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

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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

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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

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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

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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

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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

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Human serum albumin (HSA) is the most abundant protein in the systemic circulation, with comprising 60% in plasma. This protein can play a dominant role on the drug disposition and the efficiency. The pharmacokinetics and pharmacodynamics of any drug depend, largely, on the interaction with HSA. Hence, in present study, the interaction between new designed Pd(II)-complexes, 2,2′-bipyridin ethylglycinato Pd(II) nitrate, 2,2′-bipyridin butylglycinato Pd(II) nitrate and 2,2′-bipyridin octylglycinato Pd(II) nitrate, anti-tumor components, with human serum albumin as a carrier protein, were studied at different temperatures of 27 and 37° C by fluorescence spectroscopy, circular dichroism (CD) spectrophotometry and differential scanning calorimetry (DSC) techniques. Results showed Pd(II)-complexes have strong ability to quench the intrinsic fluorescence of HSA through static quenching procedure . The binding parameters were evaluated by fluorescence quenching method. From enthalpy and entropy of binding, it can be concluded that hydrophobic interactions may play an important role in the binding of Pd(II)-complexes to HSA. Far-UV-CD results represented that Pd(II)-complexes induced decreasing in content of α helical structure of protein.
From above results, it can be concluded that the blood carrier protein of HSA could bind and transfer of these new anti-cancer drugs, but the stability of the protein decreased upon the interaction with this complexes.
***Keywords*:** Pd(II) complex, DSC, HAS, Quenching, Thermodynamic parameters.

3074-Pos Board B121
Molecular Aspects of Calcium Binding in NCAD12
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Cadherins are calcium dependent,transmembrane proteins, which mediate cell-cell adhesion through homophilic interactions. They play a fundamental role in embryogenesis and tissue morphogenesis. The primary structure of cadherins comprises five extracellular domains, a transmembrane segment, and a conserved C-terminal cytoplasmic region, which interacts with the cytoskeleton through catenins. It has been known that cadherins require calcium binding at the interfaces of the extracellular domains for cell adhesion and for protection from proteases. Recent literature has proposed that binding of three calcium ions (Ca1, Ca2 and Ca3) at the domain interfaces causes a conformational change, a ''closed'' to ''open'' transition, in the extracellular domain 1, which leads to cell-cell adhesion. However, to date, no solution studies have demonstrated the conformational change upon calcium binding. Despite the important function of calcium binding in cadherin mediated cell adhesion, the characteristics of calcium binding are still unclear .In order to determine the molecular aspects of binding of Ca1, Ca2 and Ca3 in cell adhesion, a comprehensive study of the calcium binding properties is required. Our work focuses on the first two-extracellular domains of neural cadherin (NCAD12). We mutated an essential residue D134 for the binding of Ca3 in NCAD12 wild type. We used Circular dichroism and Fluorescence spectroscopy in order to obtain global and local information of conformational change on calcium binding to NCAD12 wild type and D134A. These studies were repeated with a mutant, D136N, designed to probe the cooperativity between calcium binding sites.Spectroscopic and proteolytic footprinting studies of NCAD12 wild type, D134A and D136N show that these proteins behave differently in presence of calcium. The mutations significantly lowered the stability and increased protease sensitivity in the presence of calcium. These preliminary results imply that binding of Ca3 is a critical step in conformational change.

3075-Pos Board B122
Effect of Osmolytes on Protein Stability and Folding
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Cells exhibit certain cellular coping mechanisms when faced with osmotic stresses by importing or producing organic compounds called osmolytes which aid in osmotic regulation. Proline is an example of such a compound. The primary function of these compounds is to combat the effects of dehydration in the cell. Stabilization of proteins, which are particularly susceptible to osmotic stresses, is of key importance to the cell's health. Osmolytes have been shown to directly impact the stability and solubility of proteins, and certain organic osmolytes also exhibit the function of aiding in protein folding and refolding and in preventing protein aggregation. The mode by which osmolytes aid in assuring protein stability is believed to be a solvent-oriented process by which protein folding is facilitated by the preferential ordering of solvent molecules, but the exact mechanism of stabilization remains elusive. In this research project, we characterized the supramolecular structure of proline at high concentration in solution using multi-dimensional NMR spectroscopy and dynamic light scattering. The molecular mechanism underlying the stabilizing effect of proline on a protein is studied using thermal denaturation monitored by steady-state fluorescence. Results from the thermal denaturation studies indicate that the Tm (the temperature at which 50% of the molecules are in the native state) of the protein increases in the presence of increasing concentrations of proline by about 20 °C, suggesting that thermodynamic stability of the protein is enhanced upon binding to proline. Stability studies using several other osmoloytes like TMAO, glycerol, 4-hydroxy proline, and betaine show that proline is the osmolyte which stabilizes the protein to the largest extent. Two-dimensional HSQC NMR experiments were used to reveal the binding sites of proline on FGF-1. The results of this study provide useful insights on the molecular mechanism of proline.

3076-Pos Board B123
Strategy And Biophysical Tools For Developing Modern Diagnostic Assays
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Neutrophil Gelatinase-Associated Lipocalin (NGAL) is a 20 kDa monomeric protein secreted by activated human neutrophils. NGAL is believed to bind small lipophilic substances such as bacteria-derived lipopolysaccharides, siderophores, formylpeptides, and may function as a modulator of inflammation. Clinical studies have shown NGAL can serve as an early diagnostic marker for acute kidney injury (AKI). We have developed a sensitive immunoassay to measure NGAL level in patient urine. During the course of assay development, we employed a variety of biophysical methods to characterize the physical and binding properties of several anti-NGAL antibody candidates and a recombinant NGAL protein. CD spectroscopy was used to study the structure and stability of NGAL; Förster Resonance Energy Transfer (FRET) was used to determine the binding affinity of NGAL toward anti-NGAL mAbs; Dual-Color Fluorescence Cross-Correlation Spectroscopy (DC-FCCS) was used to compare the capability of two antibodies forming a sandwich with NGAL; NMR was used to identify epitopic regions of NGAL for each antibody candidate. Two antibodies, which have the highest binding affinity toward NGAL and recognize distinct discontinuous epitope regions on NGAL, were chosen as the reagents for a sandwich type microparticle based immunoassay. This work demonstrates a modern strategy and biophysical tools, which are necessary to build a sensitive and robust diagnostic assay.

3077-Pos Board B124
Recognition and Discrimination of Gases by the Signal Transducers HemAT
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The recognition and discrimination of diatomic gases that affect biological processes through the perturbation of protein function is a formidable challenge towards our understanding of the molecular mechanisms involved in signal transduction by heme-based sensor proteins (1). There is a general consensus now that internal cavities in proteins are involved in controlling the dynamics and reactivity of the protein reactions with small ligands, such as O_2, CO, and NO usually through ligand accommodation. These cavities serve as a local storehouse for ligands near the active site, thereby increasing the effective concentration of the ligand many times. In addition to having functional role in ligand binding they are also important for determining relative affinities. In the heme-based oxygen sensors such as HemAT the recognition and discrimination of the specific gas leads to either activation or inhibition of a regulated domain. The dynamic coupling between two distinct binding sites as the underlying allosteric mechanism for gas-recognition/ discrimination that triggers a conformational switch for signaling by the oxygen sensor protein HemAT will be presented (2-3).
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3078-Pos Board B125
Fluorescent-tagged Kinases: A New Assay System For Detecting And Screening For Allosteric Kinase Inhibitors
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Targeting the ATP binding pocket has been the most common strategy for inhibiting kinases. Recently, a less-conserved allosteric site was identified in some kinases (i.e. Abl, EGFR and p38α), opening up patent space for novel drug scaffolds which are more kinase specific. Kinases are typically in the active conformation (DFG-in) with the activation loop open and extended, allowing ATP and substrates to bind. Alternatively, the adjacent allosteric site is available only in the inactive conformation (DFG-out) in which the activation