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.