compositions, thereby tying together the behavior of the mutants to a small, self-consistent set of parameters.

2080-Pos Board B96

Thermodynamic Coupling Function Analysis of Allosteric Coupling between Na^+ Release and Inward-Opening in the Human Dopamine Transporter

Michael V. LeVine, Michel A. Cuendet, Asghar M. Razavi, George Khelashvili, Harel Weinstein.
Department of Physiology and Biophysics, Weill Cornell Medical College of Cornell University, New York, NY, USA.

Allostery plays a crucial role in the mechanism of neurotransmitter-sodium symporters, such as the human dopamine transporter (hDAT). To investigate the molecular mechanism that couples transport-associated inward release of Na^+ from the Na2 site to conformational changes associated with inward-opening, we applied a novel combination of our recently developed thermodynamic coupling function (TCF) theory of allostery and Markov State Model (MSM) analysis to a 50-microsecond dataset of Molecular Dynamics trajectories of hDAT, in which multiple spontaneous Na^+ release events were observed. Our TCF approach reveals a complex landscape of thermodynamic coupling between Na^+ release and inward-opening, and identifies diverse, yet well-defined roles for different Na^+-coordinating residues. In particular, we identify a prominent role in the allosteric coupling for the Na+-coordinating residue D421, where mutation has previously been associated with neurological disorders. Our results highlight the power of the TCF theory and analysis to elucidate the molecular mechanism of complex allosteric processes in large biomolecular systems.

2081-Pos Board B97

Leveraging Cooperativity for Pocket Detection

Justin R. Porter, Gregory R. Bowman, Katelyn E. Moeder.
Biochemistry & Molecular Biophysics, Washington University in St. Louis, St Louis, MO, USA.

Cryptic pockets—transient pockets that are invisible to conventional structural techniques—are the subject of considerable interest, as they are appealing for drug design against difficult targets. A nontrivial first step is identifying the pocket, as they are by definition difficult to find. Although molecular dynamics can sample these open states, traditional pocket identification algorithms search for concavities at a protein's surface, yielding tens or hundreds of pockets per frame. Consequently, synthesizing predictions from that multitude of hits requires numerous subjective choices.

To overcome this limitation, we operationalize the notion of a pocket differently: we define a pocket to be a group of residues that demonstrate cooperative solvent exposure. Our method then only requires choices of the solvent probe size, which can be used to tune the desired pocket size, and the extent of solvent exposure that constitutes the exposed state. Using these parameters, we compute the mutual information of all pairs of amino acid sidechains' exposure/burial states and use that as input to affinity propagation clustering. The resulting clusters are pockets. Combined with the pocket definitions, we can leverage Markov state models over the featurized space to make comparisons with experiment.

This method could serve as a simple, nearly turn-key first step in a drug development pipeline targeting cryptic allosteric sites.

2082-Pos Board B98

The Rheostatic Response of Dynamic Allosteric Residue Couples (DARC) Spot Mutations

Paul Campitelli[1], Liskin Swint-Kruse[2], Banu Ozkan[1].
[1]Center for Biological Physics, Arizona State University, Tempe, AZ, USA, [2]Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS, USA.

Distinguishing causal from non-causal relationships between sequence variation and functional consequence lies central to disease prediction and presents a major challenge in biology and genomics. The need to better define the link between variation and functional impact has grown dramatically as unprecedented advances in sequencing complete exomes have yielded tens of thousands of non-synonymous single nucleotide variants (nSNVs), leading to missense variants (i.e mutations) on the human proteome. Currently, no methods consistently predict effects of missense mutations at non-conserved amino acid positions. Here, we present a method to aid in this prediction called the *dynamic coupling index* (*dci*). This technique analyzes molecular dynamics trajectories to quantify the coupling strength between selected amino acids. We have applied our approach to variants selected from a set of >1000 mutations of LacI/GalR homologues for which experimental outcomes are known. In this dataset, mutations at many non-conserved positions produce ''rheostatic'', or progressive effects on function spanning several orders of magnitude. This contrasts with mutations at evolutionarily conserved positions, which produce toggle-switch (on/off) behavior in these proteins. We show that *dci* provides insight regarding how different amino-acid substitutions at dynamic allosteric residue coupling (DARC) spots induce different dynamic responses, leading to changes in conformational dynamics of DNA binding sites. Moreover, *dci* can aid in the ability to predict a toggle site versus a rheostatic site, particularly when coupled with machine learning and evolutionary data.

2083-Pos Board B99

Dynamic Communities in Proteins: Allosteric Hotspots and Functional Modules

Sambit Kumar Mishra[1], Gaurav Kandoi[2], Robert L. Jernigan[1].
[1]Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA, USA, [2]Department of Electrical and Computer Engineering, Iowa State University, Ames, IA, USA.

Dynamic Communities in Proteins: Allosteric Hotspots and Functional Modules

Dynamic communities in proteins are cohesive units that exhibit rigid body motions. These communities can model allostery in proteins. Previous studies have shown that mutations to key community residues can hinder the transmission of allosteric signals among communities. Previously molecular dynamic simulations ($\sim$ 100 ns or longer) were used to obtain these communities - a demanding task for large multi-domain proteins. In the present study, we propose a method which uses coarse-grained Gaussian Network Model (GNM) and hierarchical clustering to obtain protein dynamics communities. We evaluate our method by comparing the communities obtained from GNM with those from MD, for a set of 45 proteins. At certain clustering levels, we observe strong correspondence between the communities from the two methods. We hypothesize that these can inform us about the number of functional communities in proteins. In another study we identified the allosteric hotspots for effector ligand binding for the same proteins with a previously trained predictive machine learning model.

2084-Pos Board B100

Transient Pocket Identification and Evaluation of their Role for Allostery

Denis Schmidt[1], Christopher Pfleger[1], Susanne M.A. Hermans[1], Markus Boehm[2], Holger Gohlke[1].
[1]Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Düsseldorf, Germany, [2]Pfizer Worldwise Research & Development, Cambridge, MA, USA.

It is widely accepted that allosteric modulators have a huge potential to overcome typical hurdles in drug design. Our group recently showed that the ''Constraint Network Analysis'' (CNA) approach is capable of identifying allosteric pathways by means of rigidity analysis. CNA hence has the potential to predict the allosteric effect of new ligands. Even in the absence of a defined binding site, allosteric regulation might be possible via so-called ''cryptic sites'' (''transient pockets''). Such cryptic sites are closed in the *apo* state due to their lipophilicity. Consequently, they are hard to identify, although they are predicted to exist in many proteins. Molecular dynamics simulations have been proposed as promising approach to sample cryptic sites in their open states. Here, we systematically investigated the influence of different organic solvents on the opening of cryptic sites during molecular dynamics simulations, starting from the *apo* state. We identified phenol as the best solvent to foster the opening of transient pockets. In five out of seven test cases, we not only measured the opening of the cryptic site but we were also able to validate the pocket conformations by redocking the crystal ligand. Unlike perturbation-based approaches, equilibrium simulations do not require an *a-priori* knowledge of the location of the binding site. After their identification, we employ molecule surrogates (''fuzzy ligands'') to fill the cryptic sites. This way, CNA is able to quantify the allosteric potential of a ligand in this binding site. The generated fuzzy ligands subsequently serve as seed for virtual screening. The cryptic site identification in combination with the ''fuzzy ligand'' approach and CNA yields a powerful workflow to prospectively identify new sites, evaluate their allosteric potential and ultimately predict new allosteric compounds.

2085-Pos Board B101

Weak Domain Stability and Higher Ca^{2+} Binding Affinity Contribute to Allostery between the D/E Linker and N-Helix of Cardiac Troponin C

Mayra A. Marques[1], Adolfo H. Moraes[2], Jerson L. Silva[1], José R. Pinto[3], Guilherme A.P. de Oliveira[1].
[1]Medical Biochemistry Institute Leopoldo de Meis, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [2]Institute of Exact Sciences, Chemistry Department, Federal University of Minas Gerais, Minas Gerais, Brazil, [3]Department of Biomedical Sciences, College of Medicine, Florida State University, Tallahassee, FL, USA.

Hypertrophic cardiomyopathy (HCM) is an inherited myopathy caused by the production of anomalous sarcomeric proteins that can lead to severe

cardiac dysfunction. Here, we used structural and biophysical approaches to better understand the pathogenesis of a cardiac troponin C (cTnC) C84Y mutation located in the D/E linker, first reported in a 17-year-old proband, presenting with left-ventricular hypertrophy. Despite the relevance of HCM disease, little is known concerning the function of the D/E linker and allosteric phenomena governing cTnC Ca^{2+} affinity. Monitored by bis-ANS fluorescence, Ca^{2+}-titrations reveal that C84Y exhibits enhanced Ca^{2+}-binding affinity in both domains and conformational changes compared to WT. Although WT and C84Y display distinct Ca^{2+}-binding behaviors, the overall dimensional values and molecular envelopes generated by small-angle-X-ray scattering data remains similar. Using circular-dichroism, C84Y revealed significantly lower thermostability in non-Ca^{2+}-bound form compared to WT. Most of our understanding of the molecular mechanisms underlying how troponin and troponin peptides switch muscle contraction ''on'' and ''off'' has been derived using experimental NMR techniques. Currently, no experimental techniques are available that allow the understanding of protein regulatory/dynamic processes at the molecular level of large, multi-domain protein complexes. To further unravel molecular changes in C84Y, three-dimensional NMR experiments were performed for backbone assignment. The largest chemical shifts were observed in N-Helix residues and at the end of D-helix and D/E linker. NMR-derived backbone amide temperature-coefficients indicate different temperature-dependent conformational changes exist between WT and C84Y Carr-Purcell-Meiboom-Gill relaxation dispersion (CPMG-RD) and R1/R2 experiments were used to probe the population and exchanging rates of C84Y compared to WT. This work sought to elucidate: main structural components underlying this pathological mutation, novel allosteric mechanisms, and the role of D/E linker in cTnC.

2086-Pos Board B102

Role of Lys Residue at Position 87 of DREAM in Allosteric Regulation of DREAM's Interactions with K_V Channel

Samiol Azam, Jaroslava Miksovska.
Florida Int University, Miami, FL, USA.

Downstream regulatory element antagonist modulator (DREAM) is a 29kDa protein, which interacts with diverse intracellular partners and is involved in many biological processes, namely, pain sensation, gene apoptosis, and modulation of Kv4 voltage channels. Previous research in our group and elsewhere demonstrated that DREAM interacts with the helix-9 of presenilin-1 (PS1HL9), the residue 2-22 (site-1) peptide of K_v4.3, and the residue 70-90 (site-2) peptide of K_v4.3 in a calcium-dependent manner. Molecular dynamics data suggests that Lys at the position 87 forms a salt bridge with Asp 165 and directly involved in the propagation of calcium-triggered structural changes between the C- terminal and N- terminal domain. To determine the impact of Lys 87 on the interdomain communication as well as to characterize its contribution to DREAM stability, Lys 87 was mutated to Ala, and the effects of the mutation on DREAM's interaction with ''PS1HL9'', ''site-1'' peptide, and ''site-2'' were determined. The results show that Trp residue in DREAM(K87A) is more solvent exposed compared with wild-type suggesting that the absence of the salt bridge destabilizes the protein structure. The emission maximum of 1,8-ANS in the presence of DREAM(K87A) is about 10 nm redshifted indicating that the mutation influences the hydrophobic cavity between the N- and C-terminal domain. The lifetime of Trp and 1,8-ANS are significantly altered in DREAM(K87A) compared with wild-type form, which in agreement with the steady-state data. Fluorescence anisotropy titration data suggest that mutation of Lys to Ala at position 87 of DREAM completely inhibit DREAM's interaction with site-2. We do plan to investigate DREAM(K87A)'s interactions with site-1 and PS1HL9. Data presented here provide insight into the role of Lys residue at position 87 of DREAM regulating DREAM's interaction with intracellular partners.

2087-Pos Board B103

Classification of Allostery in Proteins: A Deep Learning Approach

Girik Malik[1], Andrzej Kloczkowski[2].
[1]Nationwide Children's Hospital, Columbus, OH, USA, [2]Nationwide Children's Hospital and The Ohio State University, Columbus, OH, USA.

Protein fluctuational dynamics is a key element of protein functioning. One of the most interesting manifestations of protein dynamics is allostery. Binding an effector molecule at one site often results in long-range conformational changes in protein structure. Allosteric communication mechanism plays a pivotal role in the natural regulatory processes. Our recent work [1] attempted to decipher the characteristics of residues forming the Allosteric Communication Paths, by studying the annotated proteins from the AlloSteric Database (ASD) [2], belonging to four classes (kinases, nuclear receptors, peptides, transcription factors). Central aim of the study was to decipher consistent patterns inherent in the allosteric communication subsystem (ACSS). The underlying graph-theoretic approach unveiled interesting patterns in terms of individual and collective effects. Furthering our analysis in this study we tried to automate the entire process by developing an algorithm that could find some patterns in the complex networks generated earlier and classify the dataset into the four different classes presented. Using the learned graph representations we aim to classify unknown graphs, in one of the four classes using convolutional neural network. Preliminary data obtained seems encouraging and the method could further improve by the growth in the size of ASD and using other deep neural network approaches.

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2088-Pos Board B104

An Allosteric Mechanism of Abl Kinase Activation and Catalysis

Tamjeed Saleh.
Structural Biology, St Jude, Cordova, TN, USA.

c-Abl kinase plays a critical role in coordinating responses to growth factors, cytokines, cell motility, DNA damage responses and oxidative stress. Bcr-Abl, a fusion oncoprotein and the genetic basis of chronic myeloid leukemia (CML) is constitutively active and despite intense research the basis for this activation remains unclear. c-Abl activity is regulated by the N-terminal SH3 and SH2 domains working in concert to lock the kinase domain in an inhibited conformation. However, mechanistic insight into how regulated inhibition and activation is achieved is missing. Moreover, the effect of the regulatory domain on the conformation of the kinase domain has not been elucidated. Using high resolution NMR and other biophysical techniques we characterize allosteric mechanism of regulation of Abl kinase and identify a dynamic role of the regulatory module in the activation process.

2089-Pos Board B105

Allostery in NMDA Receptors

Ryan Durham, Drew Dolino, Vasanthi Jayaraman.
University of Texas Health, Houston, TX, USA.

Ionotropic glutamate receptors, of which the N-methyl-D-aspartate receptor (NMDAR) is one subtype, play a crucial role in excitatory neurotransmission within the mammalian central nervous system. In order to fully understand the role the NMDAR plays in neurotransmission, the conformational states and transitions that the receptor undergoes must be understood. Although the structures of the various conformational states of the NMDAR are known, the conformational changes by which the NMDAR transitions from state to state are not well understood. One question concerning NMDAR dynamics that remains to be answered is that of the nature of allosteric communication between the glycine binding sites and glutamate binding sites on the various subunits of the heterotetrameric receptor. In order to understand this allosteric communication, we utilized both electrophysiology and single molecule Förster Resonance Energy Transfer (smFRET) to examine the changes in the ligand binding domain (LBD) brought about by the binding of glycine and/or glutamate. The electrophysiological studies showed that there is negative cooperativity between the glycine and glutamate binding sites. The smFRET studies allowed the observation of the various states that a single receptor adopts under various ligand conditions. These single molecule experiments demonstrated that the conformational dynamics of the NMDAR LBD change when the receptor is in the presence or absence of various ligands; the NMDAR exhibits altered conformational dynamics when it is bound to glycine, glutamate, both, or neither. This data illustrates a potential mechanism for the negative allosteric effects between the glycine and glutamate binding sites that was observed in the electrophysiological studies.

2090-Pos Board B106

Allostery Advocates in Monoclonal Antibody Engineering towards Antigen Binding

Chinh Su.
Bioinformatics Institute, A*STAR, Singapore, Singapore.

Current therapeutics antibodies, such as Trastuzumab and Pertuzumab, have marked significant success in disease treatment, particularly for Her2 positive