3514-Pos  Board B609
Md ±qM Calculations Explore the Origins of Differences amongst the Red Fluorescent Proteins
J. Nathan Scott1, Patrik R. Callis2,3
1Saint Francis University, Loretto, PA, USA, 2Montana State University, Bozeman, MT, USA.
We present a number of theoretical results concerning the properties of and differences amongst several red fluorescent proteins (RFPs). RFPs are an extraordinarily useful group of fluorescent proteins that fluoresce at red-shifted wavelengths compared to GFP, thereby extending the color palette of fluorescent proteins for use in applications like FRET and multi-channel imaging. This redder fluorescence is caused by the extension of the pi-conjugated chain through oxidation of a second backbone bond of either Phenylalanine or Isoleucine, depending on the particular RFP. Our work has focused on the RFPs mCherry, mPlum, and DsRed, which share a common chromophore. Since these RFPs share a chromophore, differences in their photophysical properties must be due to differences in their chromophore environments. We performed a series of 100 ns molecular dynamics simulations followed by ZINDO semi-empirical quantum mechanical calculations in an effort to explore variables such as excitation wavelength, quantum yield, and photostability in these RFPs. The ZINDO calculations were performed on MD simulation frames at 1 ps resolution. This fine resolution allowed for the observation of rare conformational states that suggest reasons for the known differences in quantum yield amongst the proteins. Notably, the electric field contributions from every non-chromophore protein atom and ion, and the ~14,000 simulation cell water molecules, were used as a perturbation in the ZINDO calculations, which allowed us to query the electrostatic environmental influence on the chromophore.

3515-Pos  Board B670
Femtosecond Fluorescence Dynamics of Trp-Trp Dipeptide in Water and its Interaction with DNA
Yan Chen1, Haifeng Pan2, Zhandong Tao3, Menghui Jia2, Sanjun Zhang2, Jianhua Xu2, Jay Knutson1
1Tongji University Affiliated to Tongji University, shanghai, China, 2East China Normal University, Shanghai, China, 3National Institutes of Health, Bethesda, MD, USA.
We reported here the systematic measurements of fluorescence decay and quenching of tryptophan dipeptide Trp-Trp in water, and its interaction with DNA by using an ultraviolet upconversion fluorescence measurement system with time resolution better than 100 fs together with a time correlated single photon counting apparatus on the 100 ps to 30 ns time scale. We analyzed the effect of acidity and phosphate ion etc upon Trp-Trp fluorescence decay profiles. Fluorescence data reveal multiplexponential decay of Trp-Trp dipeptide in water, and Trp-Trp can interact with DNA. It is shown that Trp-Trp is subject to ultrafast quenching with DNA and also senses nearby water dynamics. The time constant for the bulk water “solvent relaxation” remains near 1–2 ps, but a new ultrafast decay, fit to an exponential time constant of ~10 ps with associated amplitude positive or negative (>380 nm) depending on the emission wavelength, has been found. Candidate mechanisms (including ET to nearby acceptors and/or collisional quenching etc.) and the analysis of time resolved emission spectra will be discussed.

3516-Pos  Board B671
Phospholipase CB β Binds to C3PO and its Components that Orchestrates RNA Interference
Shriya Sahu, Finly Philip, Suzanne Scarlata.
Stony Brook University, Stony Brook, NY, USA.
Phospholipase CB (PLCβ-4) is a family of signaling enzymes coupled to G protein Gzq. When activated by Gzq, PLCβ hydrolyses phosphatidylinositol 4,5 bisphosphate to produce two second messengers that lead to activation of protein kinase C and an increase in intracellular calcium. While the majority of PLCβ resides on the plasma membrane, a small but significant population is present in the cytosol and nucleus. Recently, our lab identified translin-translin associated factor X (TRAX) to be a cellular binding partner of PLCβ with a binding constant only 10 fold lower than Gzq. TRAX is known to bind to translin forming the complex C3PO which assists in RNA interference, along with a number of other nuclear functions. Generally, our lab showed that PLCβ can reverse the down regulation of specific genes (GAPDH) presumably through its interaction with TRAX thus linking PLCβ with gene regulation. Here, we have characterized the complexes formed between PLCβ and TRAX, translin and C3PO. Using FRET, we show that PLCβ binds to C3PO with Kd similar to PLCβ and Trax (8nM) whereas the Kd for binding to translin is much weaker (40 nM). We also found that translin, Trax and C3PO occur in multiple oligomeric states as shown by FCS and in native gels. Anisotropy studies using labeled oligonucleotides and translin and C3PO show that PLCβ inhibits a nucleotide binding mode of the proteins. Studies to characterize the change in the composition and stoichiometry of C3PO and translin by PLCβ in the presence and absence of various oligonucleotides using FCS and Photon Counting Histogram (PCH) analysis are currently underway. This work was supported by NIH GM053132.

3517-Pos  Board B672
Ultrafast Fluorescence Spectroscopy and Cell Imaging Techniques in Exploring the Caffeine Mediated Dissociation of Mutagen from Biomimetics, Nucleic Acids and Cells
Soma Banerjee, Samir Kumar Pal.
SNBNCBS, Kolkata, India.
We report a systematic investigation of caffeine induced dissociation of ethidium (Et) cation, a potential mutagen from nucleic acids and biomimetic systems. These studies are consistent with a mechanism where caffeine-Et complex formation in bulk solution drives the dissociation of DNA-bound Et. Caffeine induced extraction of Et from whole cells were also performed on squamous epithelial cells collected from the inner lining of the human mouth, A549 (lung carcinoma), A375 (human skin), RAW (macrophage) and Vero (African green monkey kidney epithelium) cell lines. Interestingly, the efficiency of caffeine in extracting Et has been found to be dependent on cell types. Our state steady and picosecond resolved spectroscopic studies on the detachment of Et from various biomimicking micelles of different charges reveal the specificity of caffeine molecule for carrying out such dissociation. The picosecond resolved Förster resonance energy transfer (FRET) studies between a DNA minor groove binder dye Hoescht 33258 (H258, donor) and Et (acceptor) have been employed to investigate the alteration in their association in presence of caffeine in the molecular level. Finally, our fluorescence micrographs of epithelial cells validate the alteration of FRET efficiency between the donor and the acceptor due to the caffeine mediated release of the latter. Our results both in-vitro as well as ex-vivo provide important clues about efficiency and mechanism of caffeine as a potential anti-mutagenic therapeutic agent.

Vibrational Spectroscopy

3518-Pos  Board B673
DFT-Level Calculations of IR, VCD and Raman Spectra for 13C-Labeled Beta Sheets
William R.W. Welch1, Jan Kubelka1, Timothy A. Keiderling2
1University of Wyoming, Laramie, WY, USA, 2University of Illinois at Chicago, Chicago, IL, USA.
With the aid of theoretical models, optical spectroscopy has proven valuable for elucidating secondary structural features in protein systems including β-sheets, which form a large portion of protein assemblies in amyloid systems. For such large protein systems, computational methods must be both efficient and robust in order to achieve accurate predictions in a timely manner. Quantum chemical methods are in principle the most robust methods available, but suffer from high computational cost that usually makes them impractical for large biomolecular assemblies. In this study optical spectra for β-sheets composed of 5-5 strand, ten-amides each, 5x10, were obtained by transferring parameters calculated using DFT for smaller 3x3 sheet segments. Sensitivity of spectra to variations in conformations was examined for parallel, anti-parallel, twisted and flat sheets. Some effects of having strands shifted out of register were also explored. Predicted amide I IR spectra show classic features for parallel and anti-parallel sheets and some new possibilities for discerning between the two are observed, primarily in IR and VCD spectra, while Raman amide I spectra was predicted to be less useful for structural discrimination. Predictions for particular isotopic labeling schemes show clear IR patterns that might be used in elucidating shifts in register for anti-parallel sheets.

3519-Pos  Board B674
Expanding the Utility of 4-Cyano-L-Phenylalanine as a Vibrational Reporter of Protein Environments
Christopher Bazewicz, Melanie Liskov, Kevin Hines, Scott Brewer.
Franklin and Marshall College, Lancaster, PA, USA.
The ability to genetically incorporate amino acids modified with spectroscopic reporters into proteins with high efficiency and fidelity has greatly enhanced the ability to probe local protein structure and dynamics. We have synthesized the unnatural amino acid (UAA), 4-cyano-L-phenylalanine (pCNPhe), containing the nitrile vibrational reporter and three Isotopomers (13C15N, 13C13N, 15N) of this UAA to enhance the ability of pCNPhe to study local protein environments. Each pCNPhe isotopic variant was genetically incorporated in an efficient, site-specific manner into superfolder green fluorescent protein (sfGFP) in response to an amber codon with high fidelity utilizing an engineered, orthogonal aminoacyl-tRNA synthetase. The isotomers of 4-cyano-L-phenylalanine