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.

1891-Pos Board B28
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.

1893-Pos Board B30
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

the molecular behavior during the trajectory is a substantial challenge that has received relatively little attention. Here, we introduce the sigma-r plot, a plot of the standard deviation of intermolecular distances as a function of that distance. This representation of global dynamics contains within a single, one-dimensional plot, the average range of motion between pairs of atoms within a macromolecule. Comparison of sigma-r plots calculated from 10 nsec trajectories of proteins representing the four major SCOP fold classes indicates significant diversity of dynamic behaviors which are recognizably different among the four classes. Differences in domain structure and molecular weight also produce recognizable features in sigma-r plots, reflective of differences in global dynamics. Plots generated from trajectories with progressively increasing simulation time reflect the increased sampling of the structural ensemble as a function of time. Single amino acid replacements can give rise to changes in global dynamics detectable through comparison of sigma-r plots. Dynamic behavior of substructures can be monitored by careful choice of interatomic vectors included in the calculation. Comparison between the sigma-r plots calculated from MD simulations and from wide angle x-ray solution scattering data is also feasible with the potential for providing direct experimental tests of the approximations required for coarse-grained MD simulations. These examples provide demonstrations of the utility of the sigma-r plot to provide a simple measure of the global dynamics of a macromolecule.

1897-Pos Board B34

Computational Modeling of the FcαRI Receptor Binding in the Fcα Domain of the Human Antibody IgA: Corse-Grained Molecular Dynamics (MD) Methods

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FcαRI receptor binding in the Fcα domain of the antibody IgA triggers immune effector responses such as phagocytosis, antibody-dependent cell-mediated cytotoxicity, respiratory burst and cytokine release in eukaryotic cells. Fcα is a dimer of heavy chains of the IgA antibody and each Fcα heavy chain which consisted of two immunoglobulin constant domains, C_H2 and C_H3, can bind one FcαRI molecule at the C_H2-C_H3 interface forming a 2:1 stoichiometry which is unique to the human IgA. Experimental evidences confirmed that FcαRI binding to the Fcα C_H2-C_H3 junction altered the kinetics of HAA lectin binding at the distant IgA1 hinge and distant Fab region.

Given the importance of residues near the C_H2-C_H3 junction for receptor binding that were predicted experimentally by binding energetic analysis, our focus in this computational research was to understand the conformational changes and the residue-pairs in long-range communication which co-ordinate the receptor binding dynamics of the Fcα dimer complex.

We computed the principal collective motions by using the corse-grained structure based molecular dynamics trajectories performed on the high resolution crystal structure of Fcα-FcαRI 2:1complex of PDB ID 1OW0 to understand the functional dynamics in Fcα. We used three distinct Fcα conformations namely free Fcα, Fcα-FcαRI 1:1 asymmetric and Fcα-FcαRI 2:1 symmetric complexes to comparatively study the functional dynamics induced upon receptor binding.

Our findings confirmed that FcαRI binding, either in asymmetric or symmetric complex with Fcα, propagated long-range conformational changes across the Fc domains, potentially also impacting the hinge and Fab regions.

Key words: IgA antibody, single-basin structure-based coarse grain MD simulation, principal component modes, long-range interaction, ligand-induced conformational changes

1898-Pos Board B35

Computer-Aided Drug Discovery Approach Finds Calcium Sensitizer of Cardiac Troponin

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Defects in the contractile machinery can lead to heart failure. Weakened contraction of the heart will lead to diminished blood supply of the organs in the human body. Thus, in the fight against heart failure, therapeutics that have the ability to increase the contractile power of the heart are urgently needed. One possible route of action to improve heart contractile power is increasing the calcium sensitivity of the thin filament. From a pharmaceutical standpoint, calcium sensitizers have the distinct advantage of not altering cardiomyocyte calcium levels and thus have lower potential for side effects. Small chemical molecules have been shown to bind to the interface between cTnC and the cTnI switch peptide and exhibit calcium sensitizing properties, possibly by stabilizing cTnC in an open conformation. Building on existing structural data of a known calcium sensitizer bound to cardiac troponin, we devised a combined computational and experimental drug discovery approach. We used Molecular Dynamics to sample a range of troponin structure conformations and accounted for receptor flexibility by running virtual screens into several conformational states. The most promising compounds were then tested using solution NMR titration assays. We were able to identify a novel calcium sensitizer 4-(4-(2,5-dimethylphenyl)-1-piperazinyl)-3-pyridinamine (NCI147866) which binds to cTnC and the cTnC-$cTnI_{147\text{-}163}$ complex. Its presence increased the affinity of switch peptide to cTnC by approximately a factor of two. This action was comparable to that of known levosimendan analogues and served as an excellent starting point for targeted compound improvement aimed at higher affinity and calcium sensitization.

1899-Pos Board B36

A Coarse-Grained Langevin Equation for Protein Dynamics: Global Anisotropy and a Mode Approach to Local Complexity

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We utilize a multi-scale approach where molecular dynamic simulations are performed to obtain quantitative structural averages used as input to a coarse-grained Langevin Equation for Protein Dynamics, which can be solved analytically. The approach describes proteins as fundamentally semiflexible objects collapsed into the free energy well representing the folded state. The normal mode analytical solution to this Langevin equation naturally separates into global modes describing the fully anisotropic tumbling of the macromolecule as a whole, and internal modes which describe local fluctuations about the folded structure. Complexity in the configurational free energy landscape around the folded state of the macromolecule leads to a renormalization of the internal modes, while the global modes provide a basis set in which the dipolar orientation and global anisotropy can be accounted for when comparing to experiments. Fundamental to this approach is the inclusion of internal dissipation which is absent in any rigid-body hydrodynamical modeling scheme. This simple approach predicts the dynamics of both global rotational diffusion and internal motion from the picosecond to the nanosecond regime, and is quantitative when compared to time correlation functions calculated from molecular dynamic simulations and in good agreement with Nuclear Magnetic Resonance relaxation experiments. Results for several well-characterized globular proteins are presented, suggesting our method describes the relevant dynamics around the global minimum well. Use of non-equilibrium simulation techniques such as metadynamics to sample the full free-energy landscape of the protein, and extension of the theoretical treatment to describe the dynamics into the biologically interesting microsecond to millisecond regime, will be discussed.

1900-Pos Board B37

Looking at Estrogen Receptor from Small Angles

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The estrogen receptor (ERα) functions as a hormone-activated transcription factor. The protein is multidomain and highly flexible. To date, however, it remains unclear how various domains interact with one another within the functional ER homodimer. Here, we show via a computational-experimental study that binding of ligand and DNA can allosterically act on the ER's domain-domain organizations and interactions. First, a set of putative conformations are identified from enabling simulations that search exhaustively all possible domain-domain interactions. Second, multiple major conformations are identified on the basis of experimental synchrotron-based measurements using SAXS and footprinting data that are best-interpreted by computational results from simulations. Finally, data from chemical cross-linking are used to verify the identified ER conformations in solution. This tight integration of multi-technique measurements provides unique insight into the function of ER that dynamically changes its conformations in response to ligand and DNA binding, both of which play critical roles in the development and progression of breast cancer.

1901-Pos Board B38

Study of Proton Transfer in Escherichia Coli Photolyase

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Photolyase is a flavoenzyme which utilizes blue-light energy to repair UV-light damaged DNA. The catalytic cofactor of photolyase, flavin adenine