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

conformational changes involved in the centrin-Sfi1p$_{21}$ complex formation by FT-IR spectroscopy, two dimensional correlation spectroscopy and isothermal titration calorimetry. The binding was exothermic and the thermodynamic data for Hcen1-Sfi1p$_{21}$ was the following: N 1.33 ± 0.0165, Ka 1.59 x10^7 ± 2.48 x10^6 M, ΔH −1.72 x10^4 ± 301.1 kcal/mol and ΔS −23.8 kcal/mol. We have also established the relative stability of these proteins by differential scanning calorimetry. Our experiments address key questions underlying the molecular basis of this complex interaction.

3089-Pos Board B136

Unraveling Integrin Antagonists' Target-Recognition Mechanisms

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Background: Pharmaceutical blockade of the platelet αIIbβ3 integrin receptor has reduced mortality from cardiovascular disease. However, gaps in mechanistic understanding limit clinical efficacy and delay new drug development.
Objectives: Integrating surface plasmon resonance (SPR) and dynamic force spectroscopy (DFS), we aim to measure the strength of integrin:ligand bonds, determining their weakest link and identifying new therapeutic intervention routes.
Methods: cHarGD, a cyclic peptide structurally similar to eptifibatide, a widely used antiplatelet drug, served as a model ligand, one readily coupled to biosensors and AFM tips. SPR provided kinetic, equilibrium, and transition state thermodynamic parameters for αIIbβ3:cHarGD complex formation, while DFS measured their mechanical stability. cHarGA, lacking the aspartate required αIIbβ3 binding, served as a negative control.
Results: SPR demonstrated that integrin binding to immobilized cHarGD was rapid (k_{on} ~ 7 × 10^3 L/mol-sec at 25 °C), readily reversible (k_{off} ~ 10^{-2} sec^{-1}), and specific (100-fold smaller signals with cHarGA). Eyring and van't Hoff analyses indicated that after overcoming an entropic barrier ($\Delta G_a^{o\ddagger}$ 12 kcal/mol), both enthalpy and entropy favored assembly of the αIIbβ3:cHarGD complex (ΔG° - 8 kcal/mol). Preliminary DFS experiments (12 nN/sec loading rate) indicated that the rupture force of cHarGD:αIIbβ3 is about 300 pN. In control experiments, where the tip was functionalized with cHarGA or albumin, lower rupture forces of 225 pN and 170 pN were observed.
Conclusions: Our SPR data indicate that entropy plays a major role in target recognition by integrin antagonists, a property shared by ~25% of drug:receptor interactions. Our DFS data suggest that integrin:ligand interactions are stabilized by multivalent contacts between clustered receptors and pharmaceutical inhibitors.This study will provide the first complete picture of the landscape for integrin:ligand interactions, using temperature and force as thermodynamic variables to determine the energetics and nm scale on which bond disruption occurs.

3090-Pos Board B137

Human Liver Fatty Acid Binding Protein: Solution Structure and Ligand Binding

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Liver Fatty Acid Binding Protein (L-FABP), a small (14 kDa) abundant cytosolic protein, may perform several functions in cells, including intracellular transport of fatty acids, nuclear signaling, and regulation of intracellular lipolysis. Among the members of the intracellular lipid binding protein (iLBP) family, L-FABP is very unique in its ability to bind two molecules of FA and a variety of other bulky ligands such as bilirubin. To help understand the promiscuous binding and transport properties of L-FABP, we have applied multi-dimensional homonuclear and heteronuclear NMR spectroscopy for studies of its structure and ligand binding. The overall conformation of human L-FABP, as determined from NOE-derived distance restraints, shows a β-clam motif comprised of a 10-stranded anti-parallel β-sheet that is covered by 2 short nearly parallel α-helices. Ligand binding to L-FABP is being studied by NMR titration experiments with two types of ligands. In the case of oleic acid, which is the primary physiological ligand of L-FABP, 2D HSQC spectra with different binding stoichiometries showed two binding sites with different affinities. In addition, two 13C-labeled bilirubin analogs are being studied to assess binding of bulky ligands. We hypothesize that the unique binding of bulky hydrophobic ligands enables the L-FABP to undergo a conformational change that is different from the other FABPs.

3091-Pos Board B138

A Novel Domain Implicated in the Interactions between pre-mRNA Splicing Factors

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Splicing Factor 1 (SF1) and U2 snRNP auxiliary factor (U2AF^{65}) form an essential protein complex that recognizes the 3′ splice site during the initial stages of pre-mRNA splicing. A ~100 amino acid domain of SF1 located between an N-terminal region that is necessary and sufficient U2AF^{65}-interactions, and a C-terminal RNA-binding domain. Despite high sequence conservation from yeast to mammals, the structure and function of this SF1 'mystery' domain is currently unknown. Here, we demonstrate that the SF1 'mystery' domain participates in the SF1 / U2AF^{65} interface by comparing heat capacity changes and chemical shift differences for U2AF^{65} association with deletion variants of SF1. Heat capacity changes for association of SF1 with the U2AF^{65} interacting domain (UHM) are significantly greater than those observed for association with a SF1 peptide composed of the minimal U2AF^{65}-interacting region. In contrast, the heat capacity changes for SF1 peptide/U2AF^{65}UHM association closely matched those predicted from the buried surface area of the complex. Given that heat capacity changes often correlate with the amount of surface area buried by complex formation, one possible explanation for this difference was that additional regions of SF1 participate in the U2AF^{65}UHM interface. To investigate this possibility, the HSQC spectra of ^{15}N-labeled U2AF^{65}-UHM in complex with SF1 C-terminal deletion variants were compared. Chemical shift differences imply that residues from conserved 'mystery' domain of SF1 participate in the U2AF^{65}UHM interface. The influence of this SF1 domain on affinity and cooperativity of pre-mRNA recognition by the SF1 / U2AF^{65} is further investigated by calorimetry and fluorescence anisotropy. These studies aid in elucidating the structural and thermodynamic means for 3′ splice site recognition by the essential SF1 and U2AF^{65} complex.

3092-Pos Board B139

A Molecular Approach to Ligand-Receptor Interaction

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We have studied a human delta-opioid receptor interacting with two agonists, Clozapine and Desmethylclozapine. Delta-opioid receptors belong to the family of G protein-coupled receptors, that transduce an intracellular biological signal upon activation via interaction with a ligand in the transmembrane domain. Although Clozapine and Desmethylclozapine only differ by a methyl group, experimental data have evidenced a more efficient action of Desmethylclozapine in the treatment of refractory schizophrenia. A molecular analysis may help to clarify issues related this difference. Molecular Dynamics simulations help to elucidate the microscopic mechanism of the interactions between the ligand and the receptor identifying features barely seen in experiments. However, as in our case, the time scale of the processes of interest is often too long to be approached by standard MD techniques. Thus, for our study we have used a recent technique, the metadynamics, that accelerates MD runs extending simulation times. Our results pointed out different routes of the drugs inside the receptor: Clozapine touches a larger number of competing minima far from the putative receptor active zone than Desmethylclozapine. This latter spends most of its time inside the receptor close to the residues of the active zone, inducing noticeable structural modifications. Additionally, the simulation of the entrance has provided evidence of a stronger interaction with the receptor of Desmethylclozapine than Clozapine, resulting in a more frequent entrance of the former. Clozapine exhibits a preferential interaction with the membrane because of its enhanced hydrophobicity. The free energy surfaces extracted from the simulations have been used for kinetic Monte Carlo simulations to obtain reliable residence times of the drugs inside the receptor. The whole results helps to understand how microscopic details can remarkably affect efficiency and activity of compounds, supporting the idea of a bottom-up strategy in the drug design.

3093-Pos Board B140

Understanding the Mechanism of the Anti-angiogenic Activity of Suramin

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Angiogenesis is a cellular process that involves the sprouting of new blood vessels from pre-existing ones. Fibroblast growth factors (FGFs) play a crucial role in the regulation for angiogenesis and tumor metastases. Therefore, intensive research efforts are on to develop drugs that can specifically inhibit FGF-induced angiogenesis. FGFs exhibit their cell proliferation activity by binding to the extracellular D2 domain of their cell surface receptor. Suramin has been previously shown to inhibit FGF-induced tumors. In this context, in the present study, we investigate the interaction of suramin with the extracellular D2 domain of the FGF receptor (FGFR). Results of the isothermal titration calorimetry (ITC) experiments suggest that suramin binds to the D2 domain of FGFR with a reasonably high affinity (K_d ~ 10^{-6} M). ITC experiments, carried out at various salt concentrations, show that suramin-D2 domain interaction is mostly stabilized by ionic interactions. Limited trypsin digestion experiments and ANS binding experiments reveal conformational changes in the D2 domain