reporter. Using the second methods we found out that the UCS domain but not the central domain prevents the thermal aggregation of the myosin motor domain. We conclude that while both the UCS domain and the central domain bind the myosin head, only the UCS domain displays chaperone activity.
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3413-Pos Board B141
Poly-(R)-3-Hydroxybutyrate-Modified Proteins
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Poly-(R)-3-hydroxybutyrate (PHB), a linear polymer of R-3-hydroxybutyrate (R-3HB), is a universal constituent of biological cells. Short-polymers of PHB (<15 residues) are covalently attached to certain prokaryotic and eukaryotic proteins (PHBylation) - primarily proteins of membranes and organelles. PHB is an amphiphilic, water-insoluble, lipid-soluble molecule that may form hydrophobic, hydrogen and coordinate bonds. The PHB backbone is highly flexible at physiological temperatures but becomes increasingly more rigid as temperatures are lowered. These physical properties indicate that PHBylation plays important roles in the targeting, folding, structure and function of PHB-modified proteins, and also promotes interactions between proteins and polyanions such as RNA, DNA and inorganic polyphosphate (polyP).

3414-Pos Board B142
GFP Variants with Alternative Strands: Protease Sensor Design and their Thermodynamic Analysis
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Green fluorescent protein (GFP) variants with one extra strand 10 (s10) can fold with either one or the other s10, and the composition of the two bound forms can be unambiguously distinguished by their resulting colors (Do & Boxer, 2013 JACS). The composition can be systematically modulated by mutating the residues on s10 or by changing the lengths of the two inserted linker sequences that connect each s10 to the rest of the protein. We have applied thermodynamic analysis on the composition to obtain insight into folding of the variants, and discovered robust empirical rules that accurately predict the product ratios of a given construct. Ratiometric protease sensors were designed from the construct by recombinantly adding a protease cleavage sequence into one of the inserted loops, where the bound s10 is replaced by the other upon protease cleavage and irradiation with light, which is based on a photodissociation phenomenon previously reported (Do & Boxer, 2011 JACS). Since the conversion between the two bound forms involves a large spectral shift, the sensor can display a very high dynamic range. The variants can serve as a platform to provide useful insight into protein folding in general, and further engineering of this class of proteins will enable their application as light-dependent modulators.

Enzyme Function and Regulation

3415-Pos Board B143
Steered Molecular Dynamics Simulations of NAD Unbinding from GAPDH and LDH
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Protein-ligand interactions play an important role in understanding biophysical processes including the glycolytic pathway. Calculation of the energy profile of ligand unbinding is essential for understanding possible substrate channeling of nicotinamide adenine dinucleotide (NAD) between lactate dehydrogenase (LDH) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Herein, steered molecular dynamics (SMD) simulations elucidate the process of NAD unbinding from LDH and GAPDH. Brownian dynamics (BD) simulate, using the energy reaction criterion, NAD diffusion towards the binding site of GAPDH or LDH to identify potential residues where strong protein-ligand coulombic interactions exist. These residues are used to design several dissociation pathways for the SMD simulations. Simulations either apply a harmonic guiding potential or a constant force SMD to perform center of mass (COM) pulling of the NAD. The two ligands in the tetrameric GAPDH protein are successfully released from the binding pocket using a force constant $k \geq 5000$ kJ/mol/nm^2 or a constant force $F \geq 600$ pN, within the first 4.2 ns of simulation time. A constant force of 600 pN is enough to pull out three of the four ligands from their corresponding LDH binding sites within the first 0.5 to 1.2 ns of simulation time. Upon releasing the ligand from the binding site, NAD conformational changes are traced, starting with a stretched, open conformation in the binding site and ending with a bent structure in solution. The bent structure is consistent with previous experimental and simulation data of NAD free in solution. The unbinding free energies associated with the NAD release along the proposed pathways are calculated using the Jarzynski equality, in the stiff-spring approximation of pulling.

3416-Pos Board B144
Computational and Experimental Study of Ketoreductase Enantioselectivity
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Enzymes are powerful enantio-, chemo-, and regioselective catalysts. They are highly efficient, operate under mild conditions, and are environmentally benign. These attributes make them great synthetic tools for chemical manufacturers. Ketoreductases (KREDs) asymmetrically reduce ketones to alcohols and are the most commonly used enzymes in industrial drug synthesis. Various mutants of L. Kefir KRED have been generated to enantioselectively reduce the substrates dihydrothiophen-3(2H)-one and dihydrofuran-3(2H)-one, and the alcohols produced can be incorporated into pharmaceuticals such as sulopenem and fosamprenavir. These substrates vary by only one atom, sulfur versus oxygen, and yet in general the KRED mutants reduce them to different enantioselectivities. In many cases mutants that produce good to excellent enantiomeric excess (ee) for one substrate have no enantioselectivity for the other. Other mutants give opposite enantioselectivities for the two substrates. In this work, the physical origins of this subtle and perplexing example of specificity are determined using several quantum mechanical and force field based computations. These methods reveal that small changes in the size, shape, and hydrophobicity of the active site resulting from mutations in as few as 1 residue can significantly effect enantioselectivity. Furthermore, the ability of various enzyme design methods to predict selectivity is evaluated through comparison to experimentally determined selectivities. This work will enable further development of KRED mutants for industrial drug synthesis and aid enzyme design efforts.

3417-Pos Board B145
In Support of Nitric Oxide Dioxygenase Function: Algal Hemoglobins and their Reduction Partners
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The ubiquity of hemoglobins as a superfamily to life has enthused the field with renewed vigor. Reactions like oxygen binding and nitric oxide (NO) dioxygenation appear to be characteristic to the hemoglobin superfamily, as revealed from investigation of recombinant globins, irrespective of whether they are associated to any particular function like oxygen transport/storage, sensing, electron transport, protection against hypoxia and other possibilities. **NO dioxygenase** reaction, common in vitro, however, was limited by lack of report of specific enzymes that can convert ferric hemoglobin, formed during reaction of oxy hemoglobin with NO, into ferrous hemoglobin - the species that reacts with NO. Absence of a known cognate reductase would prevent reduction of ferric species of hemoglobin to ferrous form and the oxidation-reduction cycle would be incomplete for NO related function to be fruitful. Assignment of NO dioxygenase activity as a physiological function requires the design of experiments that address reduction mechanisms. We used ***Chlamydomonas reinhardtii*** as model system since we have identified 12 globins and 3 putative genes that can potentially function as reductase of ferric hemoglobin. Organism database annotated these reductases as **dihydrolipoamide dehydrogenase**, **cytochrome b5reductase** and **monodehydroascorbate reductase**. So far, we have characterized 3 hemoglobins and 3 putative cognate reductases using biochemical and biophysical methods. Spectroscopic studies reveal that *Chlamydomonas* contains both pentacoordinate and hexacoordinate hemoglobins. The enzymes were found to contain flavin domain and could reduce ferric *Chlamydomonas* hemoglobins *in vitro* to their functional ferrous state. The interactions between hemoglobins and these reductases might support NO scavenging/detoxification function of globins with potential implications in biotechnology.

3418-Pos Board B146
Mapping the Substrate Binding Sites of the Integral Membrane Methyltransferase ICMT by Mutational Analysis
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The eukaryotic enzyme isoprenylcysteine carboxylmethyltransferase (ICMT) catalyzes the attachment of a methyl group onto the carboxylate of a lipid-modified cysteine at the C-terminus of its protein substrates. This is the final

processing step for proteins that contain a 'CAAX' motif, including Ras. Because inhibition of ICMT limits Ras-induced oncogenic transformation, ICMT is a potential target for cancer therapy. Human ICMT is predicted to have eight transmembrane helices and has no discernable homology with soluble methyltransferases. An outstanding question in understanding the mechanism of this enzyme is how both its water-soluble substrate (AdoMet) and lipophilic substrate (a prenylated cysteine) are recognized in the active site. A crystal structure of an integral membrane methyltransferase from a prokaryotic organism was recently determined. The prokaryotic methyltransferase has sequence similarity with ICMT in the region that binds AdoMet, but methylates different substrates, which are currently unknown. In order to identify regions of ICMT that are important for catalytic activity and delineate the CAAX binding site, we combined scanning mutagenesis with methyltransferase assays and interpreted the results with regard to the available structure. Of the 153 mutated positions, 61 reduced catalytic activity by $> 50\%$. Mutation of the conserved residues lining the AdoMet binding site reduced or eliminated catalytic activity, suggesting a similar mode of AdoMet recognition in the prokaryotic methyltransferase and ICMT. In addition, our studies have identified several residues, primarily aromatics, that may line the CAAX binding site. When substituted to alanine, the mutant enzyme is inhibited by higher concentrations of the lipophilic substrate. We propose that substrate inhibition results because these mutations alter the shape of the lipid-binding tunnel causing the substrate to bind nonproductively. Our results offer novel insights into the substrate binding sites of this unique enzyme.

3419-Pos Board B147

Regulation of Creatine Kinase by ASB9

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Creatine kinases (CKs) regulate ATP levels at sites of fluctuating energy demands. Reduced CK activity has been implicated in diseases such as heart failure and multiple sclerosis, however little is known about CK regulation. Ankyrin repeat and SOCS box-containing protein, ASB9, (part of an E3 ubiquitin ligase) has been shown to promote proteasomal degradation of CKs. We hypothesize that ASB9 and its splice variants control the levels and activity of CK at sites where energy is critically needed. Our results show that the ankyrin repeat domain of ASB9 (ASB9-ARD) binds CKB with very high affinity (nM) and 1:1 stoichiometry. Comparison of binding data from several N-terminal truncations of ASB9-ARD suggests that residues 19-35 of ASB9 contribute to the binding affinity. In addition, binding of ASB9-ARD to CK abolishes the enzymatic activity of CK. Hydrogen deuterium exchange mass spectrometry (HDXMS) has revealed that only one region in CKB (residues 182 - 203), was protected upon binding of ASB9 when the deuterium incorporation into CKB peptides alone was compared to those in complex with ASB9-ARDs. This region is right in front of the active site of the enzyme, and was shown previously to undergo a conformational change upon binding of creatine and ADP-NO3 to rabbit CKM. Results from the CK inhibition assay together with the HDXMS data strongly suggest that ASB9 binds directly to CKs and inhibits CK activity by altering regions near the active site of the enzyme. A model of this interaction generated using docking and computational modeling agrees with the binding and HDXMS data. We intend to completely characterize the ASB9-CK interaction and discover inhibitors that would disrupt the interaction, resulting in increased amounts of active CK to treat diseases in which there is a an increased energy demand and decreased CK function.

3420-Pos Board B148

Understanding Functional Evolution in the Alkaline Phosphatase Superfamily

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Over the past 40 years, it has been demonstrated that many enzymes are capable of promiscuous catalytic activities, facilitating the turnover of more than one chemically distinct substrate. This has been argued to play an important role in enzyme evolution, with highly promiscuous progenitor enzymes evolving under evolutionary pressure to modern day specialists, while still retaining some level of their former promiscuous activities[1]. This theory has been extensively tested by different experiments using *in vitro* evolution[2]. The alkaline phosphatase superfamily members provide a particularly attractive showcase for studying enzyme promiscuity, as they often show reciprocal promiscuity, in that the native reaction for one member is often a side-reaction for another[3]. While deceptively similar, their catalyzed reactions (cleavage of P-O and S-O bonds) proceed *via* distinct transition states and protonation requirements[4,5]. We present detailed computational studies of the promiscuous catalytic activity of three evolutionarily related members: the arylsulfatase from *Pseudomonas aeruginosa*[6], and the phosphonate monoester hydrolases from *Burkholderia caryophili*[7] and *Rhizobium leguminosarum*[8]. By tracking their structural and electrostatic features, and comparing to other known members of the superfamily, we provide an atomic-level map for functional evolution within this superfamily.

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3421-Pos Board B149

Did Class 1 and Class 2 Aminoacyl Trna Synthetases Descend from Genetically Complimentary, Catalytically Active ATP-Binding Motifs?

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We examined the contribution of protein evolution to the beginning of life by performing a direct test of the Rodin and Ohno hypothesis that the two Aminoacyl tRNA synthethase (aaRS) classes descended from opposite strands of a single gene. Class I and Class II aaRS catalyze synthesis of aminoacyl-tRNA molecules for use in codon-directed protein synthesis. Validating that hypothesis would thus substantially simplify the origins of an elemental process necessary for life to occur. The test of the hypothesis was set up by constructing two genetically engineered genes for 46-residue ATP binding portions of Class I and II synthetases that are coded by fully complementary nucleic acid strands. I measured the catalysis of the amino acid activation reaction by these two simple active sites. Using experimental variations of amino acid concentration, peptide concentration and time of reaction, I confirmed that the observed rate of reaction was significantly higher than the background reaction and proportional to the concentration of catalytic peptide. We observed significantly higher catalytic activity from the Class I ATP binding peptide, as had been observed previously for the wild-type Class I sequence. Activity increased with time and with increase in amino acid and peptide concentrations. The enhanced rate of amino acid activation seen for the two classes of genetically altered peptides coded by opposite strands, thus supported Rodin and Ohno's hypothesis. Ancestral 46-residue peptides coded by opposite strands of a single RNA gene might therefore have helped launch protein synthesis by activating amino acids. This work suggests that the earliest peptide catalysts might have been quite short. Sense/antisense coding would have introduced unexpected diversity from a single gene. Supported by NIGMS 78227 to C. W. Carter, Jr, and the ABS.

3422-Pos Board B150

Analyses of the Interaction Between Lipocalin-Type Prostaglandin D Synthase and Substrate or Product

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Lipocalin-type prostaglandin D synthase (L-PGDS) catalyzes the isomerization of PGH_2 to PGD_2, which acts as a endogenous somnogen in the brain. This enzyme belongs to the lipocalin superfamily which consists of transporter proteins for lipophilic substances in the extracellular space. Our previous studies suggested that L-PGDS consists of a β-barrel structure with a hydrophobic pocket. The active thiol group of the Cys65 residue is located in this pocket and faces to the inside of the pocket.

A number of biochemical studies for L-PGDS, as a drug target for sleep disorders, have been reported in attempts to understand its catalytic mechanism, and several substrate recognition models of L-PGDS have been proposed. However, details of the mechanism by which L-PDGS recognizes its substrate are obscure, since essential information, such as its binding affinity and stoichiometry, of the interactions between L-PGDS and substrates remains unclear. Therefore, isothermal titration calorimetry (ITC) experiments were carried out to characterize the binding properties, including binding affinity and stoichiometry, of L-PGDS with respect to its substrates and products. In addition, to obtain thermodynamic information regarding the substrate and product binding, the enthalpy (ΔH) and entropy (ΔS) of the binding reactions were calculated.

The results of ITC experiments revealed that both the substrate and the product bind to L-PGDS with a stoichiometry of 2 to 1 and showed two binding sites