FA1-MS11-T01

Structure of the LKB1 tumour suppressor. Daan van Aalten. *College of Life Sciences, University of Dundee.*
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The LKB1 tumor suppressor kinase regulates activity of the AMPK family of kinases. LKB1 is activaty is regulated by the pseudokinase STRADa and the scaffolding protein MO25a through an unknown, phosphorylation-independent, mechanism. Here we describe the 2.65 Å structure of the heterotrimeric LKB1/STRADa/MO25a complex, revealing an unusual allosteric mechanism of LKB1 activation. STRADa adopts a closed conformation typical of active protein kinases, and binds LKB1 as a pseudosubstrate. STRADa binding, promotes the active conformation of LKB1, which is further stabilized by MO25a interacting with the LKB1 activation loop. This represents a previously undescribed mechanism of kinase activation that may be relevant to understanding the evolution of other pseudokinases. The structure also reveals how mutations found in Peutz-Jeghers syndrome and other cancers impair LKB1 function.
The body text font is Times New Roman (size 9) with single spacing and full justification. The total allowed area is 8 x 24 cm.

Keywords: tumour suppressor, kinase, cancer

FA1-MS11-T02

Angiotensinogen adjusts its shape to complex with renin and modulate blood pressure Aiwu Zhou[a], Robin W Carrell[b], Michael P Murphy[c], Zhenquan Wei[a], Yahui Yan[a], Peter L.D. Stanley[1], Penelope E Stein[b], Randy J Read[a]. [a] *Departments of Haematology and* [b]*Medicine, Cambridge Institute for Medical Research,* [c]*University of Cambridge, and MRC Mitochondrial Biology Unit*[c]*, Hills Rd, Cambridge, CB2 0XY, UK.*
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Blood pressure is critically controlled by angiotensins, vasopressor peptides specifically released by the enzyme renin from the tail of angiotensinogen, an inert member of the serpin family of protease inhibitors. Although angiotensinogen has long been regarded as a passive substrate, the crystal structures solved here to 2.1Å resolution show that the angiotensin cleavage site is inaccessibly buried in its amino-terminal tail. The co-ordinated conformational rearrangement that makes this site accessible for proteolysis and hence initiates the processes controlling blood pressure is revealed in a 4.4Å structure of the complex of human angiotensinogen with renin. The binding of renin is seen to displace a peptide loop in the body of angiotensinogen accompanied by a shift of the terminal tail of the angiotensinogen into the active cleft of renin, the two movements being linked by a labile disulphide bridge. We show that the oxidised sulphydryl-bridged form of angiotensinogen circulates in a near 1:1 ratio with a reduced unbridged form, which interacts less effectively with renin to release angiotensin. We propose that this reversible transition of angiotensinogen from the reduced to a more active oxidised form is a newly identified modulating mechanism at the commencement of the processes raising blood pressure in man. The demonstration that the transition is responsive to redox changes and potentially influenced by nitrosylation has relevance to the known, but ill-understood, association of oxidative stress with the onset of hypertension and in particular with the hypertensive crises of late pregnancy (pre-eclampsia).

Keywords: hypertension, angiotensin, renin

FA1-MS11-T03

Chemokine Binding Protein from Orf Virus Modulates Immune Function- a new twist on an old motif. Kurt L. Krause[a], Rafael Counago[a] Stephen Fleming[b], Andy Mercer[b], [a]*Biochemistry, University of Otago, Dunedin. New Zealand.* [b]*Microbiology and Immunology, University of Otago, Dunedin, New Zealand.*
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Chemokine binding proteins (CBPs) are viral proteins that modulate inflammation by interfering with host chemokine signaling. CBPs bind to their cognate partner with picomolar affinity via an extended beta sandwich structure. Here we describe the structure of a new class of CBP from the parapoxvirus, Orf virus. The crystals of this protein were challenging to produce and optimized significantly through the use of somewhat surprising additives. Crystals occupy Space Group $P6_522$ with unit cell parameters of a = b = 75.62, c = 282.49 Å,α = 90, β = 90, γ = 120 ^{o}. The structure was phased using MAD methodologies and currently the 2.1Å structure is undergoing refinement. Early analysis indicates that it is a member of the β-sandwich family but it is quite distinct from other family members when superimposed. Additionally the crystal structure is consistent with a physiologic dimer and displays a very broad β sheet on its surface containing contributions from more than 10 β strands. The dimeric nature of this CBP appears to be a unique property of its class and may be key in explaining how it is able to bind different chemokines from at least two distinct chemokine classes.

Keywords: protein crystallography, chemokine , virology

FA1-MS11-T04

Structural basis for CRM1 nuclear export complex assembly. Thomas Monecke[a], Thomas Güttler[b], Piotr Neumann[a], Nicole Doelker[c], Clement Blanchet[d], Achim Dickmanns[a], Dirk Görlich[b], Helmut Grubmüller[c], Dmitri Svergun[d], Ralf Ficner[a]. [a]*Abteilung für Molekulare Strukturbiologie, Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany.* [b]*Abteilung Zelluläre Logistik, Max-Planck-Institut für biophysikalische Chemie, Am Fassberg 11, 37077 Göttingen, Germany.* [c]*Abteilung für theoretische und computergestützte Biophysik, Max-Planck-Institut für biophysikalische Chemie, Am Fassberg 11, 37077, Göttingen, Germany.* [d]*European Molecular Biology Laboratory (EMBL), Hamburg Outstation, Notkestraße 85, 22603 Hamburg, Germany.*
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Nuclear transport is essential for eukaryotic life. It proceeds through nuclear pore complexes (NPCs), which are embedded in the nuclear envelope (NE). Most nuclear transport pathways are mediated by importin β-type transport receptors, which include nuclear export receptors (exportins), as well as importins. In nuclear export, typically, a ternary complex is formed in the nucleus, consisting of the exportin, the cargo and the molecular switch RanGTP. This complex traverses the nuclear pore and is disassembled in the cytoplasm upon hydrolosis of the Ran-bound GTP molecule and the action of additional factors. CRM1 (exportin1 in yeast) is probably the most versatile exportin as it exports a huge variety of different RNAs, RNPs and proteins. Potential cargoes of CRM1 contain a so-called "leucine-rich" nuclear export signal (NES) that confers CRM1-mediated nuclear export. How CRM1 is able to recognize this wide range of different NESs has been unknown so far. Here, we present the 2.5 Å crystal structure of a CRM1·RanGTP·cargo ternary complex [1]. CRM1 exhibits an overall toroid-like structure that engulfs the Ran molecule and binds the cargo, snurportin1 (SPN1), through its outer surface. Three parts of SPN1 contact CRM1: The N-terminal α-helix, resembling a canonical nuclear export signal (NES), the m_3G-cap binding domain and the C-terminal tail. The structure shows how CRM1 can specifically return the RNA-free form of SPN1 to the cytoplasm and suggests that RanGTP promotes cargo-binding to CRM1 solely through long-range conformational changes in the exportin. Additionally, SAXS-experiments in combination with molecular dynamic simulations were performed to shed light on the structure of apo-CRM1. Unexpectedly and in contrast to other known nuclear transport receptors, the overall superhelical conformation of CRM1 does not change significantly when Ran and cargo are released.

[1] Monecke T., Güttler T., Neumann P., Dickmanns A., Görlich D., Ficner R., *Science*, 2009, 324, 1087.

Keywords: nuclear transport, nuclear export receptor, macromolecular complexes

FA1-MS11-T05

Structure and biochemical analysis of the heparin-induced E1 dimer of the amyloid precursor protein APP. Manuel E. Than, Sven O. Dahms, Sandra Hoefgen, Dirk Roeser. *Leibniz Institute for Age Research – Fritz Lipmann Institute (FLI), Protein Crystallography Group, Jena, Germany.*
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The amyloid precursor protein (APP) is the key player in Alzheimer's disease pathology, yet APP and its analogues are also essential for neuronal development and cell homeostasis in mammals. We have determined the crystal structure of the entire N-terminal APP-E1 domain consisting of the growth factor like and the copper binding domains at 2.7 Å resolution and show for the first time that E1 functions as a rigid functional entity. The two subdomains interact tightly in a pH dependent manner via an evolutionarily conserved interface area. Two E1-entities dimerize upon their interaction with heparin, requiring eight to twelve sugar rings to form the heparin-bridged APP-E1-dimer in an endothermic and pH dependent process that is characterized by a low micromolar dissociation constant. Limited proteolysis confirms that the heparin-bridged E1 dimers obtained in solution correspond to a dimer contact in our crystal, enabling us to model this heparin-$[APP\ E1]_2$ complex. Correspondingly, the APP based signal transduction, cell-cell- and/or cell-ECM interaction should depend on dimerization induced by heparin, as well as on pH, arguing that APP could fulfill different functions depending on its (sub)cellular localization.

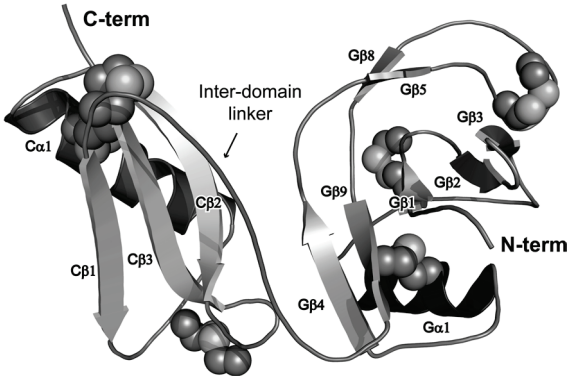

Fig. 1: Cartoon representation of the APP E1 domain, depicting the GFLD (right) and the CuBD (left). Secondary structure elements are labeled using the prefixes 'G' for the GFLD and 'C' for the CuBD.

[1] Dahms, S. O., Hoefgen, S., Roeser, D., Schlott, B., Gührs, K.-H. & Than, M. E. *Proc. Natl. Acad. Sci.* 2010, 107, 5381-5386.

Keywords: Domain-domain-interaction, Alzheimer's Disease (AD), Crystal structure