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**1239-Pos Board B149**
**Binding of the Allosteric Effector Inositol Hexaphosphate (IHP) Modulates the Dynamics of Carbonmonoxyhemoglobin on Multiple Length Scales**
**Jyotsana Lal**, Nancy T. Ho, Chien Ho, Marco Maccarini, Peter Fouquet, Lee Makowski.
The allosteric effector IHP binds deoxy-Hb and HbCO, albeit at different sites, resulting in both cases in a lowered oxygen affinity. The manner in which these interactions impact oxygen binding is unclear and may involve changes in structure, dynamics or both. Wide-angle x-ray solution scattering has been used to determine that IHP binds to two positions, 43 Å apart on the surface of HbCO, but results in little if any change in structure. Neutron spin echo (NSE) measurements show that the binding of IHP to HbCO results in a decrease in the average rate of motion of individual residues and an increase in the rate of coordinated motions of subunits relative to one another. The allosteric regulation of oxygen affinity by IHP may thus originate directly from the modulation of subunit-subunit motion induced through binding.

**1240-Pos Board B150**
**Biophysical Characterization of the Igg Binding Domains of Protein a In Staphylococcus Aureus**
**Andrew Hagarman**, William R. Franch, Terrence G. Oas.
Staphylococcus aureus is a gram positive bacterium found in the skin flora of most humans. Some strains of S. aureus produce enterotoxins that can cause toxic shock syndrome, food poisoning, and sepsis. The emergence of multi-drug resistant staph infections has become a significant health problem in hospitals. Part of S. aureus' virulence comes from Protein A, which binds to the Fc region of antibodies and inhibits opsonization. Protein A from Staphylococcus aureus has five homologous antibody binding domains, arranged in tandem repeats in the N-terminal half of the protein (SpA-N). Due to the highly repetitive nature of the gene, we synthesized and cloned it into an E. coli expression vector. Our previous studies of the B-domain of Protein A (BdpA) show that the folded state lifetime is ~10 milliseconds, meaning that BdpA samples its unfolded state 100 times per second. We hypothesize that this cycle of unfolding and refolding may confer flexibility to Protein A, which is crucial for the migration of the protein from the ribosome to the cell wall. To test this, we have designed a new approach to measure protein flexibility: Dynamic Shear Force Microscopy (DSFM). Along with DSFM , we have conducted preliminary biophysical analysis of SpA-N using methods such as analytical ultracentrifugation, circular dichroism spectroscopy, and fluorescence measurements.

**1241-Pos Board B151**
**Visualizing Adenylate Kinase Catalysis Through the X-ray Lens**
**Young-Jin Cho**, Dorothee Kern.
* Conformational dynamics of Adenylate kinase is a key to understand the catalysis of this enzyme: a phosphoryl transfer reaction. Adenylate kinases maintain the level of nucleotides by transferring a phosphoryl group from ATP to AMP, which results in 2 ADPs in equilibrium. The active site locates where the ATP-lid and AMP-lid meet on top of the core domain. It has been proposed that various kinds of conformational stages exist by opening and closing the ATP- and the AMP-lids. However, only 4 characteristic types of structures so far have been reported: Open (without substrates, both lids open), Closed (with inhibitor, both lids closed), the AMP domain closed (with AMP, ATP-lid open without substrate) and the ATP domain closed (mutant $Adk_{yst}$ with ATP, AMP-lid open without substrate). We have determined crystal structures of *Aquifex*Adenylate kinases with various substrates at high resolution up to 1.24 Å revealing conformational substates that include 1) a quasi-opened ATP-lid structure with substrates bound to both lids; 2) different conformations of substrates coinciding with movement of essential arginine residues; 3) a partially occupied metaphosphate intermediate structure.
* From these snapshots we suggest the role of arginine side chains in the active site with respect to the phosphoryl transfer step. By linking a series of these structures, we visualize the choreography of adenylate kinase catalysis in crystallographic view. NMR experiments characterizing the dynamics between these substates buttress our mechanistic interpretation of the x-ray snapshots.

**1242-Pos Board B152**
**Empirical Method For Calculation of Pka Values of Internal Ionizable Groups in Proteins**
**Carolyn A. Fitch**, Michael J. Harms, Bertrand García-Moreno.
Internal ionizable groups are essential for energy transduction processes. They usually titrate with highly perturbed $pK_a$ values; the molecular determinants of these $pK_a$ values are poorly understood. Structure-based calculation of the $pK_a$ values of internal ionizable groups is extremely challenging and of high importance owing to the functional roles of these groups. We have used a large set of variants of staphylococcal nuclease with internal Lys, Asp and Glu of known $pK_a$ to examine the performance of a variety of standard methods (FDPB, MCCE, PROPKA, Karlsberg) for $pK_a$ calculations, and to guide the development of an empirical computational protocol useful to calculate $pK_a$ values for these challenging cases. Empirical methods such as PROPKA are currently not appropriate for $pK_a$ calculations of internal groups. Calculations with standard continuum methods in which the protein is treated with a low internal dielectric constant (4-5) can be useful, especially when structural waters are treated explicitly and allowed to relax in the field of the internal charge. The most useful empirical protocol we have found, the one that minimizes the difference between measured and calculated $pK_a$ values, involves calculations with a standard finite difference Poisson-Boltzmann electrostatics method with the protein treated with a dielectric constant of 10 and using averaging over structures obtained with MD trajectories calculated with the internal ionizable groups in the neutral state. With this protocol convergence appears to be achieved with MD simulations on the order of 1 ns. However, the fact that the protein needs to be treated empirically with high dielectric constants near 10 despite relaxation with MD simulations suggests there are important relaxation processes not being captured with the relatively short MD simulations.

**1243-Pos Board B153**
**Molecular Origins of HIV-1 reverse Transcriptase Resistance to Efavirenz**
**Daniel J. Deredge**, Kathryn J. Howard, James M. Seckler, Mary D. Barkley, Patrick L. Wintrode.
Efavirenz (EFV) is a nonnucleoside reverse transcriptase inhibitor (NNRTI) used in the treatment of HIV/AIDS. HIV-1 reverse transcriptase (RT) is a heterodimeric protein composed of p66 and p51 subunits. Crystallographic and solution studies have shown that EFV binds the p66 subunit of the p66/p51 RT heterodimer with 100 nM binding affinity. EFV binding has been linked with dimer stabilization as well as reduction of the conformational flexibility of regions as far as ~50Å away from the NNRTI binding pocket. These effects have been hypothesized to play a role in the inhibition of RT. Several mutants of RT display resistance to EFV and other NNRTIs. K103N is a common drug resistance mutant, which confers high level resistance to EFV and other NNRTIs. The mutant residue, K103N, is located near the binding pocket, but is not a drug contact residue.
We have used analytical ultracentrifugation, equilibrium dialysis, and hydrogen exchange mass spectrometry (HXMS) to determine the effects of EFV on K103N RT proteins. EFV enhances dimerization of K103N p66/p66 homodimer 16-fold and p66/p51 heterodimer 2-fold, but not p51/p51 homodimer detectably. The moderate enhancement of dimerization by EFV contrasts the dramatic enhancement of subunit equilibrium association constants observed in the wild type upon EFV binding. In addition, we have used HXMS to probe the dynamics of HIV-RT K103N in the presence and absence of EFV. Again, it was determined that the K103N mutation significantly dampens the rigidity induced by EFV binding on the conformational flexibility of the wild type. Overall, our results suggest that EFV resistance conferred by the K103N mutation is not due to a loss in drug binding but rather to the reduction of long range effects induced by drug binding in the wild type.

**1244-Pos Board B154**
**Network Analysis of the Communication Pathways in HIV-1 Envelope Proteins For Mechanistic Understanding of Immune Escape**
**Anurag Sethi**, Cynthia Derdeyn, Bette Korber, S. Gnanakaran.
Allosteric mechanisms play a key role in the binding of the envelope protein (Env) on HIV-1 to CD4 and CCRX5 receptors on human cells, which is essential for viral entry into these cells. Env protein, gp120, has been the focus of antibody based vaccine. Phylogenetically corrected statistical methods have been used to identify amino acid signature patterns in gp120 that are differentially sensitive to neutralization by the well-characterized gp120-specific monoclonal antibodies and, recently from sequences derived from serum covering a wide range of neutralizing potency. Escape mutations/signatures that are a direct consequence of antibody binding are easier to interpret. But, in many cases escape signatures are manifestations of indirect regulation of antibody access due to the conformational variability, quaternary nature and allostery of Env protein. Here we develop theoretical approaches to identify some of these indirect mechanisms by which immune escape may occur. We have performed long time simulations of three different crystal structures of gp120 representing two clades. We compare the patterns of coupled motions of different regions of gp120 using covariance, principal component and network theory analyses. We find that dominant coupled (allosteric) motions of spatially separated regions in the gp120 core are preserved across clade.