MS43 EXTRACELLULAR PROTEINS AND CELLULAR ADHESION
Chairpersons: Adrian Goldman, Sthanam Narayana

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Integrins, Focal Adhesions and all That
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The integrin family of cell adhesion molecules provide a mechanical link between the extracellular matrix (ECM) and the cytoskeleton, and form the nuclei of structural and signaling complexes that regulate cell migration, proliferation and survival, typically in concert with receptors for soluble ligands. Integrins are initially activated by intracellular ("inside-out") signals; following ligation to the ECM, "outside-in" signals lead to reorganization of the cytoskeleton and activation of intracellular signal transduction pathways. Recent studies have demonstrated a critical role for the cytoskeletal protein talin, which binds to the integrin β subunit cytoplasmic tail, disrupting αβ tail association and promoting a conformational change in the extracellular domains that leads to enhanced affinity for ECM proteins, and the subsequent clustering of integrins on the cell surface. So what activates talin? Recent evidence points to a prominent role for the enzyme, phosphatidylinositol phosphate kinase type 1-γ (PIPKIγ), which forms complexes with both Src and talin at focal adhesions. PIPKIγ synthesizes the lipid phosphatidylinositol 4,5 bisphosphate ($PtdIns(4,5)P_2$), a key activator of proteins involved in focal adhesion assembly, including talin and vinculin. Our latest structural studies on integrin, PIPKIγ, talin and vinculin and their complexes, will be discussed in this context.
Keywords: cell adhesion, integrins, intracellurar signals

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The Chaperone-usher Pathway of Pilus Biogenesis: Structural Basis of the Assembly Process and of Host Recognition
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Pili are cell surface organelles and essential virulence factors responsible for host recognition by gram-negative bacterial pathogens. Type P and 1 pili of uropathogenic *Escherichia coli* target bacteria to the kidney and bladder, respectively, through their specific interaction with host surface receptors. They are assembled by the chaperone-usher pathway of pilus biogenesis, which involves a chaperone which ferries pilus subunits through the periplasm and an usher which forms the site of assembly at the outer-membrane. Structural biology work in my lab has characterized the interactions between chaperone and subunits and shown that pilus subunits have truncated Ig fold where the 7^{th} strand is entirely missing. The chaperone "donates" one of its strands to complement the truncated Ig fold of the subunits. At the usher, the donated strand is substituted with the N-terminus extension of the subunit coming next in the assembly process. This substitution process is termed "donor-strand exchange". Recent biochemical work has provided details of the donor-strand exchange reaction and shown that it proceeds via a zippering mechanism. Finally, the interactions of the pilus with the host receptor have been characterized. This work provides fundamental insight into the first event in a bacterial infection i.e. host recognition/attachment and provides the basis for designing novel antibiotics targeting specifically virulence factors.
Keywords: pilus biogenesis, host recognition, bacterial infection

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Structural Relays in Adhesion Signaling
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The formation of adherens junctions or focal adhesions relies on the interactions of the cytoskeletal proteins talin or α-actinin with vinculin, which binds to actin. Vinculin contains a head (Vh1) domain that interacts in an intramolecular fashion with its tail (Vt) domain, and this interaction clasps vinculin in its inactive state [1]. The signal(s) that disrupt the Vh1-Vt interaction to activate vinculin were unknown. Surprisingly, our crystal structures of full-length, inactive vinculin [1], and of the vinculin:talin [2,3] and vinculin:α-actinin [4] complexes, and our biochemical and biological studies, have revealed that talin and α-actinin trigger vinculin activation. Specifically, talin's and α-actinin's vinculin binding sites (VBSs) activate vinculin by displacing Vt from a distance, by provoking a totally new alteration in protein structure coined helical bundle conversion [2]. Strikingly, our structure of α-actinin's VBS (αVBS) in complex with vinculin established that this VBS must first unravel to bind and activate vinculin. αVBS then binds to vinculin's Vh1 domain in an inverted orientation compared to talin's VBSs, and provokes unique changes in the conformation of full-length vinculin, opening up far-distant regions in the molecule [4]. Collectively, these findings suggest that adhesion signaling involves a chain reaction of structural signals that is triggered by α-actinin and talin, which then activate vinculin.

[1] Borgon R.A., *et al.*, *Structure*, 2004, **12**, 1189. [2] Izard T., *et al.*, *Nature*, 2004, **427**, 171. [3] Izard T., Vonrhein C., *J. Biol. Chem.*, 2004, **279**, 27667. [4] Bois P.R.J., *et al.*, *Mol. Cell. Biol.*, *in press.*
Keywords: vinculin, talin, adhesion junctions

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Complementing Pathogens or Structural Insights into Pathogen Evasion of the Complement System
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The innate immune system is the body's first line of defense against infection acting to destroy and remove anything perceived as foreign. To cause prolonged disease a pathogen must evade this defense. We are using structural methods to study pathogen systems which act to evade innate immunity in a variety of ways (i) the complement regulator acquiring surface proteins [1] of *Borrelia burgdorferi* (ii) complement regulatory proteins secreted in the saliva of soft ticks and (iii) the type three secretion system of *Shigella flexneri* [2] used to facilitate entry of the bacterium into host cells, so hiding it from the immune system. Recent data will be presented.

[1] Cordes F., Roversi P., et al., *Nat. Struct. Molec. Biol*., 2005, doi: 10.1038/nsmb902. [2] Cordes F., et al., *J. Biol. Chem*., 2003, **278**, 17103-7.
Keywords: complement regulation, lyme disease, *shigella*

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Factor XI Structure reveals a Novel Receptor Mediated Activation Pathway
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Factor XI (FXI) is an essential component to normal blood hemostasis and inherited deficiency is associated with excessive bleeding complications after surgery or trauma. FXI functions to cleave factor IX within the intrinsic coagulation pathway. The FXI protease is activated through a unique mechanism of binding to the leucine rich repeat receptor Glycoprotein Ib (GpIb) on the surface of platelets. It is then cleaved by thrombin also bound to a different region of the GpIb receptor.

We have crystallised the intact recombinant FXI zymogen and determined the structure to 3Å resolution. Each FXI monomer has four homologous subunits called apple domains (designated A1, A2, A3, and A4, from the N terminus) which mediate protein-protein interactions. At the C-terminus there is a serine protease with a typical catalytic triad. The structure reveals a remarkable "flying saucer" quaternary arrangement with the four apple domains forming a ring