of the activation energy barrier, and $\Delta x_U$, the distance to the transition state. For all protecting osmolytes we measure $\Delta G_U$ increases, demonstrating that the I27 protein is stabilized. More striking is the measurement of $\Delta x_U$. Unfolding the I27 protein in water gives a $\Delta x_U = 2.5$ Å, a distance similar to the size of a water molecule. Water molecules have been identified as integral components of the unfolding transition state of the I27 protein, forming a solvent bridge between two β-strands. By varying osmolyte molecule size we rigorous test this solvent bridging hypothesis. We find that $\Delta x_U$ correlates with osmolyte size for molecules ranging in size from 2.5 Å to 5.6 Å. However, for larger molecules ($> 5.6$ Å) $\Delta x_U$ remains unchanged relative to the value measured in water, suggesting these osmolytes do not participate in solvent bridging in the transition state. These studies uniquely probe the length scales over which solvent molecules can modify the molecular architecture of the unfolding transition of a protein, an area which remains beyond the reach of other experimental techniques.

**373-Pos Board B252**

**Site-Specific Folding Dynamics of Isotopically Labeled Peptides Studied by Time-Resolved Infrared-Spectroscopy**

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Peptides with well-defined secondary structure are ideal model systems for study of protein folding dynamics for specific, unique structures. IR techniques provide the necessary time resolution as well as have structural sensitivity, which arises from coupling of sequential residues, normally evidenced as a splitting or frequency shift of the amide centered transitions. The amide I region, mainly the C=O stretching vibrations of the polypeptide backbone, is the prime target band for secondary structure. Isotopic labeling of individual amide 13C=O groups can induce site-specific frequency shifts and provide insight into local structure. A nanosecond laser is used to excite the solvent and induce a fast temperature jump (~10 C), and relaxation dynamics are probed with a diode laser tuned to selected, structurally sensitive wavenumbers across the amide I absorption. Site-specific dynamics have been monitored for the thermal unfolding of an isotopically labeled beta-hairpin peptide, a 12-mer tryptophan zipper peptide, which has a hydrophobic core formed by four Trp residues, by use of cross-strand coupled 13C=O labeled variants [1]. Data for single labeled peptides provided a control. Mutants of this sequence with just two Trp residues were introduced to destabilize the hairpin selectively near the termini or near the turn. Differences in kinetic behavior have been found for the loss of beta-strand and the gain of disordered structure. The isotope-edited kinetics vary with labeling position along the hairpin backbone and the mutations show consistent patterns depending on position. Our data supports a multistate folding mechanism for this hairpin structure. Similarly obtained data for other model peptides provide useful basis for interpretation of the observations.

[1] Hauser, K., Krejtschi, C., Huang, R., Wu, L., Keiderling, T.A., J. Am. Chem. Soc. 130 (2008) 2984- 2992.

**374-Pos Board B253**

**Linear Response of Biomolecules To External Perturbations: Revisit Induce-fit**

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Atoms in proteins, viewed as interlinked hubs, communicate with each other in complying with governed physical forces. The deviations of their positions from the mean, known as fluctuations, are essential in mediating functionally relevant biological processes maintaining one's daily life. The coupled fluctuations between pairs of atoms, the fluctuation covariance, can be determined analytically by Normal Mode Analysis, or numerically by MD simulations. Our study considers how this network of atoms reacts in response to external perturbations. Effects of such perturbations are exemplified by ligand- or (another) protein-induced conformational changes as well as the appreciated structural distortion of crystalline structures from its solution conformers. We assume the response of the system has linear departure from the mean under small perturbations on the Hamiltonian at equilibrium state. The formulated linear response theories, either time-dependent or -independent [1], says that the positional change of a given atom i is the accumulative sum of fluctuation covariance ij, at unperturbed state, multiplied by the force exerted on atom j. The time-dependent response function determined from MD simulation of carbonmonoxy myoglobin is used to track time-dependent conformational changes upon photo-dissociation of CO. The consequently obtained understanding of perturbation propagation is compared with experimental results. Also, the structural distortion of X-ray-characterized ubiquitin from its solution conformer can be well explained by the induce-fit theory, in a wider sense, while population-shift does not account for such a deviation.

[1] Ikeguchi M, Ueno J, Sato M, and Kidera A. (2005) Phys Rev Lett, 94, 078102.

**375-Pos Board B254**

**Dynamic Allostery In Proteins**

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Allostery or signalling between two sites of a molecule is a widely present phenomenon in nature. Classically it is believed that the communication between distant sites proceeds via a series of conformational changes. Recently allosteric proteins without conformational change have been observed. This cannot be accounted for with the classical theory and therefore we have built a model that explains the long distance signalling in such cases. We believe that the signalling proceeds via a change in dynamic behaviour of the protein and we illustrate the feasibility of such an explanation on a model system, the Catabolite Activator Protein (CAP).

CAP displays negative cooperativity without conformational change upon binding two cAMP ligands. We have built a coarse grain model of multiple slow and fast modes and demonstrated how negative cooperativity can arise without conformational change. The slow, global modes are responsible for the allosteric behavior and result in a purely entropic contribution to the allosteric free energy (ΔΔG). Multiple slow modes need to be included to achieve the experimentally observed free energy values. In the real system compensating entropic and enthalpic terms are observed. Fast modes, despite being localized can couple to the slow modes and assist allostery. We show that they account for the split of ΔΔG into the entropic and enthalpic parts. The value of ΔΔG is proportional to the number of slow modes. The size of the compensating enthalpic and entropic terms increases with number of enslaved fast modes. We can therefore estimate how many slow and fast modes are taking part in the allosteric signaling.

**376-Pos Board B255**

**Impact of Hofmeister Salts on Structural Dynamics of Photoactive Yellow Protein**

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Water is known as the lubricant of life. Without water, most proteins would lose their biological activities. Extensive studies have been carried out on how aqueous solutions with high concentration salts alter the stability and solubility of proteins. Such effects are thought to be mediated largely via salt-water interactions and water-protein interactions. This classic research field is known as the Hofmeister Series. We report the effects of Hofmeister salts on the structural dynamics of proteins. Photoactive yellow protein (PYP), a bacterial blue light photoreceptor protein, is employed as a model system in this study. Time-resolved FTIR spectroscopic techniques were used to probe the protein structural changes of PYP in response to blue light excitation. Our data demonstrate that high concentration salt solutions have profound effects on functionally important motions of PYP, including (1) the light triggered proton transfer pathway in the active site and (2) the large conformational changes associated with PYP receptor activation. We will discuss the significance of our study in relation with protein crystallization, and with other properties of the Hofmeister series.

**377-Pos Board B256**

**Linking Enzyme Conformational Dynamics To Catalytic Function With Single-molecule FRET**

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Many enzymes endure sizable conformational remodeling on a timescale comparable to their catalytic cycle. In adenylate kinase (AK) from E. coli, this involves large-amplitude rearrangements of the enzyme's lid domain, which may be critical to the enzymes' catalytic function. We applied high-resolution single-molecule FRET developed in our laboratory to follow AK's domain movements on its catalytic timescale. This was achieved by recording and analyzing data photon by photon to rigorously account for counting noise, background, and cross talks. By utilizing a maximum entropy-based approach to remove photon-counting noise, the enzyme's entire conformational distribution was quantitatively recovered without a presumed model. Armed with precise single-molecule FRET dynamics measurements and comprehensive bulk kinetic

studies of the mechanism, we were able to quantitatively reconstruct the elementary steps as well as the energetic pathways along the AK's enzymatic cycle. The mechanistic roles of AK's stochastic lid dynamics were found to engage in conformation gating, shuffling of reaction pathways, and dynamical induced fit.

**378-Pos Board B257**
**Two-colors Photo-Switching of E222Q-GFPMut2 Mutant by Fluorescence Correlation Spectroscopy**
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GFP mutants display complex photodynamics whose properties can be tuned even by single mutations of the chromophore or the protein backbone. Some GFP mutants can be photo-activated (paGFP) or photo-switched (E222Q mutants). Although photo-activable mutants are valuable tools in nanoscopy studies and have been already applied in this field, the photo-switching behavior of some GFP mutants has not been yet exploited in biological imaging.
We report here the characterization of the two-color enhancement of the E222Q mutant of the GFPMut2 protein aimed to its application in cellular imaging. The anionic fluorescence output is enhanced when the GFP is irradiated simultaneously at 390-440 nm. By fine tuning the 488 nm direct anionic *excitation* and the UV-blue *irradiation*, the GFP mutant emission can be enhanced up to 2.5 times. The maximum switching efficiency occurs at 420 nm and display a marked pH dependence.
Moreover, we have characterized the activation time of this process by modulating the irradiation or excitation beams. By means of Fluorescence Correlation Spectroscopy methods under modulated irradiation in the UV-blue range of the spectrum, we are able to measure the activation times of the switching process that lie in the 10-100 ms range. We present a simple two states model and analyze it by Laplace Transform methods to obtain a validation of the proposed model and a direct estimate of the activation times.
Finally we discuss possible applications of this behavior in fluorescence imaging and direct studies of intracellular dynamic processes.

**379-Pos Board B258**
**Conformational Transitions Of Disordered Proteins Associated With Different Redox States Of Di-thiol Pairs**
Samuel W. Fan, Richard A. George, Naomi L. Haworth, Lina L. Feng, Jason Y. Liu, **Merridee A. Wouters**.
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Because of their enhanced reactivity, physiologically important redox-active disulfides are also more susceptible to cleavage/oxidation under non-physiologic conditions. For instance, redox-active disulfides are prone to cleavage by synchrotron radiation during the process of X-ray structure determination. Here we mined the Protein Data Bank for highly similar proteins that have been solved in multiple redox states - i.e. disulfide-bonded in one structure and reduced in another. Some of these protein pairs exhibited order/disorder transitions. Disorder-to-order transitions have previously been observed upon binding of ligands. Acquisition of order upon binding of ligands concomitant with disulfide formation was apparent for the oxidoreductase gdhB, where the disulfide straddled part of the PQQ binding site, and the RNA sulfuration enzyme *Ec*TrmU, where the disulfide straddles the tRNA-binding site. However, the reverse was true for the *Thermotoga maritima* tRNA-processing enzyme, Psi55s, where significant disorder of the protein chain concomitant with disulfide reduction occurred upon binding of the tRNA substrate fragment. The introduction of disorder may facilitate further co-operative binding of the RNA and protein after the initial docking step. A subset of Redox Pair proteins exhibiting order/disorder transitions correlated with disulfide redox status may contain regions of disorder in excess of 20% of the protein chain. All the proteins in this group exist as dimers with the other monomer being more ordered. The proteins may adopt a physiologically-relevant Molten Globule state as part of their function. The oxygen-rich sequences of the disordered regions of proteins of the Redox Pair dataset seem to represent a novel type of disordered sequence not previously recognized. Finally, disordered regions are posttranslationally modified by acetylation, glycosylation, methylation and phosphorylation which may regulate the order/disorder transition. Redox-activity of disulfides should be added to this list of posttranslational modifications.

**380-Pos Board B259**
**"Arrhenius Approach to Study Kinetics of Fresh Egg Protein"**
**Dipti Sharma**.
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This study explores an interesting denaturing kinetics of fresh egg proteins following Arrhenius behavior. Fresh egg white-protein (2mg) was used in a sealed cell for scanning using calorimetric technique. Heating scans were performed from 10° C to 100° C at different heating ramp rates varying from 1 to 20° C/min. All environments were kept identical for all runs to compare parameters (temperature, enthalpy, heat energy). An endothermic peak was found on heating scan showing denaturing of protein. As heating ramp rate increases, the denature peak shifts towards higher temperature. This peak shift follows Arrhenius behavior and shows an activated denaturing kinetics of the white egg protein. This peak was also compared with the water to avoid water effects. Cooling scan and second heating scan were also performed for the samples and no residue of peak was found which clarifies that the protein was completely denatured after first heating. The denaturing peak shifts linearly with the ramp rate and temperature and gives activation energy of this transition. Behavior of denaturing peak can be explained in terms of Arrhenius theory.

**381-Pos Board B260**
**NMR Dynamics Of PSE-4 β-lactamase: An Interplay Of ps-ns Order And μs-ms Motions In The Active Site**
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Class A beta-lactamases are involved in antibiotics resistance, a persistent phenomenon in medicine and agriculture. Many kinetics and structural studies have been reported. However, comprehension of their serine-based mechanism is incomplete. Studying the dynamics of these enzymes and relating it to the considerable structural and functional data available could provide more insights. Indeed, dynamics on different timescales has been shown to be central to proteins function.
Beta-lactamases TEM-1 and PSE-4 are studied by NMR and molecular dynamics (MD), both atomistic methods to protein dynamics. TEM-1 is a traditional class A beta-lactamase for which a dynamic study by NMR has been reported recently. On the other hand, PSE-4 is a member of the subclass of carbenicillin hydrolyzing beta-lactamases. Both enzymes share high identity (42%) and structural homology (1.3 Å backbone RMSD).
We present an overview of the work done on PSE-4 by NMR. This includes amide exchange as well as 15N spin relaxation data. Analysis is performed using the Lipari & Szabo model-free formalism. Moreover, the assessment of datasets consistency, a prerequisite for united data analysis, is discussed. Finally, comparisons are made with the homologous TEM-1. It turns out that both beta-lactamases share high backbone order on the picosecond-nanosecond timescale, especially around the active site. Moreover, evidence of slow microsecond-millisecond motions around the active site points toward important dynamics arising on the catalysis timescale.
In the near future, relaxation dispersion experiments will aim at quantifying the slow microsecond-millisecond motions detected with backbone 15N spin relaxation. Moreover, experiments will be performed to assess the influence of substrate (or inhibitor) binding on the dynamics. Finally, clinically-relevant mutants will be studied to link their increased activity to possible changes in dynamics.

## Protein Assemblies

**382-Pos Board B261**
**SEDPHAT - An Analysis Platform for the Biophysical Analysis of Reversibly Assembled Multi-protein Complexes in Solution**
**Patrick H. Brown**.
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Multi-protein complexes are ubiquitous in cellular activities, and found in metabolism, transcription control, and intracellular signaling and motility. In many areas, intense research is devoted to understanding the functional mechanism of these complexes, for example, dissecting the energetics of the total assembly process from the individual components to form the functional active multi-protein complex. This is particularly difficult for complexes that are only transiently assembled by relatively low-affinity interactions. Our research is devoted to developing new biophysical methods for the characterization of the number, size and hydrodynamic shape of protein complexes as well as quantifying the dynamics of their assembly and disassembly from purified components in solution. One approach utilized is to make measurements from orthogonal perspectives of the reaction coordinate and to integrate these data in a global analysis. To this end, we have developed the analysis software platform SEDPHAT that allows us to analyze globally multiple data sets collected from one of several different biophysical techniques. We present here an overview of the analytical tools available in this software.

**383-Pos Board B262**
**Probing the Heterogeneity in the Distribution of Binding Properties of Immobilized Surface Sites through Bayesian Analysis**
**Huaying Zhao**, Inna I. Gorshkova, Juraj Svitel, Faezeh Razjouyan, Peter Schuck.