Protein Dynamics and Allostery III

2669-Pos Board B99
Proposed Thermodynamic Basis for Synaptotagmin Response
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The synaptic vesicle protein Synaptotagmin I is the calcium ion sensor for neurotransmitter release. Synaptotagmin are comprised of C2 domains, calcium ion dependent, membrane-binding domains. They contain multiple C2 domains and all C2 domains are tethered to the membrane. Our recent work has redefined Synaptotagmin I has consisting of a series of nearly ordered domains that enable the extent of calcium ion and type of phospholipid bound to be cooperatively communicated through the protein. Through applications of thermodynamics to define free energies of stability and of binding, we find C2 domains not only from Synaptotagmin I but also in general to have a common thermodynamic signature of weak free energies of stabilities in solution and stabilities that are lipid composition dependent. This plasticity in structure is proposed to underlie the plasticity in function as responsive calcium ion sensors whose response is defined by its local membrane environment.

2670-Pos Board B100
Allosteric Functional Switch in Poliovirus 3C Protease
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Viral genomes are very efficient; they are typically compact but nevertheless encode numerous elements that are essential for regulation of both its own replication and packaging, and of the host cell’s machinery. Viruses have developed successful strategies to overcome their biological information storage problem. For example, the 3C protease from the picornavirus family of positive-strand RNA viruses is responsible for binding of RNA control sequences to regulate translation and replication, interacting with phosphoinositide lipids (PI) to regulate the maturation of virus replication organelles, and acting as the main protease to cleave host and virus proteins to further regulate host and virus processes. 3Cpro can also be found as a domain in the 3CDpro polyprotein. 3C pro by itself and 3CDpro have different protease specificities, and likely different RNA and PI binding capabilities. The domains in 3CDpro are tethered by a flexible linker and do not make specific 3Cpro-3Dpol interactions. Surprisingly, we have found that by extending the C-terminal tail of 3C with just a few amino acid residues, the RNA and PI binding properties alter dramatically. These functional changes are accompanied by changes in the structural dynamics of 3C, as measured by NMR relaxation methods. We propose that these findings have critical bearing on 3C function; proteolytic processing of the C-terminus is the switch from 3CDpro to 3Cpro (by itself) activities. Such a simple, but elegant, mechanism does not require any additional domain-domain interactions in the 3CDpro polyprotein to regulate 3Cpro function, and can help explain functional differences between 3Cpro and 3CDpro that have confounded virologists and structural biologists.

2671-Pos Board B101
The Structural Basis of ATP as an Allosteric Modulator
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1Pathophysiology, Shanghai JiaTong University, School of Medicine, Shanghai, China, 2Cancer and Inflammation Program, Leidos Biomedical Research, Inc., Frederick National Laboratory, NCI, Frederick, MD, USA. Adenosine-5’-triphosphate (ATP) is generally regarded as a substrate for energy currency and protein modification. Recent findings uncovered the allosteric function of ATP in cellular signal transduction but little is understood how ATP influences the formation and conformational changes of cellular proteases. Herein, we report the structural basis of ATP as an allosteric modulator of WW domains. We have redefined a substrate-WW binding energy surface of WW domains not only from Synaptotagmin I but also with WW domains in general to have a common thermodynamic signature of weak free energies of stabilities in solution and stabilities that are lipid composition dependent. This plasticity in structure is proposed to underlie the plasticity in function as responsive calcium ion sensors whose response is defined by its local membrane environment.

2672-Pos Board B102
Geometric Description of Dynamin Induced Membrane Fission
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The terminal step during the process of clathrin-mediated endocytosis is the scission of the connection between the nascent bud and the parent membrane. Narrowing of the neck is driven by proteins or their complexes, most prominently dynamin, which assemblies into rings and spirals that constrict the connection and are believed to generate sufficient force onto the membrane to induce fission. To advance our understanding of the underlying mechanism, in this work we present a geometric framework to study the conformation of a semi-flexible polymer adhering to, or confined by an axially symmetric membrane. The rotational symmetry of the membrane is exploited to obtain a first integral of the fourth order Euler-Lagrange equation describing the polymer equilibrium states. In particular, we examine and characterize closed and helix-like curves with right-hand chirality, lying on surfaces with the shape of a cylinder and a catenoid. For the cylindrical case, the additional translational symmetry allows to integrate the Euler-Lagrange equation once more, obtaining a quadrature. In this framework the stresses transmitted by the polymer onto the membrane are determined entirely in terms of the local geometry of the combined system of the helical-dynamin coat wrapping around the membrane neck, allowing us to analyze the force and torques involved during the constriction process.

2673-Pos Board B103
Two Pathways Mediate Inter-Domain Allosteric Regulation in P11 Jingjing Guo, Xiaodong Pang, Huan-Xiang Zhou
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Allostery is an essential means for regulating biomolecular functions and provides unique opportunities for drug design, yet our ability to elucidate allosteric mechanisms remains limited. Here, based on extensive molecular dynamics simulations, we present an atomistic picture of the pathways mediating the allosteric regulation of the PPlase domain of P11 by its WW domain. Two pathways jointly propagate the action of substrate-WW binding to produce closure and rigidity of the PPlase catalytic-site loops. One pathway preexists in the apo protein but remains dormant until substrate-WW binding completes the second. The reduction in conformational entropy and preorganization of the catalytic-site loops observed here may explain why substrate-WW binding enhances ligand affinity and catalytic activity of the PPlase domain, and suggest a combination drug therapy for P11-related diseases. Whereas the traditional view of allostery has emphasized conformational transition, our study uniquely identifies a distinct role of conformational dynamics in eliciting allostery.

2674-Pos Board B104
The Impact of Perturbing Dynamic Amino Acid Networks in a β/α/β Barrel Enzyme
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Amino acid networks describe the web of noncovalent interactions between residues spanning an enzyme. These networks may be responsible for the propagation of regulatory signals across the protein that influence conformation, binding of substrate(s), and catalysis. Trypsinophan synthase (TS), the final enzyme in the tryptophan biosynthetic pathway, is a tetramer consisting of a pair of alpha and beta heterodimers arranged in a linear conformation. Two alpha and beta subunits are connected by a 25 A˚ intramolecular tunnel that channels indole, a product from the alpha reaction, to the active site in the beta subunit. In addition to this tunnel, the conformational states of these subunits are held coordinately making TS an ideal and heavily studied model for substrate channeling and enzyme-enzyme interactions. We used nuclear magnetic resonance chemical shift covariance analysis to delineate amino acid network structures in the working state (under active catalytic turnover). The alpha subunit of TS by 25A˚ to the beta subunit of TS. The second network, the substrate-WW binding energy landscape, is only observed when substrate-WW binding of substrate-WW binding enhances ligand affinity and catalytic activity of the PPlase domain, and suggest a combination drug therapy for P11-related diseases. Whereas the traditional view of allostery has emphasized conformational transition, our study uniquely identifies a distinct role of conformational dynamics in eliciting allostery.

2675-Pos Board B105
Inhibition of Protein-Protein Interactions by Allosteric Modulators
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Amino acid networks describe the web of noncovalent interactions between residues spanning an enzyme. These networks may be responsible for the propagation of regulatory signals across the protein that influence conformation, binding of substrate(s), and catalysis. Trypsinophan synthase (TS), the final enzyme in the tryptophan biosynthetic pathway, is a tetramer consisting of a pair of alpha and beta heterodimers arranged in a linear conformation. Two alpha and beta subunits are connected by a 25 A˚ intramolecular tunnel that channels indole, a product from the alpha reaction, to the active site in the beta subunit. In addition to this tunnel, the conformational states of these subunits are held coordinately making TS an ideal and heavily studied model for substrate channeling and enzyme-enzyme interactions. We used nuclear magnetic resonance chemical shift covariance analysis to delineate amino acid network structures in the working state (under active catalytic turnover). The alpha subunit of TS by 25 A˚ to the beta subunit of TS. The second network, the substrate-WW binding energy landscape, is only observed when substrate-WW binding enhances ligand affinity and catalytic activity of the PPlase domain, and suggest a combination drug therapy for P11-related diseases. Whereas the traditional view of allostery has emphasized conformational transition, our study uniquely identifies a distinct role of conformational dynamics in eliciting allostery.