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**240-Pos Board B119****The  $\gamma$ -Crystallin Fold May Have Evolved To Protect Conserved Tryptophan Residues From UV Radiation Damage Through Efficient Quenching** Jiejun Chen<sup>1</sup>, Patrik R. Callis<sup>2</sup>, Jonathan King<sup>1</sup>.<sup>1</sup>Massachusetts Institute of Technology, Cambridge, MA, USA, <sup>2</sup>Montana State University, Bozeman, MT, USA.

Crystallins, the major proteins of the vertebrate eye lens, are important for maintaining transparency and the proper refractive index gradient. The set of lens proteins responsible for these functions include the two-domain Greek key  $\beta$ - and  $\gamma$ -crystallins. Each  $\beta$ -sheet domain of  $\beta$ - and  $\gamma$ -crystallins contains two highly conserved buried tryptophans (Trps) whose fluorescence is efficiently quenched in the native state. Absorption of UV light by the lens crystallins protects the retina from UV photodamage, but subjects the crystallin Trps themselves to covalent damage. Experiments with Trp>Phe mutants of human  $\gamma$ D- and  $\gamma$ S-crystallin show that two donor Trps transfer their excited state energy to two other recipient Trps, by Förster resonance energy transfer. The two recipient Trps have very short-lived excited states [Chen, Toptygin, Brand & King, *Biochemistry* 2008, 47 (40): 10705-21]. This quenching of the Trp excited state may protect the lens crystallins from photo-damage. Hybrid quantum mechanical-molecular mechanical simulations indicate that the efficient quenching is due to fast electron transfer from the excited state Trp indole ring to its amide backbone. This charge transfer quenching mechanism is enabled by the Trp side chain conformation, two bound waters, and nearby favorably charged groups. These are conserved in most of the solved crystallin structures. This conservation, coupled with the observation of Tallmadge and Borkman [*Photochem Photobiol*, 1990, 51, 363-368] that the quenched Trps in bovine  $\gamma$ B-crystallin were protected from photochemical damage relative to the more fluorescent Trps, strongly suggests that the quenching is an evolved property of the protein fold that allows it to absorb ultraviolet light while suffering minimal photodamage. Selection for UV radiation resistance may have contributed to the recruitment of the crystallin fold as a lens protein.

**241-Pos Board B120****Mapping Proximity Within Proteins Using Fluorescence Spectroscopy: New Advances In Distance-dependent Tryptophan-induced Fluorescence Quenching**Steven E. Mansoor, Mark A. DeWitt, **David L. Farrens**. Oregon Health & Science University, Portland, OR, USA.

We previously showed that the dramatic quenching of bimanane fluorescence by Trp can be exploited to study protein structure, dynamics and protein-protein interactions. Here we report our efforts to extend this approach to four other probes: monobromotrimethylammoniumbimane (qBBr), lucifer yellow (LY), BODIPY 507/535 (BDPY), and Atto-655 (Atto). We attached each probe to a unique reactive cysteine at four different sites on T4 lysozyme, and measured the effect of a nearby Trp on the fluorescence intensity and lifetime. We also used a relatively novel yet simple approach to calculate the relative fraction of each probe forming a static, non-fluorescent complex with the Trp.

The different probes show markedly different sensitivities to quenching by the Trp, most likely due to their differences in size, rotational flexibility, length of attachment linker, and efficiencies of quenching photochemistry. Together, our data can be used to approximate the distance-dependent "sphere of quenching" for each probe. The smaller probes (bimanes and BODIPY) show substantial quenching and static-complex formation only when they are very close to the Trp (< 10Å away). Thus, these probes are best suited for measuring short-range intramolecular distances or conformational changes in proteins. In contrast, the larger probes (lucifer yellow and Atto-655) show substantial quenching and static-complex formation with the Trp over longer distances. Thus, these latter probes are less selective for monitoring local changes, but are useful for monitoring longer-range distances within a protein, or inter-molecular distances between proteins. We anticipate the broad excitation and emission range covered by these four probes (380 nm - 650 nm), combined with the ability to determine

the amount of static complex formation with Trp, should expand the use of Trp-induced fluorescence quenching as a method for studying protein structure and dynamics.

**242-Pos Board B121****Mutations In Transhydrogenase Change The Fluorescence Emission State Of Trp72 From <sup>1</sup>La To <sup>1</sup>Lb.**Jaap Broos<sup>1</sup>, Karina Tveen Jensen<sup>2</sup>, Giovanni B. Strambini<sup>3</sup>, Margherita Gonelli<sup>3</sup>, Baz Jackson<sup>4</sup>.<sup>1</sup>University of Groningen, Groningen, Netherlands, <sup>2</sup>University of Birmingham, Birmingham, United Kingdom, <sup>3</sup>CNR, Pisa, Italy, <sup>4</sup>University of Birmingham, Birmingham, United Kingdom.

The dI component of *Rhodospirillum rubrum* transhydrogenase has a single Trp residue (Trp(72)), which has distinctive optical properties, including short-wavelength fluorescence emission with clear vibrational fine structure, and long-lived, well-resolved phosphorescence emission. We have made a set of mutant dI proteins in which residues contacting Trp(72) are conservatively substituted. The room-temperature fluorescence-emission spectra of our three Met(97) mutants are blue shifted by approximately 4 nm, giving them a shorter-wavelength emission than any other protein described in the literature, including azurin from *Pseudomonas aeruginosa*. Fluorescence spectra in low-temperature glasses show equivalent well-resolved vibrational bands in wild-type and the mutant dI proteins, and in azurin. Substitution of Met(97) in dI changes the relative intensities of some of these vibrational bands. The analysis supports the view that fluorescence from the Met(97) mutants arises predominantly from the (1)L(b) excited singlet state of Trp(72), whereas (1)L(a) is the predominant emitting state in wild-type dI. It is suggested that the sulfur atom of Met(97) promotes greater stabilization of (1)L(a) than either (1)L(b) or the ground state. The phosphorescence spectra of Met(97) mutants are also blue-shifted, indicating that the sulfur atom decreases the transition energy between the (3)L(a) state of the Trp and the ground state. (Lit.: *Biophys J.* (2008) 95, 3419).

**243-Pos Board B122****MD Simulations of the Time-Dependent Red Shift in the Fluorescence of Trp in Protein GB1**

Dmitri Toptygin, Thomas B. Woolf, Ludwig Brand. Johns Hopkins University, Baltimore, MD, USA.

Time-dependent red shift (TDRS) in the emission of Trp after impulse excitation reports the dynamics of solvent and protein relaxation. TDRS of the single Trp in the B1 domain of streptococcal protein G (GB1) has been measured experimentally [Toptygin, Gronenborn and Brand, *J. Phys. Chem. B* **2006**, *110*, 26292]. The present work compares the results of Charmm MD simulations with the experimental data. The protein in a box of explicit TIP3P water was equilibrated with the ground-state charges on Trp atoms. At t=0 the charges on Trp atoms were changed to those characteristic of the excited state <sup>1</sup>L<sub>a</sub>. TDRS was calculated from a series of 2000ps excited-state MD trajectories using first-order QM perturbation theory. TDRS from a single trajectory is overwhelmed by random noise. Averaging over 100 trajectories produced a result qualitatively consistent with the experimental data. The amplitudes of the calculated and measured TDRS during the time interval from 200ps to 2000ps are in good agreement. However, at t<200ps MD results differ from the experiment, which may have several explanations. Alternatively TDRS was also calculated from one long equilibrium trajectory (with the excited-state Trp atom charges) using the autocorrelation  $C(t) = \langle \Delta E(t+\xi) \Delta E(\xi) \rangle - \langle \Delta E(\xi) \rangle^2$  of random fluctuations in the ground-excited state energy gap  $\Delta E$  and the relation  $h\nu(t) - h\nu(\infty) = C(t)/(k_B T)$  [Nilsson and Halle, *PNAS* **2005**, *102*, 13867]. This produced a TDRS curve containing less high-frequency noise, but more low-frequency noise as compared to the result of averaging over 100 short nonequilibrium trajectories. We thank Patrick R. Callis for accurate excited-state Trp atom charges. Supported by NSF grants MCB-0416965, MCB-0719248.