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

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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

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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.

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

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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).

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

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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.

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

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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.