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