

MS57 LARGE PROTEIN ASSEMBLIES - FORMING AND ANALYSING COMPLEXES**Chairpersons:** Kunio Miki, David Stuart**MS57.28.1***Acta Cryst.* (2005). A61, C75**Intermediate Filaments: from the Elementary Dimer Structure to the Complete Filament Architecture**

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Together with microtubules and microfilaments, the ~11 nm wide intermediate filaments (IFs) constitute the interconnected, dynamic cytoplasmic 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. Furthermore, 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). A61, C75**Structural Basis of Actin Filament Nucleation and Processive Capping by a Formin Homology 2 Domain**

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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). A61, C75**Active Site Coupling in Multienzyme Complexes**

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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, namely E3. 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). A61, C75**The Structure of the RC-LH1 'Core' Complex from *Rhodospseudomonas palustris***

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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 intermediate 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.

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[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). A61, C75-C76**3D Rearrangement of Proteins in the Tail of Bacteriophage T4 on Infection of its Host**

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