obtained using phosphate buffer (pH 7.0, 1.75Å diffraction, R_{factor}=18%) showed the presence of a bound phosphate anion interacting with His87, a key residue in cluster release (1). The phosphate also interacts with the N-terminus of a symmetry-related dimer (Figure). None of these interactions were previously reported (2-4). We attribute the increased stability to the novel interactions, suggesting that the rate of cluster release could be modulated by interaction with a phosphate or phosphate moiety, such as a phosphorylated protein or peptide.

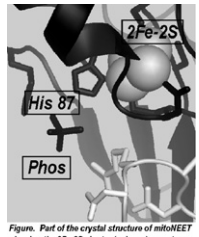

Figure. Part of the crystal structure of mitoNEET showing the 2Fe-2S cluster (spheres), a cartoon representation of the backbone (black is one dimer and white a symmetry related dimer) and specific amino acids (as labeled) that interact with the bound phosphate anion (black tetrahedron).

(1) Wiley *et al.* (2007) *J Biol Chem.* **282**, 23745-23749.
(2) Paddock *et al.* (2007) *Proc Natl. Acad. Sci USA* **104**, 14342-14347.
(3) Lin *et al* (2007) *Proc. Natl. Acad. Sci USA* **104**, 14640-14645.
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*Supported by NIH GM 41637,GM54038 and DK54441.

2283-Pos Board B253
Potent Inhibitors Of c-Myc-Max Dimerization Through Multivalent Binding To The Intrinsically Disordered c-Myc Monomer
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We have shown that selective inhibitors of dimer formation between the oncogenic basic-helix-loop-helix-leucine zipper (bHLHZip) trancription factor c Myc and its bHLHZip partner protein Max act by binding to the intrinsically disordered (ID) c Myc monomer. Multiple, independent sites for inhibitor binding were found along c-Myc bHLHZip. We exploited the multiplicity of these sites to generate novel compounds capable of multivalent binding to c-Myc. Despite their disordered protein target, these molecules bind purified c-Myc with low nano-molar affinity, which is orders of magnitude tighter than that of c-Myc's obligate heterodimerization partner Max. The inhibitors effectively disrupt c-Myc-Max dimerization and specific DNA binding; they also inhibit growth of c-Myc overexpressing cancer cell lines in vitro.

2284-Pos Board B254
Dynamic Analysis of Beta-lactamase Ligand Recognition
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A serious public health threat today is the emergence of pathogens that are resistant to commonly used antibiotics. One of the mechanisms of acquired drug resistance is the bacterial production of beta-lactamases, which break down these antibiotics. Currently used beta-lactamase inhibitors are not effective at targeting the 700 types and new mutants of beta-lactamases. Beta lactamase is therefore an important drug target in combating antibiotic resistance. Beta lactamase inhibitory protein (BLIP) is an effective inhibitor of TEM-1 and SHV-1, but binds and inhibits the two variants with different affinities. We hypothesize that elucidating the mechanism whereby the differential binding results will guide the design of new peptide inhibitors based on the BLIP structure. Molecular dynamics simulations are performed to examine the binding properties of BLIP and BLIP based peptides to TEM-1 and SHV-1 beta lactamase. These simulations on the complex will guide the design of new peptides.

2285-Pos Board B255
The Role Of Dynamics On Binding Specificity And Promiscuity
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Protein - protein interactions regulate and control a wide range of physiological processes. Protein interaction networks abound with proteins that have only a few connections while a small number of proteins, which are called hub proteins, are highly connected. Recent reports suggest that hub proteins are structurally and dynamically different from non-hub proteins. Hub proteins tend to be more disordered than other proteins. Further, binding partners of hub proteins are also observed to have higher levels of disorder. Here we perform a detailed examination of hub and non-hub proteins using the Gaussian Network Model (GNM) to elucidate the role of intrinsic dynamics for binding diversity and promiscuity.

2286-Pos Board B256
Phospho(enol)pyruvate Binding to Thermus thermophilus Phosphofructokinase
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ATP-dependent type 1 prokaryotic phosphofructokinase (PFK) catalyzes the conversion of fructose-6-phosphate (F6P) and MgATP to fructose-1,6-bisphosphate and MgADP. This enzyme is allosterically inhibited by phosphoenolpyruvate (PEP) and exists as a homotetramer with the four active sites formed along one dimer-dimer interface and four allosteric sites formed along the other. The overall structures of PFK's from E. coli, Bacillus stearothermophilus, and Lactobacillus delbruekii are very similar, and the sequences have high percent identity and similarity with Thermus thermophilus PFK. Thermus thermophilus PFK (TtPFK) comes from a gram negative bacterium that is an extreme thermophile. One of the interesting features of TtPFK is an unusually tight PEP binding (Kd=1.3 ± 0.02mM at 250C pH 8) compared to PFK's from E. coli (300 ± 10mM), Bacillus stearothermophilus (60 ± 4mM), and Lactobacillus delbruekii (24 ± 2mM). Since no structural data are available for TtPFK to date, a structure of a highly homologous PFK from a moderate thermophile Bacillus stearothermophilus was used to predict the residues important for PEP binding. From the sequence alignment and the PEP-bound D12A BsPFK structure, three non-conserved residues were identified in the allosteric binding site that may be responsible for tight binding: R55, N59, and S215. To quantify the contribution of these residues to PEP binding, single and double mutations were made to the corresponding residues in BsPFK, EcPFK, and LbPFK. Kinetic data showed that these mutations weakened the binding of PEP to different extents. In case of N59D/S215H, the PEP binding affinity was 10-fold lower N59D showed a 100-fold decrease in PEP binding affinity and R55G showed a 700-fold decrease in PEP binding. Furthermore, these mutants showed a slight increase in coupling, which means that although the binding of PEP becomes weaker, its ability to inhibit was somewhat improved. Supported by NIH grant GM33261 and Welch Foundation grant A1543.

2287-Pos Board B257
Distinguishing Interactions Responsible for Phospho(enol)pyruvate Binding from Interactions that Communicate Allosteric Inhibition in E. coli Phosphofructokinase
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The notion that substrate affinity is modified via interaction of effector ligands is a fundamental characteristic of allostery. In order to further understand the complexities of this phenomenon, this study investigates the role that functional groups in phospho-enol-pyruvate PEP play in ligand binding and allosteric propagation. Thermodynamic linkage analysis enables the nature and magnitude of allostery to be determined, allowing allosteric action to be distinguished from allosteric ligand binding affinity. By using this type of analysis to compare the inhibition caused by PEP to the inhibition imposed by PEP analogs upon binding to E. coli PFK (EcPFK), the importance of specific functional groups of PEP to ligand binding and/or allostery can be ascertained. EcPFK displays a much weaker binding affinity for the PEP analog phosphoglycolate (PG) compared to PEP. However, PG is still able to inhibit EcPFK to an extent comparable to that of PEP. This finding suggests that the methylene group that is absent in PG plays a larger role in ligand binding than in allosteric propagation. The chemical structure of phosphonoacetic acid (PA) is very similar to that of PG except for the deletion of the oxygen that bridges the phosphate group to the rest of the molecule. When the inhibition of EcPFK by PA is examined, a binding affinity comparable to PG is observed but a substantial loss in allostery is also observed. These data suggest that the bridging oxygen in PEP contributes substantially to the propagation of allosteric signal. Funding is provided by NIH grant GM33261 and Welch Foundation grant A1543.

2288-Pos Board B258
Weak Binding of Phospho(enol)pyruvate to Phosphofructokinase from Lactobacillus delbrueckii
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Most prokaryotic phosphofructokinases (PFKs), like those from Bacillus stearothermolphilus (BsPFK) and E.coli (EcPFK), exhibit both tight binding affinity for the allosteric inhibitor phosphoenolpyruvate (PEP) and strong inhibition. PFK from Lactobacillus delbrueckii subspecies bulgaricus (LbPFK), which is similar in structure and sequence to BsPFK and EcPFK, exhibits weak PEP binding, with the strength of the inhibition being indeterminate. D12, found along the active site interface, is conserved in over 150 prokaryotic PFKs,

and it forms hydrogen bonds with highly conserved residues across this interface. Mutating D12 to alanine in BsPFK enhances PEP binding by 100-fold with no change in the extent of PEP inhibition. When D12A was introduced into LbPFK only a 5-fold enhancement in PEP binding was observed. Crystal structures of D12A BsPFK and D12A LbPFK were solved to 2.4 Å resolution. Comparison of D12A and wild-type BsPFK with fructose 6-phosphate bound shows a quaternary shift along the active site interface, breaking the hydrogen bonds involving D12. By contrast, D12A LbPFK exhibits no major change in structure relative to wild type BsPFK. In hopes of further enhancing PEP binding, the following mutations of non-conserved residues in the allosteric site were made to the corresponding residues in either EcPFK or BsPFK, respectively. H59D, E55Y, D187E and S319Y combined showed no enhancement in PEP binding. S211R, D214K and G216S alone and in combination also had no effect on PEP binding. All these mutations suggest that the diminished PEP binding affinity to LbPFK is the consequence of more that just the residues in the allosteric site, likely involving the resistance of this enzyme to undergoing the quaternary shift. Funding came from NIH grant GM33216, NIH CBI training grant, and the Welch Foundation grant A1543.

2289-Pos Board B259

Kinetics and Thermodynamics of the Interaction of ANS with Proteins

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1-anilino-naphtalene-8-sulfonate (ANS) is a fluorescent probe widely used in protein folding and conformational transitions studies. The fluorescent features of ANS, a blue shift of the emission maximum and the increase of quantum yield and lifetime, are generally attributed to the binding at hydrophobic sites. Despite the interaction of ANS with proteins has been extensively studied since the early works of Gregorio Weber, few high-resolution structures of proteins complexed with ANS have been resolved. In this work the binding of ANS to BSA was analyzed at equilibrium and pre-equilibrium conditions. The combined analysis of fluorescence, near UV circular dichroism and isothermal titration calorimetric data provided a detailed description of the binding mechanism. Three ANS molecules bound to BSA in 100 mM phosphate pH 7 at 25°C. Pre-equilibrium experiments allowed to determine the affinity and the relative quantum yield at each binding site by fitting a microscopic model to the fluorescence time-course data. This analysis unambiguously indicated that the binding of ANS to BSA occurs at two different and independent binding sites with similar quantum yields and affinities (ΔG° @ -35 kJ/mol). The binding of ANS to the first site is thermodynamically favored by similar contributions of the enthalpic (ΔH = -16.3 kJ/mol) and entropic terms ($-T\Delta S$ = -19.4 kJ/mol), while the binding to the second site is enthalpically driven (ΔH = -36.6 kJ/mol; $-T\Delta S$ = 4.3 kJ/mol). Complementary information from molecular docking showed 3 ANS molecules bound at hydrophobic cavities in BSA subdomains IIA and IIIA with binding affinities in the order of those found experimentally. The sulfonate group of ANS was oriented towards clusters of polar residues, a common feature in the reported crystal structures of other ANS-protein complexes.
Supported with grants from UBA, CONICET and ANPCyT.

2290-Pos Board B260

Investigation of the Ligand-binding Mechanism of Methionine Sulfoxide Reductase A of *E.coli*

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The rise of free oxygen in the atmosphere over 2.5 billion years ago made it possible for large land-based plants and animals to thrive. But oxygen, and the energy it provides, comes at great cost. Aerobic metabolism generates highly reactive intermediates and by-products in the form of hydroxyl radicals, superoxide anions and hydrogen peroxide. Within the cell, these reactive oxygen species attack biological macromolecules, producing covalent modifications that can affect both function and structure. One amino acid residue in proteins that is particularly sensitive to oxidation is the sulfur-containing side chain of methionine. Fortunately, methionine oxidation can be reversed by the actions of peptide methionine sulfoxide reductase (MsrA), which reduces methionine sulfoxide back to methionine and restores function to damaged proteins. We have used multiple spectroscopic techniques to investigate the mechanism by which MsrA recognizes and binds to a wide range of oxidized substrates in need of repair. Substrates studied include proteins, peptides and the non-steroidal anti-inflammatory drug Sulindac. Competition experiments with the fluorescent reporter ANS suggest the existence of weak, but specific, hydrophobic interactions between MsrA and unstructured and/or hydrophobic ligands.

2291-Pos Board B261

Characterization of and Kinetics studies on Lipid Extraction of GM2AP Tryptophan Mutants using Intrinsic Fluorescence and a Dansyl-Based Fluorescence Assay

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GM2AP is an accessory protein that functions as a co-factor in degradation of the GM2 ganglioside to GM3. This non-enzymatic lipid transfer protein solubilizes GM2 from intralysosomal vesicles for reaction with HexA. The precise molecular interactions and method of extraction of the GM2 ganglioside from the lipid membrane are not yet known. GM2AP contains four disulfide bonds and three tryptophan residues (W5, W63, W131) with two of these (W63, W131) located in putative membrane binding loops. In this report, the intrinsic tryptophan fluorescence of a series of single and double TRP mutants (W5A, W5AW63A and W5AW131A) of GM2AP is used to characterize protein in solution and in the presence of lipid vesicles. Additionally, results from quenching experiements are shown, where the fractional accessibility of each tryptophan is determined for both neutral and acidic solutions. The kinetics of lipid transfer of each of the tryptophan mutants were also assayed for their ability to extract and transfer dansyl-labeled lipids from liposomes. Removal of the TRP moieties from the putative membrane binding loops results in slower lipid extraction rates, implying that these residues are important in the membrane binding of GM2AP.

2292-Pos Board B262

Binding of Organochloride and Pyrethroid Pesticides To Estrogen Receptors α and β: A Fluorescence Polarization Assay

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Several agricultural pesticides and industrial chemicals, such as the organochlorides DDT and methoxychlor, have been shown to cause both endocrine disruption in humans and binding to the estrogen receptor. Estrogen receptor-α (ERα) and -β (ERβ) are ligand-activated nuclear signaling proteins with widespread presence in the body. Binding of the hormone estradiol to the ER can affect development with an activation profile that is subtype specific. This investigation measures the ability of pesticides to bind *in vitro* to human ERα and ERβ by observing their ability to displace a fluorescent estrogen homologue from the receptor. Eight pesticide related compounds were assayed: four in the DDT family and four based on the natural insecticide pyrethrin. The organochlorides tested were: DDT, HPTE, and two DDT metabolites: VF77-1and VF72-1. The four synthetic pyrethroids tested were permethrin, deltamethrin, bifenthrin and fenvalerate. While all of the molecules in the DDT family that we tested showed strong binding to ERα, the pyrethroids showed either extremely weak (fenvalerate) or no binding (permethrin, deltamethrin, bifenthrin) to ERα under our experimental conditions. ERβ exhibited a different binding profile: high affinity binding to the DDT family of molecules AND to permethrin, lower affinity but still strong binding to deltamethrin and fenvalerate, and no binding at all to bifenthrin. These results suggest that permethrin, in addition to the DDT based molecules, could potentially have the ability to disrupt the estrogen hormone pathway through binding to ERβ. Permethrin's binding to ERβ is notable, particularly in light of its widespread use in home pet care products such as pet shampoo and flea and tick repellants. The results also suggest that the binding affinity of ERβ is similar to but less discriminating than that of ERα.

2293-Pos Board B263

Characterization of the Ca^{2+} Binding Affinity and Coordination Site of the LIN-12/Notch-Repeat

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Notch receptors are transmembrane glycoproteins of a highly conserved signaling pathway that regulate cell growth, differentiation, and death in multicellular organisms. Notch activation requires two successive ligand-induced proteolytic cleavages that enable the intracellular Notch to translocate to the nucleus and regulate gene transcription. Notch proteins exhibit a highly conserved modular architecture, which includes three tandem LIN-12/Notch-Repeats (LNRs) responsible for maintaining the receptor in its resting conformation prior to ligand binding. These highly conserved modules contain a characteristic arrangement of three disulfide bonds and a group of aspartate/asparagine residues that coordinate a Ca^{2+} ion, essential for the correct folding of an LNR. Outside of the Notch family of proteins, LNR modules also exist in proteins such as the PAPP-A and the stealth proteins. In our previous work, we had recombinantly expressed, purified, and refolded the first repeat of human Notch1 and used it as a model system to characterize the binding specificity and affinity of different