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

performing an energy minimization. Modeling of protein binding site flexibility is still a challenging problem due to the large conformational space that must be sampled and inaccurate energy function.
Here, we discuss a new strategy for achieving successful flexible docking of peptides to PDZ domains. Due to promiscuous behavior of PDZ domain proteins, two different sets of conformations are obtained by perturbing the unbound structure along the normal modes of elastic network model (ENM) responsible for Class I and Class II type binding. A restrained replica exchange molecular dynamics (REMD) is applied to these perturbed structures to explore the conformational space of the protein receptor. After restrained-REMD, different peptides are docked to each individual snapshots of the receptor to generate a collection of docked complexes of different stabilities.

3084-Pos Board B131
Computer Simulations of Channeling the Coenzyme Nicotinamide Adenine Dinucleotide Between Glycolytic Enzymes
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Functional protein-protein interactions are essential for many physiological processes. Some of these functional interactions have been hypothesized to play a role in substrate channeling, cofactor or coenzyme transfer, and compartmentation in glycolysis as a result of transient or dynamic interactions between glycolytic enzymes. Herein, Brownian dynamics (BD) elucidates the interactions between the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH); the transfer of the cofactor nicotinamide adenine dinucleotide (NAD) between LDH and GAPDH. BD tests the hypotheses of whether the interaction between GAPDH and LDH produces a functional complex that can efficiently and reversibly transfer the cofactor NAD(H) between both enzymes. Preliminary results suggest favorable enzyme-enzyme complexes between GAPDH and LDH involving four different binding modes. These complexes are mainly stabilized by positively charged lysine residues and negatively charged glutamates and aspartates from both GAPDH and LDH. The efficiency of transfer determined as the relative number of BD trajectories that reached any active site of LDH or GAPDH, show higher transfer efficiencies (about an order of magnitude) when the cofactor NAD is transferred from a GAPDH active site to an LDH active site as compared to transfer efficiencies of NAD from solution to each enzyme of the complex. The average transfer time of NAD from solution to the free enzymes is 500 ns as compared to 57-200 ns when NAD is transferred between active sites of a GAPDH/LDH complex. Similarly, the frequency distribution profiles of transfer times suggest a preference for channeling NAD between GAPDH and LDH as compared to diffusing from solution. Channeling transfer is more efficient that solution transfer, due to active site proximity, favorable electrostatics and complex geometry

3085-Pos Board B132
Molecular Modelling Of BCRP (ABCG2) Multidrug Resistance Protein And Docking Of New Camptothecin Analogues
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The ABC transporter superfamily is among the largest and most broadly expressed protein families. Members of this family use the energy stored in ATP molecules to actively extrude a variety of substrates from cells, including exogenous compounds such as drugs, metabolites, peptides, steroids, ions and phospholipids. So far, the best known and characterized major drugs transporters, have been studied in details with respect to their structure and function. It has been showed that ATP-dependent transporters can cause resistance in cancer cells by actively extruding the clinically relevant chemotherapic drugs. There is accumulating evidence that active export of anticancer drugs from cells by means of specific transporters is one of the major mechanism of drug resistance. Camptothecin (CPT) and its derivatives has been proven to be effective against a broad set of tumors. The CPT target is the human DNA topoisomerase I, an enzyme that changes the topological state of the DNA double helix during biological activity of the cell. Here we report a computaional study of the interaction mechanism between a set of biochemically and clinically relevant campthotecin ligands. A series of multiple docking simulations were carried out using the topotecan, gimatecan and irinotecan CPT derivatives as possible ligands, and a homology model of ABCG2 transporter was used as target molecule. Our results show that the camptothecin derivatives dock to distinct sites located in the trans-membrane region of the transporter molecule. The chemical nature of the substitutions at position A of the CPT analogues used in this study is also analyzed to identify the structural prerequisites responsible for the relative selectivity of the ligand. The structural basis of ligand binding may help design new CPT analogues with reduced side effects and higher affinity.

Protein-Ligand Interactions III

3086-Pos Board B133
NMR Spectroscopic and Kinetic Investigations of the Interaction of Protein Kinase A with Phospholamban and Phospholamban Mutants
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The Catalytic-subunit of Protein Kinase A (PKAc) mediates the phosphorylation of a number of proteins in cardiomyocytes which, in turn, governs myocardial contraction and relaxation. Although a wealth of kinetic and atomic-level structural data is available for the interactions of PKAc with standard, largely non-physiologically relevant substrates, these data are nearly absent for the interactions with substrates found in cardiomyocytes. Phospholamban (PLN) is a substrate of PKAc in cardiomyocytes, where it regulates the sarcoplasmic reticulum Ca2 ± ATPase. Phosphorylation of PLN allows the relief of its inhibitory affects on Ca2+ transport into the sarcoplasmic reticulum. Here, we investigate the interactions of PKAc with PLN using a variety of biophysical techniques which include NMR spectroscopy, isothermal calorimetry (ITC), and steady-state kinetic assays. Kinetic assays were used to define the steady-state kinetic parameters for the catalytic efficiency of phosphorylating PLN and two mutants of PLN, R9C and R14-delete. The ability of PKAc to bind these proteins was also measured using ITC to investigate any differences in binding affinity. Finally, TROSY-based NMR spectroscopy was used to observe and map the residue specific differences in the amide fingerprint of PKA-C when bound to each of these substrates. These data will be presented to model the effects of PLN mutations on the interactions with PKAc.

3087-Pos Board B134
Molecular recognition in protein/carbohydrate systems: From biophysics to anti-viral therapies
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Many cell-surface proteins are glycosylated, and the carbohydrates moieties can play important roles in the biological function of these proteins. Computational models have proven to be highly successful in providing deep insights into the functions of proteins and nucleic acids, and thus the application of similar approaches to the functional interactions of glycoproteins is promising area of research.
We have developed several new approaches to the modeling of interactions between glycoproteins, including continuum solvation models optimized for carbohydrates, and adaptions of computational protein design algorithms for application to glycoproteins. Results in simple systems show that these methods are highly efficient and robust [Green, DF, J. Chem. Phys. 2008].
Our new methods have additionally been applied to understanding the key features of carbohydrate recognition by virucidal lectins that are currently under investigation as anti-HIV prophylatics. Computional models explain the oligosaccharide specificity of cyanovirin-N, [Fujimoto, YK et al., Protein Sci. 2008] and initial results in the design of cyanovirin-N variants with enhanced efficacy are very promising.

3088-Pos Board B135
Comparative Biophysical Analysis of Centrosomal Proteins and Their Complexes
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Centrin is an EF-hand protein that plays both structural and regulatory roles in the centrosome. FT-IR spectroscopy was used to study Hcen1 and Hcen2 in the spectral region of 1700 - 1530 cm^{-1} was studied to determine the order of events during the thermal perturbation. For Hcen1 the order of events throughout the thermal perturbation is detailed as the following: alpha-helix followed by beta-sheets then glutamate and finally beta-turns while for Hcen2 the order of events: 3_{10}-helix followed by aggregation then β-turns, arginine and finally loops. A higher thermal stability was observed for Hcen1 than for Hcen2 and a pre-transition at 1.7 - 4.8 °C and the onset of the transition temperature was also observed for Hcen1 at 80.5 - 84 °C. Unlike Hcen1, Hcen2 was observed to aggregate at the temperature range of 43 - 58 °C. Therefore, we were able to establish differences in stability, conformation and dynamics between these closely related calcium binding proteins.
Furthermore, this calcium-binding protein interacts at low calcium levels with a novel 1242-amino acid protein known as Sfi1, which contains up to 23 centrin-binding sites. Coupled biophysical, structural, and dynamic analyses of the centrin/Sfi1 complex are essential to the understanding of its biological function. Using an interdisciplinary approach we have determined the