MS57 LARGE PROTEIN ASSEMBLIES - FORMING AND ANALYSING COMPLEXES
Chairpersons: Kunio Miki, David Stuart

MS57.28.1
Acta Cryst. (2005). A**61**, C75
Intermediate Filaments: from the Elementary Dimer Structure to the Complete Filament Architecture
Sergei V. Strelkov[a], Larisa Kapinos[a], Sergei Ivaninskii[a] , Laurent Kreplak[a], Harald Herrmann[b], Ueli Aebi[a], *[a]Mueller Institute for Structural Biology, Biozentrum, University of Basel, Switzerland. [b]Division of Cell Biology, German Cancer Research Center, Heidelberg, Germany*. E-mail: sergei-v.strelkov@unibas.ch

Together with microtubules and microfilaments, the ~11 nm wide intermediate filaments (IFs) constitute the interconnected, dynamic cytoplasmatic network critically involved in cell division, motility and plasticity. While the structures of microtubules and microfilaments are known at atomic detail, IF architecture is presently much less understood. The elementary 'building block' of all IFs is a rod-shaped alpha-helical coiled-coil dimer flanked at either side the 'head' and 'tail' domains.

By introducing a 'divide-and-conquer' approach, we have determined the X-ray crystallographic structures of a series of human vimentin fragments. As the result, an atomic model of the full IF dimer could be proposed. In addition, we are working on the atomic structure of the nuclear IF protein lamin, including crystallographic studies of individual lamin fragments and complexes thereof. We show that the specific head-to-tail association of lamin dimers during filament assembly is likely to be driven by electrostatic attraction. Futhermore, we are investigating the structural effect of mutations in IF proteins that have been associated with human disease such as myopathies, skin and neuronal diseases. Towards this goal, we combine X-ray crystallography with other methods such as electron and atomic force microscopies and solution small-angle X-ray scattering.
Keywords: intermediate filaments, macromolecular assemblies, human disease

MS57.28.2
Acta Cryst. (2005). A**61**, C75
Structural Basis of Actin Filament Nucleation and Processive Capping by a Formin Homology 2 Domain
Diana R. Tomchick, Takanori Otomo, Chinatsu Otomo, Sanjay C. Panchal, Mischa Machius, Michael K. Rosen, *Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, TX 75390, USA*. E-mail: diana.tomchick@utsouthwestern.edu

The conserved Formin Homology 2 (FH2) domain nucleates actin filaments and caps the filament barbed end in a manner that allows actin monomer addition and loss. Here we report the crystal structure of the Bni1p FH2 domain in complex with tetramethylrhodamine-actin. Each half of the FH2 dimer binds two actins in an orientation that approximates a short-pitch actin dimer, suggesting this structure could function as a template for growth of a new filament. Biochemical properties of heterodimeric FH2 mutants suggest the wild type protein equilibrates between two bound states at the filament barbed end that differentially permit monomer binding and dissociation. Interconversion between these states allows barbed end polymerization and depolymerization in the presence of bound FH2 domain. Kinetic and/or thermodynamic differences in the conformational and binding equilibria can explain the variable activity of different FH2 domains, and the effects of profilin-mediated recruitment of actin on FH2 function.
Keywords: actin filament nucleation, actin binding protein, binding equilibria

MS57.28.3
Acta Cryst. (2005). A**61**, C75
Active Site Coupling in Multienzyme Complexes
René Frank, Christopher Titman, Venkatesh Pratap, Richard Perham, Ben Luisi, *Department of Biochemistry, University of Cambridge.* E-mail: rawf2@mole.bio.cam.ac.uk

We present here the first crystal structure of a complex between pyruvate decarboxylase (E1) and the peripheral subunit-binding domain (PSBD) of the acetyltransferase (E2), which interact within the pyruvate dehydrogenase (PDH) multienzyme complex.

Remarkably, the PSBD uses essentially the same surface to recognize alternately the third component of the PDH assembly, namelyE3. The PSBD achieves this dual recognition largely through the addition of a network of interfacial water molecules unique to the E1-PSBD complex. These structural comparisons illuminate our observations that the formation of the water-rich interface in the E1-E2 complex is largely enthalpy-driven, whereas that of the E3-PSBD complex (from which bound water is excluded) is entropy-driven.

E1 is a thiamine diphosphate (ThDP)-dependent enzyme composed of a dimer of active sites. We present evidence that the ThDPs in the two active sites of the E1 communicate over a distance of 20 Å by reversibly shuttling a proton through an acidic tunnel in the protein [1]. This ‘‘proton wire’’ permits the cofactors to serve reciprocally as general acid/base in catalysis, which synchronizes the progression of chemical events and can account for the oligomeric organization, conformational asymmetry, and ‘‘ping-pong’’ kinetic properties of E1 and other ThDP-dependent enzymes.

[1] Frank R. A. W., Titman C. M., Pratap V., Luisi B. F., Perham R. N., *Science*, 2004, **306**, 872.
Keywords: multienzyme complex, active site coupling, thiamine diphosphate

MS57.28.4
Acta Cryst. (2005). A**61**, C75
The Structure of the RC-LH1 ‘Core’ Complex from *Rhodopsuedomonas palustris*
Richard J. Cogdell[a], Alistair T. Gardiner[a], June Southall[a], Christopher J. Law[a], Tina D. Howard[a], Aleks W.Roszak[b], Neil W. Isaacs[b], *[a]Division of Biochemistry and Molecular Biologyand, University of Glasgow, Glasgow G12 8QQ, UK. [b]Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, UK.* E-mail: R.Cogdell@bio.gla.ac.uk

The photosynthetic unit (PSU) of most purple bacteria is composed of two types of integral membrane pigment-protein complexes, called LH1 and the RC(reaction centre)-LH1 ‘core’ complex. Light energy absorbed by the LH2 complexes is transferred to the LH1 complex. The LH1 complex, which surrounds the RC, then passes the energy on to the RC where it is used to initiate photosynthetic electron transport.

The x-ray crystal structure of the RC-LH1 ‘core’ complex from *Rps. Palustris* has been determined at an intermeadiate resolution of 4.8 angstroms,[1]. The details of this structure will be described and it will be compared with models of the ‘core’ structure proposed from EM and AFM.

This work has been supported by grants from the BBSRC , the Wellcome Trust and NEDO.

[1] Roszak A.W., Howard T.D., Southall J., Gardiner A.T., Law C.J., Isaacs N.W., Cogdell R.J., *Science*, 2003, **302** , 1969.
Keywords: photosynthesis, membrane proteins, light-harvesting

MS57.28.5
Acta Cryst. (2005). A**61**, C75-C76
3D Rearrangement of Proteins in the Tail of Bacteriophage T4 on Infection of its Host
Michael G. Rossmann[a], Petr G. Leiman[a], Paul R. Chipman[a], Victor A. Kostyuchenko[a,b], Shuji Kanamaru [c], Fumio Arisaka [c], Vadim V. Mesyanzhinov[b], *[a]Dept. of Biological Sciences, Purdue University, 915 W. State Street, West Lafayette, IN 47907, USA. [b]Laboratory of Molecular Bioengineering, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, 16/10 Miklukho-Maklaya Street, 117997 Moscow, Russia. [c]Graduate School & School of Bioscience and*