monofunctional, being capable of only the adenylation of FMN to form FAD. Studies on bacterial FADS have shown considerable promiscuity in the acceptance of altered FMNs as substrates but absolutely no uptake of different nucleotide triphosphates (e.g. CTP, TTP, etc.).
Here we show that bacterial FADS (from C. ammoniagenes, CaFADS) will accept a modified ATP analog, specifically the fluorescent ATP analog aminopurine-2'-deoxyriboside-5'-triphosphate (2ApTP) for the production of dual fluorescent F-2Ap-D. Surprisingly, preliminary experiments indicate the monofunctional hFADS is not similarly promiscuous. These results implicate that this difference in substrate specificity may lead to unique biomarker applications. Understanding these enzymes may also lead to new approaches in nucleoside analog therapeutics.

2852-Pos Board B7
Effect of Two Point Mutations in Lung Surfactant Protein-D on Enhancing its Inhibition Activity Against Influenza a Virus
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Lung collectin surfactant proteins are pulmonary host defense proteins that contribute to innate, front-line defense against influenza A virus (IAV) and other inhaled pathogens. Effective pulmonary host defense requires fast recognition by lung surfactant protein D (SP-D) of glycans on the globular head of IAV hemagglutinin (HA), thereby initiating events leading to pathogen neutralization. In site-directed mutagenesis experiments, a double mutant of human SP-D, namely R343V/D325A, was found to enhance IAV neutralization activity. In order to understand the effect of the double mutation on the mechanism of SP-D recognition and inhibition of IAV HA, we performed molecular dynamics simulations on docked SP-D-HA complexes for wild type SP-D (wtSP-D) and double mutant SP-D (dmSP-D). Our simulations revealed that side chain properties of the mutated residues and modes of glycan binding lead to increased binding affinity of dmSP-D to the active site of IVA HA, increasing thereby its antiviral activity.

2853-Pos Board B8
Druggability Assessment of Allosteric Proteins by Dynamics Simulations in Presence of Probe Molecules
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Druggability assessment of a target protein is an important concept in drug discovery. Target flexibility and allostery poses challenges to structure based binding site identification and druggability assessment methods. We developed a simulation-based methodology for comprehensive analysis of the dynamics and binding properties of target proteins (1). Two distinguishing features of the methodology are: (i) simulation of the target in presence of a diversity of probe molecules selected upon analyzing functional groups on approved drugs, (ii) identification of druggable sites and estimation of corresponding maximal affinities based on the geometry and energetics of bound probe clusters. The use of the methodology for a variety of targets such as PTP1B, lymphocyte function-associated antigen 1, and vertebrate kinesin-5 (Eg5) provides shows that the method correctly captures the location and maximal affinities known bindings sites. It also provides insights into the target's structural changes that would accommodate, if not promote and stabilize, drug binding (2). Notably, the ability to identify high affinity spots even in challenging cases such as PTP1B or Eg5 shows promise as a rational tool for assessing the druggability of protein targets, and identifying novel allosteric sites for drug binding. A thorough case study of cytochrome (cyt) c druggability provides further support to the utility of the methodology for prospective targets with limited structural data. We identified the pocket facing heme and accessible in the partially unfolded form of cyt c as a sub-nanomolar druggable site. The first ever pharmacophore model developed for cyt c based on these simulations can distinguish known heme binders and is being advantageously used for discovering new drug-like inhibitors of cyt c.
1. Bakan A et al. J Chem Theory Comput. 2012 8(7):2435-47.
2. Bakan A, Bahar I. PNAS 2009 106(34):14349-54.

2854-Pos Board B9
Designing Short Peptides with High Affinity for Organic Molecules: A Combined Docking, Molecular Dynamics and Monte Carlo Approach
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We present a method to design small peptides able of high affinity binding with a preselected target molecule. Our algorithm builds on a combination of molecular dynamics, semi-flexible docking and replica exchange Monte Carlo, and performs simultaneous sampling in sequence and conformational spaces carefully selecting the degree of flexibility in the mutated peptides. The approach is used to design a decapeptide able to bind the anti-HIV drug efavirenz. The calculated binding energy of the designed peptide (approx. −12 kcal/mol) was confirmed experimentally by fluorescence measurements. NMR spectroscopy confirmed the interactions between the peptide and the efavirenz molecule predicted by the algorithm.

2855-Pos Board B10
Protein Kinase G Ligand Binding Specificity via Confine-And-Release Thermodynamic Integration
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Protein Kinase G (PKG) is a second messenger dependent protein implicated in smooth muscle relaxation. Active PKG signals for blood vessel dilation, making it an attractive target in cardiovascular drug discovery. The PKG regulatory domain consists of two, potentially druggable, cyclic nucleotide binding domains (CNBDs), which activate PKG when simultaneously occupied by cGMP. Here we contribute to the characterization of each CNBD by examining its ability to discriminate between cGMP and a highly similar ligand, cAMP. Relative binding free energies were calculated using molecular dynamic simulations and thermodynamic integration (TI). The contribution of individual residues was assessed by repetition over a series of mutant structures. In some cases, multiple ligand binding conformations (syn vs. anti about the glycosyl bond) were found to meaningfully contribute to the binding free energies. As the barrier to rotation between these conformations prevented adequate sampling on computationally feasible TI timescales, the confine-and-release method was used to account for the contribution of each conformation. This resulted in improved convergence and agreement with experiment.

2856-Pos Board B11
Computer Simulations of NAD Channeling between GAPDH and LDH
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Functional protein-protein interactions are essential for many physiological processes and may play important roles in substrate channeling, coenzyme transfer, and compartmentation in glycolysis. Herein, Brownian dynamics (BD) elucidates the interactions between the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH) and the transfer of the cofactor nicotinamide adenine dinucleotide (NAD) between LDH and GAPDH. BD channeling simulation results strongly depend on choice of reactive atom set. When the reactive atoms set comprise of atoms in the vicinity of the NAD binding site of either enzyme, short NAD trajectories between enzyme subunits occur. If the reactive atoms set were chosen from atoms belonging to NAD binding sites, the efficiency of reaching LDH decreased significantly, and even the shortest trajectories spent time equilibrating with solvent before binding the next active site. Transfer of NAD from GAPDH to LDH is sensitive to overall structure of enzyme-enzyme complex. Small variations in orientation of one enzyme relative to the other cause changes in channeling efficiency. The process of NAD release from GAPDH and LDH binding sites was studied with steered molecular dynamics. The GAPDH/LDH complex with two LDH subunits facing two GAPDH subunits was chosen as initial structure. Six NAD molecules were included - two molecules in GAPDH active subunits and 4 NAD molecules bound to each LDH subunit. External forces were applied to O3 NAD atom of each NAD molecule. MD trajectories (2 ns) with external force ($\leq$ 1000 pN) were able to pull the NAD out of GAPDH, but not out of LDH. Such strong binding of NAD by LDH is because the nicotinic moiety is buried deep inside LDH subunit globule. Additional studies are needed to confirm the hypothesis that intermolecular contacts soften GAPDH subunit and its affinity to NAD reduces.

2857-Pos Board B12
An Atomic Force Microscopy Study of Riboflavin Receptor Targeting Nanoparticles
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Riboflavin ligands present an alternative pathway for targeted drug delivery as riboflavin receptors are over-expressed in breast and prostate cancer cells. We have examined a riboflavin conjugated PAMAM dendrimer (generation 5) for