CALI and the labelling specificity that fluorescent proteins provide is very useful to avoid uncontrolled photodamage. Indeed, fluorescent proteins have been successfully used in CALI, although of the inactivation mechanisms by ROS are dependent on the fluorescent protein used and are not fully understood [2,3]. Here, we present a quantitative study of the ability of TagRFP to produce ROS, in particular singlet oxygen. TagRFP is able to photosensitize singlet oxygen with an estimated quantum yield of 0.004 [4]. This is the first estimation of a quantum yield of singlet oxygen production value for a GFP-like protein. We also find that TagRFP has a short triplet lifetime, which reflects relatively high oxygen accessibility to the chromophore compared to EGFP. Our results provide photophysical insight that allows the understanding of the mechanism behind CALI. Moreover, it has implications in improving photobleaching in fluorescent proteins.

[1] K. Jacobson, Z. Rajfur, E. Vitriol, K. Hahn, *Trends Cell Biol.***2008**, 18, 443.
[2] M. A. McLean, Z. Rajfur, Z. Z. Chen, D. Humphrey, B. Yang, S. G. Sligar, K. Jacobson, *Anal. Chem.* **2009**, 81, 1755.
[3] M. E. Bulina et al, *Nat .Biotechnol.***2006**, 24, 95..[4] X. Ragas, L. P. Cooper, J. H. White, S. Nonell, C. Flors, *submitted*.

294-Pos Board B94

Probing Local Protein Environments with the Infrared Probe: L-4-Nitrophenylalanine

Emily E. Smith, Barton Y. Linderman, Austin C. Luskin, Scott H. Brewer.

The genetic incorporation of unnatural amino acids (UAAs) with high efficiency and fidelity is a powerful tool for the study of protein structure and dynamics with site-specificity in a relatively non-intrusive manner. Here, we illustrate the ability of L-4-nitrophenylalanine to serve as a sensitive IR probe of local protein environments in the 247 residue superfolder green fluorescent protein (sfGFP). Specifically, the nitro symmetric stretching frequency of L-4-nitrophenylalanine was shown to be sensitive to both solvents that mimic different protein environments and ^{15}N isotopic labeling of the three-atom nitro group of this UAA. $^{14}NO_2$ and $^{15}NO_2$ variants of this UAA were incorporated utilizing an engineered aminoacyl-tRNA synthetase/tRNA pair into a solvent exposed and a partially buried position in sfGFP with high efficiency and fidelity. The combination of isotopic labeling and difference FTIR spectroscopy permitted the nitro symmetric stretching frequency of L-4-nitrophenylalanine to be experimentally measured at either site in sfGFP. Additional spectroscopic results exploring the utility of other isotopically labeled UAAs to probe local protein environments with site-specificity will also be presented.

295-Pos Board B95

Investigations of Effects of Cooper Ion on the Allergenicity of β-Lactoglobulin

Adeleh Divsalar, Sajedeh Ebrahim-Damavandi, Ali Akbar Saboury.

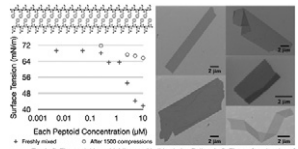
β-lactoglobulin (BLG), the most abundant whey protein in milk, governs the overall process-induced aggregation and gelation of whey protein products. BLG industrial application can be limited due to its high allergenicity. Here, we attempt to obtain new views from inducing of aggregation and then reducing allergenicity of BLG upon interaction with cooper ions. The effect of Cu^{+2} on the structure of BLG as the carrier model protein was investigated using UV-Visible, fluorescence and circular dichroism (CD) spectroscopy instruments at different temperatures of room and physiologic. Results of UV-Visible studies represent that adding Cu^{+2} to BLG solution caused increasing of turbidity in the protein solution. It is noticeable that rate of increasing turbidity is higher at upper temperature. Fluorescence studies revealed that Cu^{+2} can quench the intrinsic fluorescence emission of the protein at different temperatures. The far-UV CD studies displayed that Cu^{+2} cannot induce any changes in the secondary structures of BLG at different temperatures. The results highlight that copper ions affect the tertiary structure to change and induce slightly open structure lead to formation of supramolecular aggregates in BLG. Also, it seems that inducing of aggregation in BLG upon interaction with cooper ions may be responsible for the disappearance of conformational epitopes or masking effect on the recognition of epitopesand consequently, which reduced allergenicity of BLG.

296-Pos Board B96

Creating Peptoid Nanosheets by Buckling a Gibbs Monolayer

Babak Sanii, Romas Kudirka, Andrew Cho, Helen Tran, Li Tan, Ron N. Zuckermann.

Peptoids are N-substituted glycine analogs of peptides that can be designed to have comparable structures and functions as proteins but with much greater biological resiliency. We have designed peptoids that self-assemble into planar nano-materials as large as millimeters long yet only 2.7nm thick[1], that can survive boiling temperatures, deep vacuums and biological environments. They are held together largely by coulombic and aromatic interactions, however they aggregate due largely to hydrophobic interactions. The peptoid nanosheets are the product of irreversible buckling of a Gibbs monolayer formed at the air-water interface.



Top Left: The peptoid amphiphiles used in this study. Bottom Left: The surface tension of a solution of such peptoids increases with surface compressions. Right: The compressions produce stable, free-floating nanosheets (SEM images).

Here we report on this mechanism of peptoid nanosheet formation and explain the role of surface-area compression ratios on the macromolecular reaction kinetics and theoretical yields. This robust assembly mechanism coupled with atomic-resolution peptoid design enables free-floating planar platforms for bio-functionalization and analysis.

[1] Free-floating ultrathin two-dimensional crystals from sequence-specific peptoid polymers. Nature Materials 9, 454 (2010).

297-Pos Board B97

Osmolytes Reduce the Interfacial Affinity of Proteins

Claus Czeslik, Florian Evers.

Osmolytes are substances that affect osmosis and are used by cells to adapt to environmental stress. Here, we present a neutron reflectivity study on the effect of various osmolytes on the structure of protein adsorbates at solid-water interfaces. Ribonuclease A (RNase A) and bovine insulin were used as model proteins adsorbing at a hydrophilic silica and a hydrophobic polystyrene planar surface. From the data, neutron scattering length densities were extracted revealing the thickness, volume fraction and roughness of the protein adsorbates in the absence and the presence of urea, trehalose, sucrose, and glycerol. All data point to a clear effect of these osmolytes on the degree of protein adsorption. For example, 1 M trehalose leads to a reduction of the adsorbed amount of RNase A to 72 % on a silica surface and to 36 % on a polystyrene surface. The non-protecting osmolyte urea (2 M) suppresses RNase A adsorption to 76 % and 64 %, respectively. Sucrose was found to be of similar activity as trehalose. The changes in adsorbed protein mass can be attributed to a lower volume fraction of the protein in the adsorbate layers. Apparently, the osmolytes under study help to reduce the non-specific adsorption of proteins at interfaces.

298-Pos Board B98

Nucleic Acid Stability in Glycine Betaine Solutions: Correlating Small Molecule Interactions with Nucleic Acid Surfaces

Jeffrey J. Schwinefus, Elliot Schmidt, James Kohler, Alexandra Thomas.

This work seeks to elucidate the mechanism of glycine betaine destabilization of DNA and RNA secondary structures to enhance our understanding of the physical chemistry of folded nucleic acids. Vapor pressure osmometry was used to quantify glycine betaine exclusion from nucleoside 5′-monophosphates (5′-NMPs). The majority of the exclusion was attributed to glycine betaine exclusion from anionic oxygens on the phosphate group and aliphatic carbons and oxygens on the sugar group. Glycine betaine was proposed to accumulate at the nucleobases with the greatest accumulation around guanine. Thermal denaturation transition temperatures of GC-rich DNA and RNA duplexes decrease to a greater extent with increasing glycine betaine concentration than AT- (adenine-thymine) and AU- (adenine-uracil) rich double helices. We propose favorable interactions between glycine betaine and amino groups exposed during unfolding account for the greater destabilization of GC-rich DNA and RNA.

299-Pos Board B99

Separation of Preferential Interaction and Crowding Effects on DNA Hairpin and Duplex Formation

D.B. Knowles, N.F. Deines, A. Lacroix, M. Thomas Record.

Solutes affect protein and nucleic acid processes as a consequence of their competition with water for biopolymer surfaces that become exposed (or buried) in the process (‘‘preferential interactions’’) and through their ability to occupy space and reduce the available volume of the solution (‘‘crowding’’). Here we develop a quantitative analysis of the molecular weight dependence both preferential interaction and crowding effects of flexible coil polymers, with the capability of interpreting or predicting effects of any flexible coil polymer on any biopolymer process. We report dependences of free energies of DNA hairpin helix unfolding and duplex dissociation on PEG concentration