densities are Fourier transformation partners of the irreducible correlation functions. By fitting theoretical spectral densities to experimental data we can readily obtain the values of preexponential factors and activation energies [2]. We are currently applying our generalized model-free method to interpret the behavior of active Meta-II rhodopsin. Our aim is to establish if the local fluctuations of the ligand initiate the structural changes of rhodopsin to understand the activation mechanisms of GPCRs in general. Moreover, the results from our generalized model-free analysis method can be used in molecular dynamics (MD) simulations without the limitations of simplified motional models. [1] M.F. Brown (1982) *JCP* **77**, 1576-1599. [2] A.V. Struts *et al*. (2011) *NSMB* **18**, 392-394.

3294-Pos Board B22

Conformational Motion in Gene Regulatory Proteins

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Proteins' structures determine functions and the functions determine the structures. Yet, proteins and other biological macromolecules are dynamic systems under continuous conformational motion and the motions are keys to many of the biological processes in which they are involved. We argue that conformational motion driven by structural specificities, and not structures alone, determines biological functions. For instance conformational motion in gene regulatory proteins acts as a selective molecular switch motion in DNA binding mode and controls gene regulations. Perhaps, such action is relatively well studied in prokaryotic OxyR belonging to LysR family of transcriptional regulatory proteins. Escherichia coli Oxidative stress response genes are transcriptionally regulated by OxyR through a reversibly reducible cysteine disulfide biosensor. The redox status in these cysteines induces structural changes which are conformationally transmitted to the dimer subunit interfaces and alters DNA binding mode. However, crystal structures of Porphyromonas gingivalis OxyR regulatory domains indicate locked dimer configuration insensitive to cysteine disulfide redox status and shows only one activating mode. Conformational motion in Porphyromonas gingivalis OxyR changes dimer/tetramer convention (dimer binding to DNA or tetramer binding to DNA) and alters differentiation in gene regulations. Crystal structures along with modeled full-length Porphyromonas gingivalis, Escherichia coli and Neisseria meningitidis OxyR-DNA complexes predict different DNA binding modes in these organisms and indicate how the limited conformational motion differentiates the species. Aerobic organisms' OxyR is confined in open dimer configuration; Anaerobe's OxyR is confined in closed dimer form, while facultative organisms can conformationally switch OxyR dimer configurations. Conformational motion in aerobes and anaerobes OxyR is restricted by dimer configuration and can only change dimer/tetramer population while in facultative organisms conformational motion induces configurational switch in dimers.

3295-Pos Board B23

Differences in Troponin C Dynamics Between Cardiac and Skeletal Muscle - A Molecular Dynamics Perspective

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Troponin (Tn), part of the thin filament in cardiac as well as skeletal muscle, plays an important role in calcium signaling events in muscle contraction. It acts as a Ca^{2+}-dependent switch, activating and deactivating the myofilament leading to contraction and relaxation of the muscle cell. An important mechanism in the regulation of contraction is the opening up of the Troponin C (TnC) hydrophobic patch to allow Troponin I (TnI) to bind. Two main structural differences are observed between cardiac TnC (cTnC) and skeletal TnC (sTnC): sTnC binds two calcium ions in its N-terminal regulatory domain, while cTnC only binds one calcium ion. The calcium binding site I in cTnC is inactive due to mutations. Additionally, the hydrophobic patch between helices A and B in the N-terminal regulatory domain is open in sTnC, while it is closed in cTnC. In previous work we performed microsecond molecular dynamics (MD) simulations of cTnC in different states of calcium binding to estimate the free energy difference for opening of the hydrophobic patch. Here we present long time-scale MD simulations of sTnC in its apo, one Ca^{2+}-bound and two Ca^{2+}-bound forms. We observe a 10,000-fold increased opening frequency in sTnC compared to cTnC. The dramatic difference is caused almost entirely by changes in the binding site I dynamics upon calcium binding. Investigations of sTnC mutations shown to prevent calcium binding to site I shed further light on the differences between the TnC isoforms. Additionally, Brownian dynamics simulations are used to investigate TnI association with TnC. Simulations of a full length troponin model elucidate the dynamical interplay between the TnC, TnI and TnT subunits.

3296-Pos Board B24

The Role of Conformational Flexibility in Inhibitor Binding and Substrate Recognition for Cyp119

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Conformational flexibility of several cytochromes P450 has been observed in response to substrate and ligand binding, and thus may play an important role in catalysis. CYP119, a bacterial thermophilic protein from cytochrome P450 superfamily has been observed in three different conformations with different inhibitors bound using X-ray crystallography, but the significance of these states in solution and in the function of the enzyme is not well known. According to the crystal structures, much of the diversity in conformational states arises in F and G helices, which fold around the substrate binding site at the distal heme face. Double electron electron resonance (DEER) was used to measure the average distances and the distance distributions between spin-labels for populated conformational states in solution. Pairs of spin-labels were introduced by coupling to engineered cysteines on the protein surface, and the effects of labeling on ligand dissociation constants (K_d) and enzyme function were characterized. DEER results from three different mutants of CYP119 indicated that 4-Phenylimidazole binding results in a conformational state that is distinct substrate-free and Imidazole-bound forms. The DEER distance changes between the two conformational states were determined as 10Å for two of the mutants and 12Å for the other one. Data will also be presented for the complex between CYP119 its proposed substrate, lauric acid. These results will be discussed in terms of models for substrate recognition and gated functional behavior in these enzymes.

3297-Pos Board B25

Conformational Changes in Protein Binding Processes

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Ligand-protein and protein-protein binding processes play a crucial role in biological systems. They are often associated with conformational changes that induce effects such as signal transmission or allostery. To structurally and energetically explore the interplay between induced fit, conformational selection, and allostery, we performed molecular dynamics simulations of three selected proteins.

As a first system, we choose the cAMP binding process at the potassium channel MloK1. The obtained free energy differences between the two main protein conformations, an open and a closed state, reveal that the process is best described by an induced fit mechanism. We found that the binding affinity is mainly caused by the conformational change.

Next, we explored the structural determinants of allostery of the export protein CRM1. This ring-shaped protein plays a crucial role in the nucleocytoplasmic transport of macromolecules. We investigated what structural features and how the binding of RanGTP and cargo proteins determine the overall conformation. We found that the enforced rearrangement of a key helix due to RanGTP binding changes the stability of the overall conformation. This induces a global conformational change, which in turn causes a local conformational change in the cargo binding site. The link between global and local conformation leads to cooperative binding.

Third, we investigated the influence of ligand binding on the dimerisation of nitrate reductase. This enzyme is a key player in nitrogen fixation and binds a molybdenum containing cofactor in its active centre. We identified key motions caused by the cofactor binding, and suggest how these motions might be coupled to dimerisation.

Overall our simulations underline and explain how subtle free energy changes due to ligand/protein binding can change the overall protein free energy landscape thus causing conformational changes, which are key to the protein function.

3298-Pos Board B26

A Model for Allosteric Control of Pore Opening by Substrate Binding in the Eutl Microcompartment Shell Protein

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The ethanolamine utilization microcompartment (eut MCP) is a giant protein assembly that acts as a metabolic organelle in enteropathogenic bacteria, allowing them to proliferate in the human gut. The eut MCP consists of a polyhedral shell, reminiscent of a viral capsid, which encapsulates several sequentially

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 cardomyopathies. 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