Microsymposia

MS.22.1

Evolution and dynamics of protein complexes
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There is an abundance of data on protein interactions and protein complexes, both from conventional small-scale experiments collected over the decades, including three-dimensional structures, and more recently by large-scale functional genomics experiments. We can draw on this information to ask whether (i) non-functional protein interactions constrain protein sequences and (ii) whether protein structures harbor information about conformational changes upon binding to each other.

In order to answer the first question, we project evolutionary and systems information onto 397, 196, and 701 proteins of known structure from E. coli, S. cerevisiae and H. sapiens respectively. We find that the propensity of proteins to interact in a non-specific manner with other proteins is inversely correlated with their abundance in E. coli and S. cerevisiae. This tendency is most pronounced at surface residues, suggesting that high abundance proteins have evolved to have a less sticky surface. In E. coli and S. cerevisiae, we also find that the evolutionary conservation of an amino acid is positively correlated with the stickiness of the surface environment around it. Thus, residues in sticky surface patches are evolutionarily more constrained, possibly because they are more likely to trigger non-functional interactions if they mutate. Although significant, the impact of protein stickiness is comparatively small in shaping the physico-chemical properties and evolution of H. sapiens proteins. This suggests that promiscuous protein-protein interactions are freer to accumulate in species with a small effective population size; a phenomenon akin to junk DNA accumulation.

While non-functional interactions shape protein sequence and structure, functional protein interactions require not just sequence but also structural complementarity, which often involves conformational changes. We have analyzed the relationships between the structures of proteins and the conformational changes that they undergo upon binding.

We find that the relative solvent accessible surface area of both free and bound subunits can be used to predict the magnitude of binding-induced conformational changes. We demonstrate that the relative solvent accessible surface area of monomeric proteins is useful as a simple proxy for intrinsic flexibility and for predicting conformational changes upon binding. In addition to the predictive power of this correlation, it reveals a strong connection between the flexibility of unbound proteins and their binding-induced conformational changes, consistent with the conformational selection model of molecular recognition.

Keywords: bioinformatics, protein complexes, protein interactions

MS.22.2

Structural insight into the regulation of AMP-activated protein kinase
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The energy sensor AMP-activated protein kinase (AMPK) plays a central role in regulating cellular metabolism and energy homeostasis and is thus a major drug target for obesity, type 2 diabetes and related metabolic disorders. AMPK is a heterotrimeric enzyme composed of catalytic α-subunit and regulatory β- and γ-subunits and characterized by its ability to undergo allosteric activation upon AMP binding to the γ-subunit. However, the molecular basis for this allosteric regulation remains unclear.

The catalytic α-subunit contains an N-terminal Ser/Thr kinase domain (KD) and an autoinhibitory domain (AID). We determined the crystal structures of an unphosphorylated fragment of the AMPK α-subunit (KD-AID) from S. pombe and of a phosphorylated kinase domain from S. cerevisiae (Snf1-pKD). Structural analyses indicate that AID binds, from the ‘backside’, to the hinge region of its kinase domain and constrains the mobility of helix αC, hence resulting in an autoinhibited KD-AID with much lower kinase activity than that of the kinase domain alone. Further in vitro kinetic studies demonstrate that disruption of the KD-AID interface reverses the autoinhibition and these AMPK heterotrimeric mutants no longer respond to the change in AMP concentration.

Structural studies on AMPK core provide information on the heterotrimer formation, but the competitive binding of ATP or AMP results in little, if any, conformational changes. In contrast to previous results, our co-crystallized core structures of mammalian AMPK, in complex with either two ATP or three AMP, exhibit different nucleotide/protein stoichiometries and significant conformational differences on the γ-subunit. We set up a minimal kinetic model for AMPK regulation with two functional nucleotide binding sites and demonstrated that the predominant regulatory site on the γ-subunit binds AMP 125-fold more tightly than ATP. Together, our structural and biochemical data have shown the primary mechanism of AMPK autoinhibition and provide a relatively comprehensive view for its allosteric regulation by AMP/ATP exchange.

Keywords: AMP-activated protein kinase, autoinhibition, allosteric activation

MS.22.3

A pseudokinase mediates cell wall integrity in Mycobacterium Tuberculosis
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Prokaryotic cell wall synthesis is a dynamic process that is continuously coordinated with cell growth and division, but the mechanisms by which this process is regulated remain obscure. We define a phosphosignalling pathway in Mycobacterium tuberculosis (Mtb) that controls peptidoglycan (PG) biosynthesis in response to PG substructures. The eukaryotic-like Ser/Thr protein kinase (STPK) PknB initiates the signal, phosphorylating the putative flipase for PG precursors, FlpA. The phosphorylation site borders a novel, deeply diverged pseudokinase domain that adopts the kinase fold and forms a back-to-back, N-lobe dimer characteristic of bacterial receptor STPKs. Biochemical and structural studies show that the pseudokinase domain fails to bind ATP, lacks an ATP binding site, and has lost substrate-binding and catalytic motifs. Importantly, the phosphorylated FlpA pseudokinase binds a Forkhead Associated (FHA) domain protein, FhaA, in vitro and in vivo. The crystal structure of the pseudokinase: FHA domain complex reveals unanticipated three-dimensional contacts that augment current models of FHA domain recognition. Knocking down the essential FlpA flipase and FhaA regulator in vivo using a regulated proteolysis tag reveals that these proteins are crucial for cell-wall integrity, cell growth and normal cell morphology. Overall, our studies define a pseudokinase and FHA-domain interaction that mediates an essential bacterial STPK signaling network.

Keywords: Pseudokinase, FHA domain, kinase signalling


Structural basis for vitamin B12 uptake
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Vitamin B12 is a bacterial organic compound and an essential coenzyme in mammals, which take it up from the diet. This occurs by the combined action of the gastric intrinsic factor (IF) and the ileal vitamin-binding CUB domain and the low-density lipoprotein (LDL) receptor is obstructed by DCC binding. Our results reveal the structural basis of cargo recognition by the myosin-X MyTH4-FERM domain.

One of the most exciting processes involving myosin-X relates to the axon path-finding of neurons, which is essential for proper wiring in the brain. During neural development, axons are navigated by extracellular guidance cues such as those provided by netrins. Deleted in colorectal cancer (DCC) and neogenin are membrane proteins that function as netrin receptors. Myosin-X recognizes these receptors as cargos and redistributes to the cell periphery or to the tips of neurites, where growth cones dynamically develop filopodia.

In addition to mediating the biological function of selective cargo transportation on actin cables, myosin-X directly interacts with microtubules and plays a key role in spindle assembly during meiosis to ensure faithful delivery of replicated chromosomes to daughter cells following cell division. This surprising myosin-X function is mediated by a direct interaction between microtubules and the MyTH4-FERM cassette. However, the manner by which myosin-X recognizes microtubules has remained unclear.

Here, we report on a series of structural and biochemical/biophysical studies concerning DCC recognition by the myosin-X MyTH4-FERM cassette. We reveal the presence of a VHS-like fold within the MyTH4 domain. Our 1.9 Å-resolution structure clarifies details of an unexpected binding mode of DCC to the myosin-X FERM domain which is distinct from those found in the FERM domain of radixin that links membrane protein/plasma membrane and actin cytoskeletons. We also show that the cassette binds the C-terminal acidic tails of tubulins and that this binding is obstructed by DCC binding. Our results reveal the structural mechanism that underlies cargo recognition by the cassette and provide the molecular basis for further structural and functional investigations of biologically and medically important myosin-X, as well as of the related unconventional myosins containing MyTH4-FERM cassette.

Keywords: biomacromolecule, molecular recognition, X-ray diffraction


Structural basis of cargo recognition by the myosin-X MyTH4-FERM domain
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A large number of unconventional myosins appeared early in eukaryotic evolution and these play vital roles in diverse cellular processes including intracellular transport, organization of F-actin, mitotic spindle regulation and gene transcription. Myosins consist of three distinct regions, a head, neck and tail. The heads contain actin-based motor domains that display homology among different myosins and these structures have been extensively studied. In contrast to the tail, the heads display diversity by combination of a variety of functional domains that mediate cargo recognition, which determine the individual cellular functions of myosins. However, little is known about the tail domain structures and their specific cargo recognition.

Myosin-X is an unconventional myosin implicated in elongation of filopodia, which function as tentacles that explore and interact with cell surroundings to determine the direction of cell movement and to establish cell adhesion such as in the case of synapses. The manner by which myosin-X discriminates between cargos for transportation to the tip and how these cargos contribute to filopodial processes at the tip remains unknown. Myosin-X contains myosin tail homology 4 (MyTH4) and 4.1 and ezrin/radixin/moesin (FERM) domains for cargo recognition.

One of the most exciting processes involving myosin-X relates to the axon path-finding of neurons, which is essential for proper wiring in the brain. During neural development, axons are navigated by extracellular guidance cues such as those provided by netrins. Deleted in colorectal cancer (DCC) and neogenin are membrane proteins that function as netrin receptors. Myosin-X recognizes these receptors as cargos and redistributes to the cell periphery or to the tips of neurites, where growth cones dynamically develop filopodia.

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