**2283-Pos**
**Molecular Dynamics Simulation of Conformational Change in a Fatty Acid β-Oxidation Multienzyme Complex**
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Recent biochemical studies suggest that many enzymes are organized into multienzyme complexes in the cytoplasm or subcellular organelles. Despite importance in cellular mechanisms, the structural basis for enzyme multimerization and underlying biochemical implication have not been established yet. Fatty acid β-oxidation multienzyme complex (FOM), an $\alpha_2\beta_2$ hetero complex consisting of three component enzymes, ECH, HACD and KACT, has been a subject of intense investigation, due to the importance in the catabolic processes for fatty acids utilization. The purpose of this investigation is to clarify the mechanism of the conformational changes in FOM occurring in the process of the multi-enzymatic reactions at the atomic level by using molecular dynamics simulation, based on the crystal structures determined by Morikawa's group. In this presentation, we present results of four molecular dynamics simulations of 100 ns for (i) FOM without ligands and (ii) FOM with 2 $NAD^+$, started from each of the two crystal forms (form I & form II). Extraordinarily large conformational fluctuations were observed in the disposition of the domains in both forms, while intra-domain fluctuations were within the ordinary level. We will discuss the simulation results on the subject of a possibility of the channeling reactions between consecutive enzymes within the multienzyme complex.

**2284-Pos**
**The Effect of Osmolytes on Protein Dynamics in LDH-Catalyzed Reaction**
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Laser induced temperature jump relaxation spectroscopy was used to probe the effect of osmolytes on the dynamics of LDH-catalyzed reaction. NADH fluorescence and absorption relaxation kinetics were measured for the LDH reaction system in presence of varying amounts of trimethylamine-N-oxide (TMAO), a protein stabilizing osmolyte, or urea, a protein destabilizing osmolyte. The fluorescence kinetics show that TMAO does not significantly affect pyruvate binding/unbinding, but noticeably changes the dynamics of the active site loop, strongly decreasing its kinetic response. The nature of these changes is not yet fully understood. The lower rate of the absorption kinetics, representing mainly the hydride transfer step and to a much lesser extent the loop dynamics, becomes faster upon TMAO addition, especially at lower temperatures (17-22C). This may be explained by an enhancement of hydrogen tunneling resulting from compression of the active site. Urea, in concentrations up to 1 M, strongly diminishes the kinetic response of pyruvate binding/unbinding, while increasing its rate at higher temperatures (32-37C). Loop motions slow in presence of urea, as does the rate of hydride transfer. In presence of 2 M urea, no kinetic features arising from the reaction are observed, presumably due to expected protein denaturation.

**2285-Pos**
**Identification of Signal Transmission Pathways in F-Box Proteins Using Perturbation-Based Markovian Transmission (PMT) Model**
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As a part of ubiquitin-dependent proteolytic network, F-box proteins play important roles in almost all regulations of cellular processes, including signal transduction, apoptosis, cell cycle, and cell division. F-box proteins bind various protein substrates and tag them with multiple ubiquitin molecules, which are then further recognized by proteasome for degradation. F-box protein and Skp1 protein (S-phase kinase-associated protein 1) are key components of the SCF (Skp1, Cullin, and F-box protein) ubiquitin-ligase complexes. We study the dynamic behavior of these protein complexes, which are modeled by a connected network of Markovian transmission. The dynamics of signal transmission between the F-box motif and the substrate binding domain of the system is obtained using the Perturbation-based Markovian Transmission (PMT) model, which was recently developed and has been successfully applied to the GroEL-GroES chaperone system (Lu and Liang, 2009). In our model, the initial perturbation generated by the protein-protein interaction on the F-box motif is transmitted by a Markovian process, in which the dynamics of the probability flow is followed by solving the master equation directly. We give details of the dynamics of several Skp1 and F-box protein complexes, including beta-TrCP, Skp2, and Cdc4. The signal transmission pathways from the F-box domain to the substrate binding domain are also identified. In addition, we predict a set of key residues which are important for allosteric transition based on their distinctive dynamic properties. Our predictions are consistent to biochemical observations. Our PMT model can be applied to other large systems of biomolecules for understanding their dynamic behavior and for identifying pathways of allostery. (Lu, Hsiao-Mei and Linag, Jie, "Perturbation-based Markovian Transmission Model for Probing Allosteric Dynamics of Large Macromolecular Assembling: A Study of GroEL-GroES," PLoS Comp. Bio., 2009.)

**2286-Pos**
**Structural Basis for Regulation Specificity in Syk-Family Kinases**
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Protein kinases regulate cellular signaling pathways, and the complexity arising from multiple phosphorylation cascades necessitates tight regulation by associated modular domains. Syk and ZAP-70 are Syk-family non-receptor tyrosine kinases that fulfill critical roles in immunological signaling, and each contains a regulatory tandem of modular SH2 domains. This tandem binds to ITAM (immunoreceptor tyrosine-based activation motif) sequences on antigen-stimulated receptors (such as the T-cell receptor), resulting in activation of the downstream kinase domain and an immune response. Syk and ZAP-70 share high sequence and structural similarity, yet Syk is less tightly regulated than ZAP-70, and an understanding of its regulatory mechanism is essential for the design of therapeutic drugs targeted to diseases associated with immunodeficiency, autoimmunity, and transplant rejection. We have used atomistic, explicitly solvated, microsecond-timescale molecular dynamics simulations and free-energy calculations to show that differences in conformational flexibility resulting from selected point mutations can explain the regulatory differences of Syk and ZAP-70. We show that mutation of a single residue conserved in many SH2 domains permits the crystallographically-observed closure of an essential ligand binding pocket in one of the SH2 domains of ZAP-70, but an energetic barrier of 4 kcal/mol is revealed in Syk, consistent with the experimentally-observed ability of its isolated domain to bind ITAM, and with the greater promiscuity of the entire tandem. We also show that ITAM-bound ZAP-70 and Syk are conformationally stable. Upon ITAM disengagement, the SH2 domains of ZAP-70 are rapidly uncoupled, but this is prevented in Syk due to key mutations at the interface between SH2 domains. Greater stability of binding-competent tandem conformations and constitutively open recognition pockets in Syk provide a compelling explanation for its amplified activation plasticity. A subsequently parameterized multi-state coarse-grained model should yield a global description of regulatory equilibria in Syk-family kinases.

**2287-Pos**
**Metal-Binding Residues are Distinguished by their Lower Mobilities and Efficient Signal Propagation Properties**
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Metal-binding proteins are associated with a variety of functions. Understanding the fundamental functional mechanism of these proteins entails a thorough characterization of the structural and dynamic properties of their metal-binding sites. Recent studies have shown that proteins at equilibrium undergo collective changes in conformation, which facilitate their function, and such intrinsic protein dynamics can be characterized using elastic network models in conjunction with normal mode analysis methods.[1] The usefulness of the Gaussian Network Model (GNM) in capturing the unique architecture-induced properties of a protein has been shown in analyzing the dynamics of catalytic sites for a series of enzymes where functional sites were inferred from structural dynamics. In this study, we analyzed the equilibrium dynamics of metal-binding proteins as seen in the lowest frequency modes predicted by the GNM, to see whether any dynamic role is assumed by the metal-binding sites, in addition to their chemical role of coordinating the ligand. We also examined the communication properties of these sites using an information theoretic spectral method of signal propagation.[2] Our results demonstrate that metal-binding residues are predisposed towards having relatively smaller-scale fluctuations than other residues. Intuitively, a flexible residue would be entropically more unfavorable for binding purposes and the inherent small-scale fluctuations imply lesser loss of conformational entropy upon metal binding. Our study further shows that metal-binding residues efficiently communicate signals, suggesting that their particular locations in the structure have been evolutionarily optimized to achieve most efficient allosteric function. These properties provide insights into the functional design of metal-binding proteins.
*References:*
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2. Chennubhotla, C & Bahar, I. (2007) *PLoS Comp Biology* **3**, 1716-26.