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
Neville Y. Forlemu[1], Eric N. Njabon[2], Kathryn A. Thomasson[1].
[1]UND north dakota, Grand forks, ND, USA, [2]Chadron State University, Chadron, NE, USA.
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

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Molecular Modelling Of BCRP (ABCG2) Multidrug Resistance Protein And Docking Of New Camptothecin Analogues
Alessandro Grottesi[1], Giordano Mancini[1], Alessandro Desideri[2], Giovanni Chillemi[1].
[1]CASPUR, Rome, Italy, [2]University of Rome, Rome, Italy.
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
Larry R. Masterson, Gianluigi Veglia.
University of Minnesota, Minneapolis, MN, USA.
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
David F. Green.
Stony Brook University, Stony Brook, NY, USA.
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
Liliana D.V. Sosa, Daniel Narvaez, Ana M. Gomez, **Belinda Pastrana**.
University of Puerto Rico, Mayaguez, PR, USA.
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