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

MS57.28.1
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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 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

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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

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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, 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

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The Structure of the RC-LH1 ‘Core’ Complex from *Rhodopsuedomonas 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 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

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3D Rearrangement of Proteins in the Tail of Bacteriophage T4 on Infection of its Host
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The contractile tail of bacteriophage T4 undergoes major structural transitions when the virus attaches to the host cell surface. The baseplate at the distal end of the tail changes from a hexagonal to a star shape. This causes the sheath around the tail tube to contract and the tail tube to protrude from the baseplate and pierce the outer cell membrane and the cell wall, before reaching the inner cell membrane for subsequent viral DNA injection. Analogously, the T4 tail can be contracted by treatment with 3 M urea. The structure of the T4 contracted tail, including the head-tail joining region, has been determined by cryo-electron microscopy to 17 Å resolution. This 1200 Å-long, 20 MDa structure has been interpreted in terms of multiple copies of its approximately 20 component proteins. A comparison with the metastable hexagonal baseplate of the mature virus shows that the baseplate proteins move as rigid bodies relative to each other during the structural change.

Keywords: bacteriophage T4, infection, conformational changes

MS58 CRYSTALLOGRAPHY AND UNDERSTANDING OF CULTURAL HERITAGE

Chairpersons: Salvatore Siano, Henk Schenk

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An Archaeometric Study of Lead-White Pigment and its Production using Neutron Diffraction

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In antiquity up to very recent times lead-white pigment was widely used. However the structures of its phases as well as the interaction of the lead-white pigment with oil are not well known. In this paper we look at the different production processes of the lead-white through the ages by means of historically based reconstructions (the Dutch stack method, the German and French production processes). The products involved in the different stages of the lead white production process are characterised by means of Rietveld phase analysis of neutron diffraction data. The interaction of linseed oil, produced according to historical recipes, with the lead-white pigment as well as with the hardener materials is studied using paint reconstructions under varying external conditions. Neutron diffraction can also be used in this case to obtain information on the reacted products.

Keywords: archaeometry, phase analysis, neutron diffraction

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Powder Diffraction in Art and Archaeology

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Powder diffraction is one of the most common techniques used as a tool for non-destructive investigations of archaeological objects. It enables both the study of small specimen (extracted from easel paintings, cosmetics recipients, walls of prehistoric caves...) and the direct analysis of painted fragments. Part of our activity is to apply relevant crystallographic techniques on ancient powdered materials, particularly using synchrotron X-ray and neutron radiation, supplemented by spectroscopic and microscopic methods: identification of the crystalline phases, quantitative and microstructural analysis, structure determination… Some difficulties, due to the specificity of the samples (rare, precious, multiphased, poorly crystallised, spread on a support…) have to be controlled. We will give some relevant examples showing the applicability of powder diffraction to the study of ancient pigments[1-3]. Recent developments about data analysis routines will be presented.

[1] Walter P., et al., *Nature*, 1999, **397**, 483-484. [2] Martinetto P., et al., *Acta Crystallographica*, 2002, **C58**, i82-i84. [3] Sanchez del Rio M., et al., *Archaeometry*, *in press.*

Keywords: powder diffraction, archaeology, non-destructive analysis

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X-ray Powder Microdiffraction: Useful Tool in Investigation of Wall Painting Layers

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Identification of inorganic and organic compounds in colour layers helps in dating and restoring of historical paintings. X-ray powder microdiffraction (micro-XPRD) extends the possibilities of traditional optical microscopy and SEM/EDX used in microanalysis of mineral pigments in fragments of paints. Laboratory diffractometers with monocapillary primary optics and x,y,z-stage are now available for a routine analysis of 0.1 mm large samples, heterogeneities in fragments and colour layers in cross-sections with a roughly flat surface. Resulting diffractograms usually need no pre-treatment before their search-match analysis.

As an example, measurements of the top green layer in a sequence of colour layers in wall paints from the Plasy Monastery, Czech Republic, are shown. The sample was measured both in Bragg-Brentano geometry and micro-XPRD, and quartz, albite, celadonite, chlorite, calcite, and gypsum were found. Micro-XPRD showed that chlorite and quartz are a contamination from the sand used in plaster. Micro-XPRD also revealed that gypsum is a part of the salt efflorescence on the outer surface. Micro-XPRD identified celadonite as a green pigment in colour layer that would not be possible using spectral methods.

Keywords: micro-XPRD, mineral pigments, paintings

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Colouring and Opacifying Agents in Roman Glass: a Multi-technique Analytical Approach

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Many samples of opaque Roman glass of different typology (mosaic tesserae, game counters, vessels and artefacts) and colours (red, green, yellow, white and blue) coming from different Italian archaeological sites have been analysed to identify and characterize the colouring and opacifying agents.

The chemical analyses were carried out using wavelength dispersive electron microprobe (WDS-EMPA), the crystalline phases used as opacifiers were identified using both an automatic powder diffractometer (XRPD) and a Gandolfi camera. Secondary electron (SEM) and backscattered electron (BSE) images were performed to study the distribution and morphology of the opacifier particles, whereas their qualitative chemical analysis was obtained with an energy dispersive system (EDS). Moreover X-ray absorption spectroscopy (XAFS) and XPS studies have been performed on green and red samples to investigate the influence of coordination and oxidation state of copper atoms on colour and opacity of the glass. These analyses allowed to conclude that, in the most of the red opaque samples, copper is mainly present as metallic nano-clusters, accompanied by monovalent copper coordinated to the oxygen atoms of the glass framework, whereas in few ones, it is present as less stable cuprite crystals, leading to a more weathered glass. In green