acting metabolic enzymes that convert ethanolamine to ethanol and acetyl phosphate. The tightly-packed molecular shell surrounding the eukaryotic channel is believed to act as a semi-permeable barrier, allowing the passage of substrates, and larger cofactor molecules, while minimizing the eflux of a toxic acetaldehyde intermediate. Previous structural studies of the eukaryotic channel demonstrated that a conformational change of the EutL shell protein opens a 10-15Å pore through the shell. That observation led to a model for how the protein shell might interconvert between high and low permeability conformations, but the mechanism controlling the pore opening has remained unclear. Here we present structural and functional studies directed toward understanding how the conformational switch is regulated in EutL. The X-ray crystallographic structure of EutL bound to ethanolamine provides evidence that binding of this small metabolite stabilizes the "closed-pore" conformation by sterically blocking re-arrangement to the open conformation. Specific binding of ethanolamine to EutL was verified by isothermal titration calorimetry (ITC). Thermodynamic parameters derived from ITC experiments were rationalized through analysis of molecular contacts revealed by X-ray crystallography and molecular dynamics simulations. We show that ethanolamine binding is specific, i.e. EutL does not bind to other small molecules associated with the metabolic reactions carried out in the eukaryotic channel. Our results suggest a model for EutL function in which the presence of ethanolamine decreases the porosity of the MCP shell by modulating the interconversion between open and closed pore conformations.

3299-Pos Board B27 Covariance Ration Analysis of Molecular Dynamics Trajectories of Hiv-1 Reverse Transcriptase
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HIV-1 reverse transcriptase (RT) is a major drug target for HIV treatment, and understanding its function and inhibition would significantly improve our ability to create new anti-HIV drugs. RT can perform DNA-polymerization using either a DNA or a RNA template, and possesses an RNase activity. Elastic network modeling is a method to rapidly probe and compare protein dynamics. We have previously shown that combining elastic network modeling with hierarchical clustering of both structural and dynamics data elucidates RT functional states. Here we extend our method beyond X-ray crystallographic structural data, to structural data determined by short molecular dynamics trajectories of RT bound to a primer template and either the correct dNTP or a mismatched dNTP. This reveals that RT bound to a mismatched dNTP is capable of entering into a novel nonfunctional state after dNTP incorporation. In this state, the thumb subdomain experiences inhibited dynamics and the primer/template breaks contacts with the p51 subunit. The incorporation of the correct dNTP shields RT from this nonfunctional state, allowing polymerization to continue. In summary, surveying structural and dynamics changes that occur in molecular dynamics trajectories alongside X-ray crystallographic structural data provides novel insights into normal RT function.

3300-Pos Board B28 Spectrosopic Analysis of Channelrhodopsin and its Chromophore
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Channelrhodopsins are photoreceptors which control phototaxis in green algae. Electrophysiological experiments showed that they act as light-gated ion channels when heterologously expressed in oocytes or HEK cells. Due to this function these cation channels are widely used in the new field of optogenetics where specific nerve cells are depolarized through optical stimulation. Channelrhodopsins are already widely-used in neurophysiological applications and the channelrhodopsins are also used in the new field of optogenetics where specific nerve cells are depolarized by light. Although these optical channels are already widely used in the new field of optogenetics, the mechanism controlling the pore opening has remained unclear. Here we present structural and functional studies directed toward understanding how the conformational switch is regulated in EutL. The X-ray crystallographic structure of EutL bound to ethanolamine provides evidence that binding of this small metabolite stabilizes the "closed-pore" conformation by sterically blocking re-arrangement to the open conformation. Specific binding of ethanolamine to EutL was verified by isothermal titration calorimetry (ITC). Thermodynamic parameters derived from ITC experiments were rationalized through analysis of molecular contacts revealed by X-ray crystallography and molecular dynamics simulations. We show that ethanolamine binding is specific, i.e. EutL does not bind to other small molecules associated with the metabolic reactions carried out in the eukaryotic channel. Our results suggest a model for EutL function in which the presence of ethanolamine decreases the porosity of the MCP shell by modulating the interconversion between open and closed pore conformations.

3301-Pos Board B29 Bordetella Pertussis Adenylate Cyclase Toxin: Potential Modulator of Calmodulin Metal-Binding Properties
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Calmodulin (CaM) is a potent activator of Bordetella pertussis adenylate cyclase toxin (CyaA) in the presence or absence of calcium (Ca2+). Physiological concentrations of magnesium (Mg2+) are sufficient to fully or partially saturate CaM at resting Ca2+ levels, which may facilitate CaM-dependent stimulation of CyaA, but it remains unclear what role metal-binding plays in toxin activation. In this study, multi-dimensional nuclear magnetic resonance (NMR), dynamic light scattering (DLS), and circular dichroism (CD) were used to examine the effects of Mg2+-binding on the structure and hydrodynamic properties of CaM/CyaA complexes. NMR structural investigations of partially (2Mg2+/2Ca2+) and fully Ca2+-loaded (4Ca2+) CaM/CyaA complexes revealed that Mg2+-binding is largely localized to sites I and II of CaM. In the presence of CyaA, sites III and IV remained Ca2+-loaded, even when Mg2+ is in excess, indicating that CyaA prohibits metal exchange in the C-terminus of CaM. Moreover, interaction with CyaA stabilized Mg2+-binding at site II of CaM implying that CyaA modulates CaM’s metal-sensing properties. DLS and CD analyses showed that differences exist in the global conformations of CaM/CyaA complexes in the 2Mg2+/2Ca2+ and 4Ca2+ loaded states. The conformation and metal-binding properties of CaM’s N-terminal domain were perturbed by mutations targeting the CaM/CyaA interface. However, these mutations had no detectable structural impact on sites III and IV of CaM, confirming CyaA interaction differentially modifies the conformation of each domain. These data suggest that CyaA alters the Ca2+- and Mg2+-binding properties of CaM, which would represent an alternative, novel mechanism of toxin function within the cell.
with the goal they will be able to partially inhibit SERCA and also be imper- 
vious to dephosphorylation. Insights to these issues will provide better para-
digms 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 chro-
matography 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 
possible due to the preclusion of chromophore formation. Arg96, in particu-
lar, is an interesting residue because of its major role in defining the electro-
static environment, however, its intolerance to mutation necessitates the use of a 
split protein reconstitution with a chromophore-containing peptide harvested 
from a non-mutated donor. We use this scheme to investigate the ef-
fects 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 empir-
ical 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 thermody-
namics. Our model system is the Kempl Eliminase KE07, which was designed 
computationally to catalyze the conversion of 5-nitrobenzoxazole to cyano-
phenol, 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 statis-
tical fluctuations (entropy) that occurs at the side chain level could give us 
important leads to predict further mutations. This work also provides further evi-
dence to start looking beyond the traditional structure-function paradigm and 
corporate 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 that dynamics of the enzyme affect the catalytic ac-
tivity. 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 mo-
tions 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 capabil-
ities 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 concentra-
tions. 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 dichromism. To ensure that the structure was maintained, 
N-15 enriched human fibroblast growth factor-1 was over expressed and puri-
fied. This sample will be used to test the structural stability of hFGF-1 by Het-
eronuclear 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-features that offer an additional -CH₂- unit in the 
monomer backbone. The study of such non-natural peptides offers insight into 
general folding mechanisms. The similarities to z-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 z-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-Hala₆-Lys(H⁺) 
to the z-peptide Ac-Ala₆-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 find-
ings from simulations with experimental fingerprints from ion-mobility mass-
spectrometry and vibrational spectroscopy.
As expected, the natural z-peptide Ac-Ala₆-Lys(H⁺) is found to be mostly 
ν-helical at room temperature. For Ac-β-Hala₆-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 z-helix in natural z-peptides. 

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 therape-
utic 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 mu-
tants 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 lyso-
zyme and staphylococcal nuclease mutants. This work will not only lead to a