with the goal they will be able to partially inhibit SERCA and also be impervious to dephosphorylation. Insights to these issues will provide better paradigms with which to design therapeutic mutants of PLN for treatment of heart failure, and also demonstrate a model by which an enzyme can be controlled through tuning the allosteric regulation of an inhibitor.

3304-Pos Board B32
Protein-Chromophore Interactions in Green Fluorescent Protein (GFP) Studied by Split Protein Reconstitution
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We have utilized a split green fluorescent protein (GFP) system to generate a range of semi-synthetic proteins to study the interaction of the autocatalytically-formed chromophore with the protein environment. Using circularly permuted GFP, the alpha helix containing the chromophore has been relocated to the N-terminus and then removed by site-specific proteolysis of an engineered loop region. Following denaturation and size exclusion chromatography purification, the truncated protein and the chromophore-bearing peptide are separated. We previously showed that the truncated protein is capable of re-forming a chromophore upon reconstitution with a synthetic peptide. This work is extended to incorporate unnatural amino acids into the chromophore for studying hydrogen bonding and proton transfer. An additional application we have pursued is to use chromophore "transplantation" in order to examine the spectroscopic effects of mutations that would ordinarily be impossible due to the preclusion of chromophore formation. Arg96, in particular, is an interesting residue because of its major role in defining the electrostatic environment, however, its intolerance to mutation necessitates the use of a split protein and complementation with a chromophore-containing peptide harvested from a non-mutated donor. We use this scheme to investigate the effects that remodeling the electrostatics at this critical position have on the color tuning and quantum yield of GFP.

3305-Pos Board B33
Side Chain Entropy in Enzymes and its Role in Catalysis
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Directed Evolution is a popular biochemical engineering technique used to improve, and in some cases alter the activity of enzymes. Although many empirical strategies have been developed to carry out directed evolution, it is still an opaque process that could benefit from more rational design. In this work, we try to rationalize directed evolution based on the physical principles of thermodynamics. Our model system is the Kemp Eliminase KE07, which was designed computationally to catalyze the conversion of 5-nitrobenzisoxazole to cyanophenol, but showed poor activity in solution (kcat/KM ~12 M-1s-1). Seven rounds of directed evolution lead to a 2 order of magnitude improvement in kcat/KM but structural analysis could not explain why many of the mutations were made. Using a sequential Monte Carlo technique we have been able to calculate and identify systematic changes in the side chain entropy through the 7 rounds of directed evolution. Our technique uses a realistic energy function coupled with a rotamer library to estimate the important rotameric states. These results suggest that the functional partitioning of structure (enthalpy) and statistical fluctuations (entropy) that occurs at the side chain level could give us important leads to predict further mutations. This work also provides further evidence to start looking beyond the traditional structure-function paradigm and incorporate entropic contributions for designing/improving enzymes.

3306-Pos Board B34
Rationalizing Directed Evolution through Protein Dynamics
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Directed evolution is an experimental technique used to improve catalytic activity of low performing enzymes. However, the underlying principles behind directed evolution are not well understood. In addition to the structure of an enzyme, recent studies have shown the dynamics of the enzyme to affect the catalytic activity. We are analyzing the impact of protein dynamics on the catalytic activity of an enzyme through certain dynamic metrics such as distance and velocity time correlation functions. These metrics can give an insight into the coupling of motions of various residues with the active site. Recent efforts in directed evolution have shown very promising results for artificially designed Kemp Eliminases, in the form of a 200 fold increase in activity over seven rounds of directed evolution. Dynamic metrics can be used to rationalize the mutations that occurred over the rounds of directed evolution. These metrics can then be used as predictive tools, to predict new potential targets for mutation, thus reducing the search space for potential mutations by many orders of magnitude.

3307-Pos Board B35
Understanding the Structural Determinants for the Stability of Human Fibroblast Growth Factor
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Human fibroblast growth factor (FGF) is an important protein that plays a role in morphogenesis, angiogenesis and wound healing. Because of these capabilities this protein is of medical interest. Unfortunately the lack of stability of FGF poses a problem to further research. Understanding the structure of FGF at the atomic level is necessary for designing novel FGF variants with enhanced stability and wound healing properties. The specific aim of this study is to test and record the structural stability of FGF through a gradient of salt concentrations. Through thermal denaturation, it was shown that the stability increased slightly with the increased salt concentration. The structure of hFGF-1 has been investigated using a variety of biophysical techniques such as trypsin digestion and circular dichroism. To ensure that the structure was maintained, N-15 enriched human fibroblast growth factor-1 was over expressed and purified. This sample will be used to test the structural stability of hFGF-1 by Heteronuclear Single Quantum Coherence (HSQC) using NMR spectroscopy. The hope of this study is to identify key properties that will allow the optimization of FGF stability. These data can then be used to develop novel mutants with enhanced stability and wound healing properties.

3308-Pos Board B36
Is there a Beta-Peptide Equivalent of the Alpha-Helix?
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In organic chemistry, the concept of homologation describes the extension of a carbon chain by one methylene unit. The application of this concept to peptides gives rise to the class of β-peptides that feature an additional -CH_2- unit in the monomer backbone. The study of such non-natural peptides offers insight into general folding mechanisms. The similarities to α-peptide structure combined with stability against proteases makes such foldamers promising scaffolds with possible applications in drug design.
With regards to such applications it is remarkable that until now no β-peptide structure with the same H-bonding pattern as the α-helix was found. Hints for this structure stem from a previous theoretical study [1] and from diffraction experiments on Nylon-3 [2]. We compare the β-peptide Ac-β^2hAla$_6$-Lys(H^+) to the α-peptide Ac-Ala$_6$-Lys(H^+) which likely is helical in the gas phase [3]. The gas phase represents a cleanroom environment to study the intrinsic structural properties of peptides. We employ density-functional theory with the PBE functional corrected for dispersion effects [4] to perform extensive replica-exchange *ab initio* molecular dynamics simulations. We combine findings from simulations with experimental fingerprints from ion-mobility mass-spectrometry and vibrational spectroscopy.
As expected, the natural α-peptide Ac-Ala$_6$-Lys(H^+) is found to be mostly α-helical at room temperature. For Ac-β^2hAla$_6$-Lys(H^+) we find both helical and non-helical conformers in the low-energy regime. However, the helical conformations seem to be favored by vibrational entropy. The comparison with experiment points clearly to a helical structure with a hydrogen bond pattern i←i+4 analogous to the α-helix in natural α-peptides.
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3309-Pos Board B37
Novel Computational Methods to Design Protein-Protein Interactions
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Despite the abundance of structural data, we still cannot accurately predict the structural and energetic changes resulting from mutations at protein interfaces. The inadequacy of current computational approaches to the analysis and design of protein-protein interactions has hampered the development of novel therapeutic and diagnostic agents. In this work, we apply a simple physical model that includes only a minimal set of geometrical constraints, excluded volume, and attractive van der Waals interactions to 1) rank the binding affinity of mutants of tetratricopeptide repeat proteins with their cognate peptides, 2) rank the energetics of binding of small designed proteins to the hydrophobic stem region of the influenza hemagglutinin protein, and 3) predict the stability of T4 lysozyme and staphylococcal nuclease mutants. This work will not only lead to a