acting metabolic enzymes that convert ethanolamine to ethanol and acetyl phosphate. The tightly-packed molecular shell surrounding the eut MCP is believed to act as a semi-permeable barrier, allowing the passage of substrates, products, and larger cofactor molecules, while minimizing the efflux of a toxic acetaldehyde intermediate. Previous structural studies of the eut MCP 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 biophysical studies directed toward understanding how the conformational switch is regulated in EutL. The X-ray crystal structure of EutL bound to ethanolamine provides evidence that binding of this small metabolite stabilizes the "closed-pore" conformation by sterically blocking rearrangement 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 eut MCP. 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 from either a DNA or an RNA template, and possesses an RNase function. 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
Spectroscopic 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 meanwhile used in the new field of optogenetics where specific nerve cells are depolarized by light. Although the channelrhodopsins are already widely-used in neurophysiological applications, the mechanism how these proteins transfer ions, is still not clarified in detail. Most algae containing light-gated ion channels exhibit two different types of channelrhodopsins (ChR1 and 2) with apparent mechanistic differences.
We want to understand the processes leading to the opening of the channel, which include isomerization of the retinal after light excitation and proton transfer reactions from the Schiff base which is protonated in the ground state. Therefore, we apply time-resolved spectroscopic methods to different channelrhodopsins to determine and compare the intermediate states on a time scale from 100 ns to 5 s. Resonance Raman spectroscopy as well as retinal extraction with HPLC detection is used to derive information about the retinal structure in the ground and illuminated state.

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 (Ca^{2+}). Physiological concentrations of magnesium (Mg^{2+}) are sufficient to fully or partially saturate CaM at resting Ca^{2+} 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 Mg^{2+}-binding on the structure and hydrodynamic properties of CaM/CyaA complexes. NMR structural investigations of partially ($2Mg^{2+}2Ca^{2+}$) and fully Ca^{2+}-loaded ($4Ca^{2+}$) CaM/CyaA complexes revealed that Mg^{2+}-binding is largely localized to sites I and II of CaM. In the presence of CyaA, sites III and IV remained Ca^{2+}-loaded, even when Mg^{2+} is in excess, indicating that CyaA prohibits metal exchange in the C-terminus of CaM. Moreover, interaction with CyaA stabilized Mg^{2+}-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 $2Mg^{2+}2Ca^{2+}$- and $4Ca^{2+}$-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 Ca^{2+}- and Mg^{2+}-binding properties of CaM, which would represent an alternative, novel mechanism of toxin function within the cell.

3302-Pos Board B30
Imods: Fast Exploration of Macromolecular Collective Motions
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iModS is a web-based tool to approximate protein and nucleic acid flexibility using normal mode analysis in internal coordinates [1]. Given an input structure, the server provides a fast and powerful tool to model, visualize and analyze functional collective motions. Vibrational analysis, motion animations and morphing trajectories can be easily carried out at different scales of resolution. The server is very versatile, non-specialists can rapidly characterize potential conformational changes whereas advanced users can select between multiple coarse-grained representations and elastic network potentials. It includes advanced visualization capabilities for illustrating molecular flexibility based on affine-models and vector field representations. The visualization engine is also compatible with HTML5 and WebGL capabilities ensuring full accessibility to all devices. The web server can be freely accessed at http://imods.chaconlab.org.
1. López-Blanco JR, Garzón JI, Chacón P. (2011) iMod: multipurpose normal mode analysis in internal coordinates. Bioinformatics. 27 (20): 2843-2850.

Protein Design, Prediction, and Evolution

3303-Pos Board B31
Rational Structure-Based Design of PLN Mutants to Optimize Dephosphorylation and Tune Serca Function
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Phospholamban (PLN) is the endogenous inhibitor of the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA), the enzyme that regulates cardiac muscle relaxation. In its phosphorylated state (pS16-PLN, pT17-PLN, and pS16pT17-PLN), PLN does not inhibit SERCA. Dysfunctions in SERCA:PLN interactions and in the PLN phosphorylation mechanism have been implicated in cardiac disease, and targeting PLN is becoming a promising avenue for treating cardiomyopathies. In this study, we seek to further improve the design therapeutic PLN mutants by optimizing their functional interactions with an endogenous regulatory phosphatase of PLN, protein phosphatase-1 (PP1). PP1 is responsible for PLN dephosphorylation and the transition from its non-inhibitory to inhibitory state. Using a combination of NMR spectroscopy and biochemical assays, correlations will be built between the structural dynamics of promising therapeutic mutants of PLN and their ability to be dephosphorylated by PP1. Additionally, several new mutants of PLN are developed