fast form is initiated by sodium binding. Allosteric communication between the sodium binding site and active site is necessary for substrate recognition and enzyme function: specifically, the conversion from the slow form to the fast form upon $Na^+$ binding. Thrombin contains a water channel that extends from the $Na^+$-binding site to the active site. It has been suggested previously that this water channel may play a role in the allosteric communication between the $Na^+$-binding site and the active site. We have analyzed water channel structure and fluctuations in *apo* and *holo* ($Na^+$-bound) forms of thrombin using molecular dynamics. Our results show that the water channel of thrombin exists in three distinct states. States of large channel volume and high water occupancy are observed more often when the $Na^+$-binding site is empty. These large volume/high occupancy states include a water channel that completely connects the $Na^+$-binding site to the active site through a network of hydrogen bonds. Furthermore, the large volume/high occupancy water channel causes significant structural changes in the active site, including disruption of hydrogen bonding in the catalytic triad. Conversely, when the cation binding site is occupied by a $Na^+$-ion, the water channel has a significantly smaller volume, fewer water molecules, and does not provide a complete hydrogen bond network between the $Na^+$-binding site and the active site. We analyze these results in the context of changes in the catalytic activity of thrombin in the presence and absence of $Na^+$.

**3068-Pos Board B115**

**The Importance of Electrostatic Effects in the Recognition of NPF Motifs by the EH Domains of Mammalian EHD Proteins**

**Gillian D. Henry**, Joseph V. Dineen, James D. Baleja.
Tufts University, Boston, MA, USA.

EHD proteins are membrane remodeling proteins that control exit of receptors from the recycling endosome and their return to the plasma membrane. They consist of a nucleotide binding domain and an EH domain, which is a small $Ca^{2+}$ binding module composed of 2 EF hands. EH domains bind to asparagine-proline-phenylalanine (NPF) motifs, which are found in various proteins, often in multiple copies, throughout the vesicle trafficking pathway. The mechanism by which EH domains discriminate between individual NPF containing proteins is not well understood. There are 4 closely related EHD proteins in mammals, termed EHD1-4. We have measured the affinities the EH domains from all 4 EHD proteins for 11-residue NPF containing peptides derived from Rabenosyn-5 and Rab11-Fip2 using isothermal titration calorimetry. The highly acidic Rabenosyn peptides bind with affinities of the order of $6*10^5\ M^{-1}$; this is an order of magnitude higher than was found for the neutral Rab11-Fip2 peptide. The Rabenosyn peptide interaction was also found to be salt dependent. In a second set of experiments, we synthesized EH domains from EHD1 and EHD4 with short NPF containing sequences from derived from Rabenosyn or Rab11Fip2 tethered to the C-terminus. Fluorescence quenching experiments showed that a tryptophan residue at the base of the NPF binding pocket was protected by the tethered NPF. All tethered NPF sequences enhance the stability of the EH domain to urea denaturation, but the Rabenosyn sequence (13 $kJmol^{-1}$) is more effective than the Rab11-Fip2 sequence (9 $kJmol^{-1}$). Our results suggest that negative charges directly following the NPF sequence strongly enhance the affinity of EHD EH domains for their target sequences.

**3069-Pos Board B116**

**Analyzing Electrostatic Determinants of Affinity and Promiscuity in the HIV-1 Reverse Transcriptase System Using Charge Optimization**

**Mona Minkara**, Mala L. Radhakrishnan.
Wellesley College, Wellesley, MA, USA.

Electrostatic charge optimization (Lee and Tidor, *J. Chem. Phys.* 1997) was used to study the binding site of wild type and mutant HIV-1 reverse transcriptase (RT) complexed with the non-nucleoside inhibitors nevirapine and rilpivirine (TMC-278). Our ultimate goal is to analyze and to further understand the electrostatic determinants of tight binding and broad molecular recognition toward this rapidly-mutating target. For each inhibitor-RT mutant pair, we computed the drug's optimal charge distribution - the hypothetical charge distribution that would bind to the target variant more tightly than any other isosteric drug. By comparing these optimal charge distributions with each drug's actual charge distribution, we are able to identify potential sites of electrostatic non-complementarity that may be altered to increase binding affinity. Additionally, by comparing the electrostatic optima for a given drug toward multiple RT variants, we can gain insight into the sensitivity of the electrostatic determinants of binding to the variability in RT as a result of certain drug resistance mutations.

**3070-Pos Board B117**

**A Thermodynamic Study Of Ligand Access/escape From Protein Cavities**

**Harish Vashisth**, Cameron F. Abrams.
Drexel University, Philadelphia, PA, USA.

Insulin, a protein hormone, regulates glucose homeostasis and carbohydrate metabolism in higher organisms. Therapeutic formulations of hormone are preserved against degradation and denaturation by antimicrobial preservatives such as phenols, cooperative binding of which stabilizes hexameric complexes of insulin. Dissociation of hexameric species (on minutes to days time scale) into biologically active monomers is facilitated by rapid unbinding of phenols (on milliseconds time scale). However, a clear understanding of dissolution kinetics and determinants of the rates of ligand unbinding remains obscure, chiefly due to unresolved ambiguities in NMR results. We have used random acceleration molecular dynamics (RAMD) to identify and characterize a variety of potential ligand dissociation mechanisms. We observe three distinct exit routes for the ligand and resolve potentials of mean force (PMFs) along them by performing free energy calculations. Free energy profiles for each mechanism are computed with the help of second order cumulant expansion of Jarzynski's equality and non-equilibrium work statistics gathered from multiple independent steered molecular dynamics (SMD) simulations. Based on energetic barriers and structural properties, we suggest a plausible preferred mechanism for the ligand exchange. The most likely pathway with the lowest free energy barrier involves a leap over the ''gate'' formed by HisF5 and IleA10, with simultaneous passage of the ligand through a narrow channel existing between LeuA13, LeuH17, and the ''gate''. Free energy profiles also display several weakly-bound metastable states for the ligand during entry and exit from R6 insulin hexamer.

**3071-Pos Board B118**

**Expression of, and Preliminary Biophysical Characterization of a Minimal Binding Domain peptide of TSC2 that binds to the small GTPase Rheb**

**Paul D. Adams**, Huimin Liu.
Uninveristy of Arkansas, Fayetteville, AR, USA.

Rheb (Ras homolog enriched in brain) is a recently identified Ras protein the cycles between GTP-and GDP-bound states similarly to other Ras proteins. An important interaction between Rheb and the Tuberous Sclerosis complex proteins (TSC1/2) mediates the mammalian Target Of Rapamycin (mTOR) pathway, which regulates cell growth, energy and nutrient levels. TSC2 contains a GTPase-binding domain near its C-terminus that contains a highly conserved 15-amino acid region. Mutations in this region has resulted in altered GTPase activity towards Rheb, suggesting that the 15 amino acid conserved region is vital for this interaction.

To date, molecular details of this important Ras-effector interaction are unknown. We present here a preliminary characterization of an interaction of Rheb with a minimal GTPase-binding domain peptide derivative of TSC2. A series of peptides truncated from the C-terminal region of TSC2 encompassing the GTPase-binding domain were expressed in bacterial cell lines. A peptide of 37 amino acids (TSC2-37) has demonstrated similar binding affinity to Rheb in-vitro to a 900 amino acid C-terminal derivative of TSC2. Isothermal titration calorimetry was used to examine the binding affinity of a synthetic peptide consisting of only the15 amino acids of the conserved GTPase-binding region of TSC2 for Rheb to highlight the importance of the conserved region in binding. These results represent a first step towards the biophysical characterization of the Rheb-TSC2 complex that is expected to provide molecular details that can be translated to its biological function.

**3072-Pos Board B119**

**Biochemistry on a Leash: Confinement as a Regulatory Mechanism for Bimolecular Reaction Rates**

**Daniel Reeves**, Keith Cheveralls, Jane Kondev.
Brandeis University, Waltham, MA, USA.

We describe two mechanisms by which confinement is utilized to regulate diffusion-limited bimolecular reaction rates. The first mechanism, illustrated by the actin capping protein formin, uses a flexible polymer to tether ligand binding sites, which serve as intermediaries, to the reactive site. The second mechanism uses a potential (e.g. hard wall potential), to constrain the motion of a ligand receptor within a confining volume. We analyze both mechanisms theoretically, using a combination of analytic and numerical techniques, to obtain the steady state binding kinetics. We explore how the reaction rates are regulated by parameters of the model such as the length of the polymer tether, and use our findings to explain the key features of the formin system. Finally, we suggest other systems, both synthetic and biological, in which these mechanisms for regulating bimolecular reactions might be at play.

**3073-Pos Board B120**

**A Comparative Study On The Interaction Of New Designed Aliphatic Pd(II) Complexes With Human Serum Albumin**

**Adeleh Divsalar**[1], Mohammad Javad Bagheri[1], Ali Akbar Saboury[1], Hassan Mansoori-Torshizi[2].
[1]Instute of Biochemistry and Biophysics, Tehran, Iran, Islamic Republic of, [2]Department of Chemistry, University of Sistan & Baluchestan, Zahedan, Iran, Zahedan, Iran, Islamic Republic of.