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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. Synaptotagmins 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 disordered 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 3Cpro protein 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. 3Cpro 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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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 about this critical behavior of ATP. Through extensive analysis of ATP in solution and proteins, we found that the free ATP can exist in the compact and extended conformations in solution, and the two different conformational characteristics may be responsible for ATP to exert distinct biological functions: ATP molecules adopt both compact and extended conformations in the allosteric binding sites but conserve extended conformations in the substrate binding sites. Nudged elastic band simulations unveiled the distinct dynamic processes of ATP binding to the corresponding allosteric and substrate binding sites of uridine monophosphate kinase, and suggested that in solution ATP preferentially binds to the substrate binding sites of proteins. When the ATP molecules occupy the allosteric binding sites, the allosteric trigger from ATP to fuel allosteric communication between allosteric and functional sites is stemmed mainly from the triphosphate part of ATP, with a small number from the adenine part of ATP. The detailed mechanism presented in this study may apply to other enzymes in complex with allosteric or substrate ATP molecules, and provide important insights for the molecular basis of ATP acting as a substrate and an allosteric modulator in cell signaling [Lu S, et al. PLOS Comput Biol, 2014, 10: e1003831].

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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 assembles into rings and spirals that constrict the connection and are believed to generate sufficient force onto the membrane to induce fission. To advance our undertanding 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 Pin1

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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 PPIase domain of Pin1 by its WW domain. Two pathways jointly propagate the action of substrate-WW binding to produce closure and rigidification of three PPIase 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 PPIase domain, and suggest a combination drug therapy for Pin1-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.

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The Impact of Perturbing Dynamic Amino Acid Networks in a (β/α)8 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. Tryptophan 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. The alpha and beta subunits are connected by a 25Å 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 highly coordinated 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 networks in the alpha subunit, a (β/α)8 barrel enzyme. We have shown that these observed networks change between the resting state (in the absence of substrates) and the working state (under active catalytic turnover). Furthermore, the loss of a hydrogen bond between the dynamic β2α2 and β6α6 loops in the T183V variant significantly changes these networks and the catalytic rate seen in the wild-type. These networks were perturbed by making small Ala to Gly substitutions of surface residues correlated to the catalytic Glu49. These modifications resulted in modest decreases in the catalytic rate, although they are 25 Å away from the active site. Amino acid networks are important for the function of an enzyme and may be manipulated to tune its function.