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

loop undergoes a large conformational change. It was our aim to develop a new fluorescence-based kinase binding assay which could sense this conformational switch and screen for new allosteric kinase inhibitors.
We used crystal structures of p38α (active and inactive) to identify positions in the activation loop which undergo significant movement and could be mutated into Cys for the subsequent attachment of environmentally-sensitive fluorophores. Other solvent-exposed Cys were mutated into Ser to minimize non-specific labeling. Using acrylodan-labeled p38α, we developed the first sensitive (Z-factor = 0.85) HTS method for screening inhibitor libraries for allosteric kinase inhibitors. Endpoint fluorescence measurements can be used to directly determine the Kd of inhibitor binding. Real-time fluorescence measurements provide information about the kinetics of association and dissociation.
Adaptation of this approach to other kinases has allowed us to identify weakly binding scaffolds which bind to the allosteric site. Using structure-based drug design, we were able to further develop these smaller compounds into more potent kinase inhibitors (Kd = low nM). These new tight binding compounds have also become powerful tools for identifying which kinases can and cannot be inhibited allosterically, a critical question which remains to be answered in the kinase field.

3079-Pos Board B126
Fluorescent, Protein-Based Sensors for ADP
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ATP conversion to ADP is a central process in all living organisms and is catalyzed by a vast number of different enzymes. The energy generated can drive metabolic processes, directed transport, force-generation and movement as well as signal transduction and regulation. While ATPases generate ADP and free phosphate, kinases transfer the terminal phosphate of ATP to a wide variety of substrates, from metabolic intermediates to proteins, so controlling their activity. Hence, assays to monitor ADP concentrations have wide applications in biochemical and biomedical research, ranging from detailed understanding of mechanochemical coupling in motor proteins to screening for ATPase and kinase inhibitors.
Fluorescent, protein-based biosensors have been reported for a number of biomolecules such as sugars, amino acids, metal ions and phosphate. This approach takes advantage of the highly specific interaction of a protein with the target molecule, which can be coupled to an optical signal by attaching fluorophores in suitable positions on the protein. Following this strategy, we have developed sensors for ADP based on fluorescently labelled mutants of the bacterial actin homologue ParM. We report two ADP sensors with distinct optical properties and ADP-binding characteristics, suitable for different types of in vitro assays. A coumarin-labeled variant binds ADP tightly and fast and can detect submicromolar concentrations of ADP. The sensor is particularly useful for mechanistic studies, where high sensitivity and high time resolution are required. The second variant is labeled with two rhodamine dyes, exploiting the stacking of rhodamines to generate a signal change. This variant has a more photostable fluorophore, higher wavelength excitation and lower ADP binding affinity, making it more suitable for high-throughput screening assays.

3080-Pos Board B127
Using Molecular Dynamics to Investigate Substrate Recognition and Co-evolution in HIV-1 Protease
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Human Immunodeficiency Virus Type-1 (HIV-1) protease recognizes at least ten cleavage sites as its natural substrates. There is little sequence homology between these substrates and they are asymmetric around the cleavage site in both charge and size distribution. Thus, understanding of the molecular determinants of substrate recognition is challenging as well as of great importance in design of effective drugs. The protease-substrate complex crystal structures indicate that substrates occupy a remarkable uniform region within the binding site, which has been termed as the substrate envelope. Nevertheless, protein activity is intimately related to the dynamics, from local to global motion of the structure. To this end, an elaborated analysis on both structural and dynamic features of seven HIV-1 protease-substrate complexes have been carried out by molecular dynamics (MD) simulations. Conformations of the complex structures in time were analyzed with respect to the interaction of substrate with protease in terms of the substrate volume, changes in van der Waals contacts between the two, and dynamics of both substrate and protease. Co-evolution of substrate peptides with the drug-resistant protease variants was also analyzed. Similar analysis to those in wild-type complex structures were done for MD simulations for p1-p6 substrates (wild-type and LP1'F) in complex with protease variants (D30N, N88D, and D30N/N88D). The substrate recognition was observed to be an interdependent event and the recognition mechanism may not be the same for all natural substrates. The dynamic substrate envelope was found to be smaller than the crystal structures suggest. The substrate recognition is altered when there is drug resistance and this alteration is compensated by co-evolution. The results reveal that conservation of the peptide conformational preferences and dynamic behavior of the complex structure appears to be important for substrate recognition.

3081-Pos Board B128
Peptide Binding Site Determination Using Conformational Memories and MMC
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Conformational memories (CM) is a Monte Carlo/simulated annealing method that efficiently searches the torsional conformational space of flexible molecules.[1] This method has been recently expanded to include variable bond angles.[2] We report the application of CM to ligand/protein docking via the study of a GP-41 7mer, ELDKWAS, bound to the HIV-1 neutralizing antibody 2F5 (PDB ID: 1TJG).[3] Crucial cavity bound waters were determined using MMC and compared to the 1TJG structure.[4] CM was initiated with the peptide 53 Å above the protein and brought to the surface in 18 steps using 0.25 kcal/mol planar and 0.05 kcal/mol cylindrical restraints. At each position, 200,000 MC steps were performed with the temperature annealed from 3000K to 310K. In order to account for electrostatic screening in aqueous solutions we employed a distance dependent, non-linear (sigmoidal) screened Coulomb potential.[5] Final system energies were calculated with a constant dielectric of 1.0 or with the GB/SA solvent model for water. The GB/SA model was found to yield better results with the identification of the native energy funnel for the peptide.[6] The lowest energy structure identified had an RMSD (backbone atoms) less than a 1.5 Å compared to the peptide conformation in the 1TJG structure. [Support: NIH DA03934 and DA021358]
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3082-Pos Board B129
Inferring Protein-protein Interface From Geometry Of Unbound Structures
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Protein-protein interactions are the basis of cellular functions. Although computational methods have been developed to identify interface sites of protein-protein interaction, this remains a challenging task because of the complications such as conformation change and multiple binding partners. We develop a novel interface prediction method by incorporating information from unbound structures of both binding partners. Proteins are represented by alpha shape and the geometric characteristics of the surfaces are used to generate surface patches dynamically. Statistical interface potentials derived from the protein-protein interaction dataset are used to discriminate candidate interfaces. Protein flexibility, interface collision, and solvent accessible area are integrated in our method to model the binding process more realistically. Our new method can create surface patches very close to the native interface. Compared to the prediction results of the state-of-art meta method that combined several interface predictors, our method gives similar performance on the protein-protein interaction of Benchmark dataset, and gives better performance on more challenging targets from the CAPRI (community wide protein-protein docking competition). Furthermore, our method overcomes the limitations of existing methods and can predict multiple interfaces on proteins with multiple binding partners.

3083-Pos Board B130
Flexible Docking in PDZ domains using Elastic Network Model and Replica Exchange Molecular Dynamics
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Molecular docking is a key tool for studying protein-ligand or protein-protein interactions and for designing new drugs. Majority of the current binding/docking methods attempt to predict the bound ligand by keeping the protein (receptor) fixed and moving the target ligand around the binding site while