Symposium 1: Protein Modularity and Flexibility in Signal Transduction

12-Symp
Mechanisms Of Biological Regulation By Highly Dynamic Protein Complexes
Julie Forman-Kay[1,2], Tanja Mittag[1], Jennifer M. Baker[1,2], Joseph A. Marsh[1,2].
[1]Hospital for Sick Children, Toronto, ON, Canada, [2]University of Toronto, Toronto, ON, Canada.
Intrinsically disordered proteins play important roles in mediating regulatory interactions. While many of these proteins fold upon binding to targets, others appear to be only transiently and locally ordered, existing in a dynamic complex with protein partners. The interaction of the disordered Sic1 (a cyclin dependent kinase inhibitor) with Cdc4 (a component of an SCF ubiquitin ligase) involves the dynamic exchange of multiple linear binding motifs within Sic1 on and off of a single Cdc4 binding site. The disordered nature of the Sic1 chain enables Cdc4 to recognize multiply phosphorylated Sic1 by sensing a mean electrostatic field rather than only the field due to local binding of a linear motif. This sensitivity to multiple phosphorylations may facilitate a switch-like biological response of Sic1 ubiquitination and degradation to cyclin kinase activity. The regulatory (R) region of the cystic fibrosis transmembrane conductance regulator (CFTR) is another example, with transient and local ordering of multiple segments upon binding to multiple target proteins. The R region dynamic complex with the first nucleotide binding domain (NBD1) of CFTR appears to facilitate a rheostat-like function in activating CFTR channel conductance with an ability to integrate multiple regulatory binding inputs. We have measured NMR and SAXS structural data and used them as input to our program ENSEMBLE to generate sets of coordinates that represent significantly populated conformers within the isolated disordered states of Sic1 and the CFTR R region. The resulting ensembles can be docked to structures of the folded binding domains to further enable characterization of these dynamic complexes, leading to a better understanding of the role of disordered protein interactions in biological regulation.

13-Symp
The Modular Logic of Cell Signaling Systems
Wendell Lim.
UCSF, San Francisco, CA, USA.

14-Symp
Allosteric effects and regulation of signaling: the ubiquitin E3 ligases
Ruth Nussinov.
SAIC, NCI-Frederick, and Tel Aviv University, Frederick, MD, USA.
Allosteric communications are important for cellular signaling[1], including ubiquitin systems. In E3 ubiquitin ligases, substrate binding proteins, e.g. VHL-box, SOCS-box or the F-box proteins, recruit substrates for ubiquitination via conformational selection and population shift[2,3], accurately positioning and orienting the substrates for ubiquitin transfer. Yet, how does the E3 machinery precisely position the substrate? What is the role of allostery in regulation of signaling for ubiquitin or ubiquitin-like systems? To address these questions, we performed molecular dynamics simulations for different ubiquitin or ubiquitin-like systems. We simulated seven substrate binding proteins. All have two domains: one binds to the substrate; the other to E3 ligase modules Skp1/Elongin C. We found that in all cases the flexible inter-domain linker serves as a hinge rotating the substrate binding domain, optimally and accurately positioning it for ubiquitin transfer. We further observed that the linker flexibility could be regulated allosterically by binding events of either domain. For one of these substrate binding proteins, pVHL, a tumor suppressor protein which forms part of the E3 ubiquitin ligase complex and regulates the degradation of the hypoxia inducible factor, we designed five allosterically-stabilizing mutants to stabilize the inter-domain interface[4]. We proposed that drugs mimicking the mutants' allosteric effects may rescue pVHL function in the von Hippel-Lindau disease. Ubiquitin-like E3 ligases are also observed to allosterically regulate degradation. Sumoylation involves covalent attachment of SUMO (Small Ubiquitin-Like Modifier) to target proteins. The E3 ligase, RanBP2, was observed to allosterically regulate sumoylation process.
1. Tsai CJ, del Sol A, Nussinov R. J Mol Biol. **378**:1-11, 2008.
2. Ma B, Kumar S, Tsai CJ, Nussinov R. Protein Eng. Des. Sel. **12:**713-720, 1999.
3. Boehr DD, Wright PE. Science **320**:1429-30, 2008.
4. Liu J, Nussinov R. Proc Natl Acad Sci USA **105**:901-6, 2008.

15-Symp
Dynamic Origins of Interdomain Cooperativity in the Vav1 Proto-Oncoprotein
Michael K. Rosen, Pilong Li, Ilidio R.S. Martins, Gaya K. Amarasinghe, Bingke Yu, Junko Umetani.
UT Southwestern Medical Center at Dallas, Dallas, TX, USA.
Multi-domain signaling proteins exhibit complex behaviors due to cooperative interactions between domains. In many such proteins a core regulatory interaction, involving binding of an inhibitory domain to the active site of a catalytic domain, is cooperatively enhanced by additional intramolecular domain-domain contacts. However, the physical basis of this cooperativity, and thus the energetic construction of multi-domain systems, is not well understood. The five-domain N-terminal regulatory element of the Vav1 proto-oncoprotein is representative of this class of signaling molecules. The catalytic activity of the Vav1 Dbl homology (DH) domain is autoinhibited by binding of an adjacent helix into the enzyme active site. This core inhibitory process is cooperatively enhanced through an unknown mechanism by intramolecular contacts of the N-terminal calponin homology (CH) domain of the protein. Here we show by NMR spectroscopy that the isolated helix-DH module exists in equilibrium between a ground state where the active site is blocked by the inhibitory helix, and an excited state where the helix is dissociated. The rate of phosphorylation of the helix, an event that relieves autoinhibition, is linearly related to the population of the excited state. Thus, phosphorylation only occurs efficiently through the excited state, and internal dynamics are required for and control the rate of activation of the helix-DH module. In the full five-domain element this regulatory equilibrium is further biased 10-20-fold toward the closed state, implying that regulatory cooperativity derives from thermodynamic coupling between the helix-DH equilibrium and other interdomain binding equilibria in the protein. This explains the transforming activity of truncated Vav1 proteins lacking the CH domain, and suggests that Vav1 activation *in vivo* likely involves integration of kinase signals and signals to the CH or other domains of the molecule.

Symposium 2: Many Ways to Regulate a Molecular Motor

16-Symp
Force Dependent Regulation of Myosin-I
Michael Ostap.
University of Pennsylvania School of Medicine, Philadelphia, PA, USA.
Myosin-Is are the single-headed, membrane-associated members of the myosin superfamily that are found in most eukaryotic cells. They have been shown to play essential roles in membrane dynamics, cytoskeletal structure, mechanical signal-transduction, endosome processing, and possibly nuclear transcription. Unlike many other unconventional myosins, vertebrate myosin-Is are catalytically and mechanically active in the absence of post-translational modification, bound cargo or accessory proteins, or other signaling molecules. Therefore, it appears that recruitment of myosin-Is to specific cellular regions of high actin concentration is all that is required for catalytic activation. However, once activated, the kinetic activity of at least one isoform, myo1b, has been shown to be dramatically regulated by tension. Using an optical trap, we found that the rate of myo1b detachment from actin decreases >75-fold under tension of 2 piconewtons or less, resulting in myo1b transitioning from a low (<0.2) to a high (>0.9) duty-ratio motor. We are investigating the mechanism of this force regulation, and we are investigating the effects of these forces on the attachment of myosin-I to lipid membranes.

17-Symp
Structural Basis for The Regulation of Drosophila Myosin 7a
James R. Sellers[1], Yi Yang[1], Thomas Baboolal[2], Verl Siththanandan[1], Matthew L. Walker[2], Peter J. Knight[2], Michelle Peckham[2].
[1]National Heart, Lung and Blood Institute, Bethesda, MD, USA, [2]University of Leeds, Leeds, United Kingdom.
Drosophila myosin-7a is a single-headed myosin with a tail containing two MyTH4-FERM domains separated by an SH3 domain. Myosin-7a is often found in regions of high actin density and mutations in human myosin-7a result in deafness and blindness. We have previously shown that a subfragment-1 (S1) from this myosin has a Vmax of 1s-1 and a KATPase of 1μM. Full length myosin-7a has a Vmax similar to that of S1, but its KATPase is about 30μM. Negatively-stained processed images of myosin-7a in the presence of ATP show that the tail is tightly folded back against the motor domain forming a compact structure which obscures the normally easily recognized features of the motor

domain. Myosin-7a unfolds at either high ionic strength or in the absence of ATP, revealing a clearly recognizable motor domain, the lever arm and some features of the tail region. C-terminal truncations were made to determine which portions of the multi-domained tail are necessary for the regulation. Removal of the last 99 amino acids which are highly conserved in all myosin-7a molecules and form a subdomain (termed MyTH7) of the FERM domain, or mutation of two conserved amino acids in this region, is sufficient to prevent folding of the molecule in the presence of ATP and activates the enzymatic activity. A construct consisting of the second FERM domain binds actin in an ATP-insensitive manner with a Kd of 30μM which is similar to the KATPase value for the full length molecule. We propose that at low actin concentration myosin-7a is folded and inactive, but at high actin concentrations such exists in actin bundles, it binds first via its tail binding site which then frees the motor domain to functionally interact with actin.

18-Symp
The Ups and Downs of Smooth Muscle Myosin Regulation
Kenneth A. Taylor.
Florida State University, Tallahassee, FL, USA.
Since discovery that regulatory light chain (RLC) phosphorylation was the primary method of regulation of smooth and non-muscle myosin II, the structure of the phosphorylated and dephosphorylated forms has been a goal only partially realized. Regulation in both smooth muscle and the related scallop striated muscle myosin requires a 2-headed myosin species; single headed species are unregulated. Thus head to head interactions are key to achieving the inhibited state. A folded conformation of full length myosin, generally referred to as the 10S conformation was discovered early by conventional electron microscopy and in 1999, a structural explanation for the head-head interactions was obtained from 2-D arrays of dephosphorylated smooth muscle heavy meromyosin formed on lipid monolayers and imaged in 3-D by cryoEM. The structure was later confirmed by 2-D arrays of the 10S conformation of whole myosin. While this structure explained inhibition of the solubilized form of regulated myosins, it had not been observed in filaments. Surprisingly, the first observation of smooth muscle myosin-like head-head interactions in a thick filament was obtained from tarantula striated muscle, not from smooth muscle myosin. Even more surprising was the observation of a similar conformation in relaxed cardiac muscle thick filaments. Thus, these head-head interactions observed first in smooth muscle HMM, appear to be ubiquitous in relaxed muscle although still not confirmed for thick filaments in relaxed smooth muscle. Still to be determined is an explanation of the factors that can lead to solubilization and the location of the N-terminus of the RLC, whose location and structure has yet to be revealed, in the phosphorylated and dephosphorylated state. Theoretical modeling has provided possible explanations for several factors that affect regulation but has not yet yielded a coherent theory. Supported by NIAMSD.

19-Symp
Switching Gears with Myosin Binding Protein-C
Samantha P. Harris[1], Justin F. Shaffer[1,2], Kristina L. Bezold[1], Robert W. Kensler[3].
[1]University of California, Davis, Davis, CA, USA, [2]University of Washington, Seattle, WA, USA, [3]University of Puerto Rico, San Juan, PR, USA.
Myosin binding protein-C (MyBP-C) is a thick-filament protein in vertebrate sarcomeres that limits cross-bridge cycling kinetics and reduces myocyte power output. However, the mechanisms by which MyBP-C influences cross-bridge kinetics are not well understood. The goal of the present study was to investigate the ability of the first 4 N-terminal domains (C0-C1-motif-C2) of cardiac (c) MyBP-C to affect actomyosin interactions and interact with actin. Here we show that recombinant proteins containing the C1 and motif domains increased Ca^{2+} sensitivity of tension and increased rates of tension redevelopment (k_{tr}) at submaximal [Ca^{2+}] in permeabilized rat trabeculae. Proteins containing these domains also biphasically activated then inhibited Ca^{2+}-activated ATPase rates of heavy meromyosin and myosin S1 in solution. Cosedimentation binding assays demonstrated saturable binding of the 4 N-terminal domains to F-actin at a 1:1 molar ratio (Kd ~ 10μM). However, more than one interaction site was indicated by turbidity and electron microscope analyses that showed actin bundling in the presence of recombinant proteins. Phosphorylation of the motif or increasing pH reduced binding to a 1:2 molar ratio and abolished actin bundling. Phosphorylation reduced but did not eliminate effects of recombinant proteins to increase Ca^{2+} sensitivity of tension and k_{tr} at submaximal [Ca^{2+}] in permeabilized trabeculae. Together these results suggest that the N-terminus of cMyBP-C interacts with F-actin through multiple distinct sites, at least one site is modulated by electrostatic charge interactions, and that functional effects of the N-terminus of MyBP-C are mediated in part by phosphorylation independent mechanisms. Supported by NIH HL080367.

Platform A: Protein Conformation

20-Plat
Active Unfolding of Collagen is not Required for Collagenolysis to Occur in Solution
Ramon Salsas-Escat, Collin M. Stultz.
Massachusetts Institute of Technology, Cambridge, MA, USA.
A number of disorders such as tumor metastasis, arthritis, and atherosclerotic heart disease are related to excessive collagen degradation. Therefore methods that further our understanding of collagenolysis are of particular interest. However, as the collagenase active site is too small to accommodate the triple-helical structure of collagen and the scissile bond within collagen is not accessible to the collagenase catalytic site, the precise molecular mechanism of collagenolysis is unclear. Prior experiments have been interpreted to mean that collagenases actively unfold collagen, in a process that requires intact full length collagenase - which is typically a multi-domain protein, containing both a catalytic and hemopexin-like domain - leading to exposure of the scissile bond. Here we demonstrate that collagen types I and III can be degraded by the catalytic domain alone of either MMP1 or MMP8 at temperatures far below the melting temperature of collagen. These data argue that active unwinding of collagen is not required for collagenolysis to occur in solution. Molecular simulations further suggest that normal thermal fluctuations in the structure of the triple-helical structure of collagen lead to the protein sampling states where the scissile bond is relatively exposed and hence accessible to collagenase active site. Taken together, these data suggest that collagen degradation is the result of the interaction of preformed locally unfolded states of collagen and collagenases, rather than a mechanism that involves active collagenase-mediated unfolding.

21-Plat
Lipid Bilayer Coated Gold Nanoparticles Provide Insight Into Proteins' Conformational Changes
Stephan M. Woods[1], Katrice E. King[1], Avishek Kumar[2], Roberta E. Redfern[1], Alonzo Ross[3], Arne Gericke[1].
[1]Kent State University, Kent, OH, USA, [2]Carnegie Mellon, Pittsburgh, PA, USA, [3]University of Massachusetts Medical School, Worcester, MA, USA.
While nanoparticles have been studied and used for many years and their unique chemical and physical properties have been extensively characterized, nanoparticles are now increasingly used to investigate biological systems. Nanoparticles can be formed on a submicron scale and do not interfere with normal biological processes, thus can be used in an *in vivo* system, allowing researchers to gain insight into its inner workings. The use of nanoparticles has also proven to be quite flexible, and has provided an indispensable tool in the advancement of drug delivery, tissue engineering, and detection of biomolecules. We have previously studied conformational changes occuring within proteins upon binding to phospholipid model membranes, particularly the tumor suppressor protein PTEN. To gain a more in depth characterization of these protein conformation changes, we have developed a technique in which we have fabricated 50nm gold nanoparticles coated with an asymmetric lipid bilayer, in a manner that allows us to control the identity of the lipids making up the outer leaflet. Using Raman spectroscopy, we will take advantage of the surface enhanced Raman spectroscopy (SERS) effect, which will increase PTEN band intensities binding of the protein to the lipid covered nanoparticle. Systematic mutation of tryptophan residues within the protein will allow us to probe the binding induced conformational changes in the vicinity of the tryptophan. While SERS has been used previously to study protein conformational changes, proteins were in these cases directly immobilized onto the nanoparticles. In contrast, our novel approach immobilizes the lipids on the nanoparticles and the protein can freely interact with the target lipid. While we plan to use these methods to examine PTEN, further development of this technique will allow for better study of conformational changes in a myriad of interfacial enzymes.

22-Plat
Extended conformations in alanine peptides
Kang Chen[1], Zhigang Liu[1], Chunhui Zhou[1], W. Clay Bracken[2], **Neville R. Kallenbach**[1].
[1]New York University, New York, PA, USA, [2]Weill Cornell College of Medicine, New York, NY, USA.
Spectroscopic evidence for the presence of local order in unfolded proteins, including polyproline II (PII) structure, now appears incontrovertible. The data supporting this order relies on analysis of short chain peptides. The dimensions of unfolded chains nevertheless conform to random coils. We have re-examined the dimensional properties of short chains using paramagnetic proton spin relaxation measurements to evaluate intermediate range distances (r)