3327-Pos Board B55
Moonlighting Proteins
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Moonlighting proteins comprise a class of multifunctional proteins in which a single polypeptide chain has multiple biochemical functions that are not due to gene fusion events. Examples include cytosolic enzymes that are also transcription factors, crystallins, chaperones, extracellular growth factors, or cell surface adhesins. The variety of known moonlighting proteins, the multiple ways in which one protein can have multiple functions, the potential benefits to the organism of combining two functions in one protein, and the methods proposed for a protein to evolve a second function suggest that moonlighting proteins might be common. The ability of a protein to moonlight in different multi-protein complexes or pathways can complicate the prediction of protein function from sequence or structure and the annotation of sequence databases. To date, most moonlighting functions have been found by serendipity. There is currently no straightforward method to identify which proteins moonlight, or for determining if a protein of interest is a moonlighting protein. In addition, sequence homologues of moonlighting proteins often do not perform both functions. We are using biochemical methods and X-ray crystallography to study individual moonlighting proteins. We are also organizing information about the sequences, structures, functions and functional sites of the over 200 known moonlighting proteins into the web-based MoonProt database.

Dynamics of Ligand Binding

3328-Pos Board B56
Thiol Labeling Reveals Presence of Cryptic Binding Sites in β-Lactamase
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The field of drug design has for many years sought to design ligands for known binding pockets in proteins. Cryptic binding sites, which are unexpected openings that are invisible to normal experimental methods but can modulate activity through communication with the active site, have the ability to greatly expand the number of proteins whose behavior and activity can be easily modulated by increasing the number of druggable sites. Recent work has offered the possibility of identifying these sites from molecular dynamics simulations of proteins in the absence of ligands, allowing identification of sites for many ligands to be screened in a single experiment. To experimentally verify these computational models, we have tested β-lactamase for the presence of cryptic binding sites. We have employed a method using mutagenesis and thiol labeling to verify the transient opening of cryptic sites as well as the ability of a large, covalent adduct at these sites to alter activity of the protein. This has led us to conclude that conformations with transiently open pockets exist in equilibrium with the known structure under native conditions, and these pockets may be suitable for future drug design studies.

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Structural Dynamics Studies of Fatty Acid Binding Protein-4 by Solution NMR Spectroscopy
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Adipocyte fatty acid binding protein-4 (FABP4) is a 132-aa intracellular lipid binding protein involved in the transport of fatty acids between cell membranes and organelles. FABP4 participates in several pathways including lipolysis and lipogenesis, and is involved in lipid and energy metabolism related diseases such as diabetes. Although the x-ray structure of FABP4 has been determined and binding to several of its hydrophobic ligands well characterized, the transitions in the structural dynamics upon ligand binding has yet to be determined. Here, solution NMR experiments will be carried out on 15N and 13C labeled FABP4 to study the structural transitions between its free and bound states. Spin relaxation measurements will also be used to reveal any changes that occur upon binding of FABP4 to its hydrophobic ligands.

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Insights into the Cyclic Nucleotide Selectivity Mechanism of Cyclic GMP Dependent Protein Kinase II
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Membrane bound type II cGMP dependent protein kinase (PKG II) is a central mediator of cGMP signaling cascade, which regulates circadian rhythmicity, intestinal water secretion, bone growth and renal functions. PKG II contains an N-terminal regulatory (R)-domain, and a C-terminal catalytic (C)-domain. The R-domain contains tandem cyclic nucleotide binding domains (CNB-A and B) each with different affinities for cGMP, the second messenger that regulates kinase activity of PKG II. While it is known that PKG II needs to be highly selective for cGMP over cAMP to function properly, little is known about its cyclic nucleotide selectivity and the selectivity's role in activation. To understand its cyclic nucleotide selectivity and activation mechanism of PKG II, we first identified CNB-B to be highly selective for cGMP and solved its crystal structure with cGMP. The complex structure revealed that PKG II utilizes an arginine and two aspartate residues on the C-terminal helix to recognize the guanine moiety in cGMP. This is completely different from PKG I, where a conserved arginine from the β barrel of CNB-B specifically binds the guanine moiety of cGMP and imparts cyclic nucleotide selectivity. We are currently testing the roles of PKG II specific interactions in cGMP selectivity and activation of PKG II.

3331-Pos Board B59
Effects of Ligand Binding on the Rigidity and Mobility of Proteins: An Experimental and Computational Approach
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Cyclophilin A is an enzyme which plays a role in the folding of proteins. It also binds to and aids the function of the immunosuppressant drug cyclosporin A as well as binding to the HIV-1 capsid protein.
We expand upon a method recently used in our study of HIV-1 protease and use the computational tools FIRST and FRODA to model the flexibility and motion of cyclophilin A in the presence and absence of its principal ligands.
In order to verify the simulation process, we have also conducted hydrogen-deuterium exchange NMR (HDX) experiments, the results of which are also presented.
The computational procedure is far quicker than more in-depth approaches such as molecular dynamics, yet it yields results which are comparable with experimental data, on a timescale of CPU-minutes.
We use the computational tools FIRST and FRODA to model the flexibility and motion of cyclophilin A in order to predict the results of hydrogen-deuterium exchange NMR (HDX) experiments.
The rigidity analysis software FIRST can be used to predict the "folding cores" of proteins identified as slowly exchanging residues in HDX. This prediction is improved using the protein mobility software FRODA.
We are using these methods to investigate the effect of ligand binding on cyclophilin A computationally and experimentally.

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Bridging Simulations and Calorimetry: Computational Studies of Binding Thermodynamics and Entropy-Enthalpy Transduction
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Molecular simulations are now fast enough that we can begin to make connections to the thermodynamic data provided by isothermal titration calorimetry. Here, I will describe the thermodynamic analysis of a millisecond-duration protein simulation. A central result is that local perturbations of a protein, due, for example, to binding of small molecules, may easily induce global conformational shifts with large associated shifts in entropy and enthalpy. Thus, the global conformational shifts can effectively transduce local binding thermodynamics into quite different overall apparent thermodynamics. This phenomenon, which may be termed entropy-enthalpy transduction, likely occurs in many systems, and could make measured entropies and enthalpies of binding unreliable indicators of binding forces. Entropy-enthalpy transduction may also help explain the high experimental variance of measured enthalpies and entropies relative to free energies, and may underly many cases of entropy-enthalpy compensation.

3333-Pos Board B61
Differential Responses of Msh2/6 and Damaged DNA Probed by Molecular Dynamics
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Over the past decade, there has been a growing interest in studying the binding of DNA to the MutSalpha protein complex. This heterodimeric protein complex, the Msh2/Msh6 complex in humans, is the initial complex that can