# 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