Unexpectedly, the apo N253F mutant with ~80% of hydrolase activity but no detectable isomerase activity showed a strikingly different conformation, in which the Bβ1-Bβ2 loop in subdomain B is disordered, and the subdomain B rotates away to create an open active site. Interestingly, this mutant displays a high structural similarity to the apo sucrose hydrolase. Therefore, our DrTS-N253F structure may represent an open conformation for the apo TS, while the DrTS-Tris may represent a substrate-induced closed conformation that will facilitate intramolecular isomerization and minimize disaccharide hydrolysis.

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Biophysical Characterization of Naturally Occurring Titin-M10 Mutations

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The extreme C-terminus of titin (termed the M10 domain) binds to the N-terminus of obscurin in the M-band of skeletal muscle cells. Multiple M10 mutations are linked to limb-girdle muscular dystrophy type 2J (LGMD2J) and tibial muscular dystrophy (TMD) in humans. The high-resolution structure of M10 has been solved, along with M10 bound to an obscurin-like target. However the effect of the M10 mutations on protein structure and binding has not been thoroughly characterized. Here we express all four of the naturally occurring human M10 missense mutants and biophysically catalogue them. Three of the four mutations are severely misfolded, and are binding incompetent. One mutation, I57N (also called the Belgian mutation), shows no significant structural, dynamic, or binding differences from the wild-type domain. We suggest that this mutation is not directly responsible for muscle wasting disease, but is instead merely a silent mutation found in symptomatic patients.

Protein Dynamics and Allostery II

1892-Pos Board B29

Investigating the Mechanism of Iron Dependent Repressor (IDER) Activation and DNA Binding

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Metalloproteins form a major class of enzymes in the living system and are involved in critical biological functions such as catalysis, redox reactions and as ''switches'' in signal transductions. Iron dependent repressor (IdeR) is a metal-sensing transcription factor that regulates free iron concentration in Mycobacterium tuberculosis. IdeR is also known to promote bacterial virulence, making it an important protein for therapeutics.

In this study, we have employed molecular dynamic simulations on different binding states of IdeR in the presence and absence of iron to study its influence on protein function. Structures were investigated using hydrogen bonds and protein structure networks and displayed significant variation between the metallated and the non-metallated systems. Briefly, we could establish the role of iron in stabilizing the monomeric unit of IdeR which in turn promotes protein dimerization. Two major monomer conformations, ''open'' and ''closed'' were identified and their geometrical parameters were also quantified. Perhaps, the most striking results are obtained from the simulations of the IdeR-DNA complex in the absence of metals, where the protein subunits are seen to dissociate away from the DNA quite rapidly. Such drastic changes in the IdeR-DNA interactions not only provide molecular insights about the role of iron, but also about the mechanism of DNA binding and unbinding. Based on the ensemble structure analysis, we suggest the role of iron as a possible allosteric effector that enhances the IdeR-DNA interactions.

Our simulation results enable us to understand the sequence of events that govern IdeR-DNA binding in the presence of iron.

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Dynamic Characteristics of Allosteric Pathways in scFv Antibody Fragments

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Proteins exhibit dynamic behavior that is constrained by the hydrogen bond network (HBN). Previous work [Tong Li, et. al. PLoS ONE 9(3) 2014] on a set of six single chain F_v (scFv) anti-lymphotoxin-β receptors demonstrated that there is a redistribution of flexibility upon mutation due to changes in the HBN. The observed redistribution occurs due to enthalpy-entropy compensation in the native state ensemble. Moreover, the shifts in rigidity and flexibility follow the Le Châtelier's principle, meaning increased rigidity is offset by increased flexibility elsewhere. Extending this work further, the thermodynamic and mechanical response is calculated for localized mechanical perturbations that reduce conformational entropy along the protein backbone. At each mechanical perturbation site all other residues that have significant changes in flexibility are identified. Some perturbation sites yield no statistically significant response, and others yield a response that is spatially localized near the perturbation site. A relatively small fraction of perturbations generate strong distal responses, indicating they are putative allosteric sites. Importantly, the allosteric pathways that carry the distal changes in flexibility or rigidity are linked to fluctuations in the HBN, which also depend on the redistributions of rigidity and flexibility that occur upon mutation. Mutations induce a population shift that changes the most probable constraint networks in the equilibrium ensemble, and alter the mechanical signaling pathway through the modification of the HBN. Interestingly, a reciprocal relation is observed among conjugate response and perturbation sites, such that they can be interchanged in their role. A comparative analysis on response maps due to perturbation is made across all six mutant structures, which provide important insight into how sensitive allosteric mechanisms are within antibody fragments.

1894-Pos Board B31

Functionally Important Residues from Mode Coupling during Short-Time Protein Dynamics

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Relevance of mode coupling to energy/information transfer during protein function, particularly in the context of allosteric interactions is widely accepted. However, existing evidence in favor of this hypothesis comes essentially from model systems. We here report a novel formal analysis of the near-native protein dynamics which allows us to explore the impact of the interaction between (possibly non-Gaussian) vibrational modes on fluctutational dynamics. We show that, an information-theoretic measure based on mode coupling alone yields a ranking of residues with a statistically significant bias favoring the functionally critical locations identified by experiments.

1895-Pos Board B32

High-Speed AFM Observation of Antibody IGG Characteristic of Swinging Arms

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Antibody IgG molecule is a ''Y'' shape protein. It has two Fab regions and one Fc region. Fab regions bind to the antigens. Hinge region connects a Fab region to the Fc region.

High-speed AFM (HS-AFM), developed by Prof. Ando in Kanazwa University, can observe dynamic behavior of motor protein, myosin as movie without chemical fixing or stain treatment (1, 2).

We observed IgG in solution using HS-AFM. ''Y'' shape of IgG was imaged clearly, and Fab and Fc regions were distinguished. The Fab regions moved in torsional direction like swinging arms. This behavior depends on flexible structure of hinge regions. We analyzed the Fab swivel movements as random walks, and estimated the flexibility of the IgG hinge region.

The flexible nature of hinge region contributes for the antibody to bind to the antigen. For the first time, we have identified the swinging nature of this soft structure, which is important for antibody function. The lacking of swing movement would lead to reduce binding between antibody and antigen (3).

HS-AFM can directly observe dynamic behaviors of biomolecules as movie in solution, and reveal functions in detail.

1. T. Ando et al., Proc. Natl. Acad. Sci. USA. **98**, 12468- (2001).
2. N. Kodera et al., Nature **468**: 72- (2010)
3. J. Preiner et al., Nature Communications **5**: 4394- (2014).

1896-Pos Board B33

Visualizing Global Properties of a Molecular Dynamics Trajectory

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Molecular dynamics (MD) trajectories are very large data sets that contain substantial information about the dynamic behavior of a protein. Condensing these data into a form that can provide intuitively useful understanding of