probe the local microenvironment of the residues as well as the distance between 5-fluoroTrp residues and the mannitol binding site.

Our results show that mannitol binding induces large conformational changes in EIImtl, that the C domain shows a rigid structure and that the binding site is asymmetrically positioned in the EIImtl dimer.

239-Pos Board B118

Investigation Of Excited-State Relaxation In Single-Tryptophan-And Other Proteins Via Multidimensional Static And Time-Resolved Fluorescence

Stefanie Schwedler, Katharina Kohse-Höinghaus, Regina Brockhinke, Andreas Brockhinke.
Universität Bielefeld, Bielefeld, Germany.

In recent years, spectral relaxation has been established as a theory to explain nonexponential decays of intrinsic tryptophan fluorescence in single-tryptophan proteins. However, systematic measurements are required to account for the occurence of spectral relaxation in specific proteins. We investigated different single tryptophan proteins, varying in size, predominating secondary structure and polarity of the fluorophor environment, in order to correlate these parameters with spectral relaxation.

Multidimensional static fluorescence measurements delivers a spectroscopic fingerprint containing every single excitation and emission spectrum of the substance in question. Herewith we evaluated stokes shifts, quantum yields and stern vollmer constants, yielding information on the polarity, accessability and quenching acitivity of the fluorophor environment. Furthermore, shifts in emission wavelength at the red excitation edge indicated the presence of a relaxation process.

Fluorescence dynamics were investigated using a tunable pulsed laser (either 3 or 80ps pulse width) and an intensified streak camera as detection unit, yielding simultaneously time and wavelength resolved spectra. Detection efficiencies of the phosphorus screen were calibrated via a halogen lamp and the accuracy of the resulting lifetimes was confirmed using a matrix of different reference dyes.

The resulting measurements revealed the occurrence of spectral relaxation due to shifts of the center of gravity with time and increase of lifetime with emission wavelength. Though negative preexponential factors could only rarely be assigned, an increasing time shift of the fluorescence maximum at longer emission wavelength proved these effects to stem from an excited state process and not from different conformers in the ground state.