PS01.09.15 ANGLE-DISPERSIVE TIME-OF-FLIGHT DIFFRACTION USING THE ISIS SPALLATION SOURCE. G. Will, W. Schafer, E. Jansen, H. TietzeJaentsch, W. Kockelmann, Mineralogical Institute, University Bonn, Bonn, Germany

Using the Position Sensitive Scintilation Detektor JULIOS at the Spallation Source ISIS it is possible to measure diffraction diagrams as a function of scattering angle 21q and energy. Simultanously the experiments are installed at the ROTAX/DIF time-of-flight diffractometer at ISIS. At a typical diffraction angle of 90° (center of the detector) we can cover d- values from 0.668 to 2.707 Å, corresponding to 2 θ = 72,1° to 107,0° and λ=1.074 Å to 3.223 Å (corresponding to 3 msec to 9 msec). Two detectors are installed at present. The pulse frequency of ISIS is 20 msec, the resolution of the detector JULIOS is 5 msec (theoretical). Due to practical reasons, (because of the pulse frequency), we operate normally with the JULIOS resolution of 18 msec, corresponding to 0.0054 Å.

Numerous diffraction diagrams have been collected in the last 6 months, including diagrams for magnetic structure analysis at low temperature. A good diffraction diagram con be collected within 1 to 2 hours. Refinement of the structures is done by the Rietveld crystal and the two-step POWLS method. Examples will be given.

Combined Cryo Electron Microscopy & X-Ray Diffraction of Macromolecules

MS01.10.01 ELECTRONS AND X-RAYS WORKING TOGETHER TO VISUALIZE ANTIBODY-RHINOVIRUS INTERACTIONS. Timothy S. Baker, Thomas J. Smith, and Norman H. Olson, Department of Biological Sciences, Purdue University, West Lafayette IN 47907-1392.

Examinations of detailed interactions that occur among macromolecules within large complexes often require or benefit from combined structural information obtained from complementary techniques. Cryo-electron microscopy (cryoEM) and three-dimensional (3D) image reconstruction provide a low resolution envelope or framework for constructing a "pseudo" atomic model of the complex from high resolution structures of the components.

Our 25Å resolution, 3D reconstruction[1] of a complex between intact rhinovirus serotype 14 (HRV14) and the Fab fragment of a neutralizing monoclonal antibody (Fab17-IA) was used in this manner to dock, as rigid bodies, the separately determined X-ray structures of the virus[2] and Fab fragment[3]. This model was then used to initiate phasing to 8Å of recently obtained X-ray data from frozen, single crystals[4] of the HRV14/Fab17-IA complex.

Preliminary analysis of the X-ray structrure of the complex, after phase extension to 4Å, shows that the Fab CDR3 loop of the heavy chain adjusts its conformation to give a tighter Fab-virus interaction. In addition, the variable domain of the Fab is rotated slightly relative to the starting model in an orientation that fits the cryoEM reconstruction even better than the original model. In hindsight, the decision to dock atomic models as rigid bodies (thereby generating density overlaps) rather than allowing for conformational flexibility in the Fab or virus structures, reduced the overall quality of the initial model. Despite these errors, the initial model led to successful phase extension of the X-ray crystallographic data.

References

1. Smith, T. J., *et al.* (1993) *J. Virol.* **67:1148-1158.**
2. Rossmann, M. G., *et al.* (1985) *Nature (London)* **317:145-153.**
3. Liu, H., *et al.* (1994) *J. Mol. Biol.* **240:127-137.**
4. Smith, T. J.and E. S. Chase (1992) *Virology* **191:600-606.**

MS01.10.02 COMPARISON OF TWO STRUCTURES OF BACTERIAL LIGHT HARVESTING COMPLEXES DETERMINED BY EM AND X-RAY CRYSTALLOGRAPHY. Hugh Savage, Guillermo Montoya, Irmgard Sinning. EMBL Postfach 102209, D-69012 Heidelberg, Germany

Within the membranes of photosynthetic bacteria, up to three types of light harvesting complexes (LHI, LHII and LHIII) are found. These complexes absorb photons and transfer the excitation energy to the photosynthetic reaction centre. The LH complexes comprise pairwise-units of α and ß polypeptides with associated pigment molecules. The polypeptides each contain one transmembrane alpha-helix with bacteriochlorophyll and carotenoid molecules bound between them.

The structure of the LHII complex from *Rhodovulum sulfidophilum (RS)* has been examined using cryo-electron microscopy to a resolution of 7Å. The complex is a nonamer containing nine αß subunits. These are arranged in two radially symmetric concentric cylinders, with the nine α chains positioned in the inner cylinder and the nine ß chains forming the outer cylinder. The positions of the eighteen transmembrane helices are readily observed in the EM projection maps, along with eighteen additional peaks, attributed to the pigment molecules.

The X-ray structure of the LHII complex from *Rhodopseudomonas acidophila, strain 10050* (RA) has been determined recently (McDermott et al, 1995) and also contains nine aß subunits. Comparison of the RS and RA peak positions indicate small but significant differences The similarity of the two nonameric structures at 7Å in projection indicates that results obtained by the two methods of electron and X-ray crystallography, are directly comparable. EM analysis of 2D crystals allows a rapid determination of key structural features and the oligomeric state of the complex.The determination of further structures of LH complexes will uncover the full extent of the variability of the oligomerization states in different bacteria and also in the native membrane.

McDermott et al. (1995) *Nature* **374**, 517-525.
Savage et al. (1996) *Structure* **4**, in press.

MS01.10.03 UNDERSTANDING MUSCLE CONTRACTION BY COMBINING CRYSTALLOGRAPHY, CRYO-EM AND FIBRE DIFFRACTION. K.C. Holmes, Max Planck Institute for Medical Research, Postfach 103820, D-69028 Heidelberg, Germany

Muscle contraction comes about via the relative sliding of two sets of protein filaments, the "thick" - myosin-containing and "thin" - actin-containing filaments. The relative movement is brought about by the "Cross-bridges" which project out from the thick filaments and which by means of an asynchronous cyclical "rowing action" and concomitant hydrolysis of ATP shift the actin passed the myosin.

The atomic structure of the actin filament has been determined by combining the protein crystallographic structure of the actin monomer (1) with fiber diffraction patterns from orientated gels of actin filaments (2). The problem of refinement of this structure is discussed by Tirion [Topic 03.04]. The Structure of the myosin cross-bridge has been determined by protein crystallography and the structure of the acto-myosin complex has been determined by combining the structures of the actin filament and the myosin cross bridge with the help of cryo-electron microscopic reconstructions from actin filaments carrying a myosin cross-bridge atttached to each actin (so-called "decorated actin") (3, 4). The myosin cross bridge is "tadpole-like" in shape. the head (sometimes called the motor domain) binds to the actin whereas the tail (sometimes called the regulatory domain) does the rowing. There is a cleft in the motor domain which is thought to provide the link between ATP and actin.

The myosin cross bridge is necessarily polymorphic: to understand muscle contraction one needs to understand how the shape of the cross bridge responds to the binding and hydrolysis of nucleotides [Rayment, Topic 04.13] and to the binding of actin. EM studies of the actomyosin complex show that the tail rotates on binding ADP [Milligan, Topic 04.13]. Since the complex between myosin cross-bridge and monomeric actin has not been crystallised it is imperative to get high resolution data from cryo EM reconstructions. One method of extending the resolution is by the use of an energy-filter microscope. Images with a resolution of 15-20Å have been obtained which allow a more detailed examination of the effect of actin-binding on the myosin cross-bridge. Fiber diffraction from orientated gels of decorated actin in the presence and absence of ADP can also be used to register changes in the actomyosin structure.

1. Kabsch *et al* (1990) *Nature* **347**:37-44.
2. Holmes *et al* (1990) *Nature* **347**:44-49.
3. Rayment *et al* (1993) *Science* **261**:58-65.
4. Schröder *et al* (1993) *Nature* **364**:171-174.

MS01.10.04 COMBINING CRYO-EM AND X-RAY DIFFRACTION STRUCTURE RESULTS FOR SPHERICAL VIRUSES. Michael G. Rossmann, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907-1392

Cryo-EM structures of spherical viruses usually have a limiting resolution of around 22 Å. X-ray diffraction data are usually somewhat incomplete inside 25 Å resolution, on account of being obscured by the beam stop. Thus, phasing of the X-ray data, using the EM structure as a model, frequently lacks sufficient overlap to permit successful phase extension using the non-crystallographic symmetry. These problems can be alleviated both by carefully matching the radial scale of the EM image to the X-ray data and by assigning a crude atomic structure to the density. This can be based either on a rough structural interpretation or on suitably randomly placed atoms within the boundary of the EM density.

Interpretation of the EM density with reasonable models based on structural components of the virus requires checking for the uniqueness of the proposed fit. A variety of biological information can be used to support the results. Ross River virus (an enveloped +RNA virus) and ϕX174 (a ssDNA phage) assembly intermediates will be used as examples.

PS01.10.05 OPTIMAL MODELING OF ELECTRON MICROSCOPIC 3D RECONSTRUCTIONS USING COMPONENTS OF KNOWN ATOMIC STRUCTURE. Tang, J., Blanc, E., & Chapman, M.S.; Department of Chemistry and Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306-3015, USA

Several large complexes beyond the reach of x-ray crystallography have recently been analyzed using a combination of electron microscopy and interpretation with crystallographically derived atomic models of their smaller components. Analyses include complexes of actin and myosin, relevant to muscle function; viruses with antibodies and cellular receptor fragments. The focus of our work is the development of intuitive computational methods that optimize the agreement between model and EM based 3D reconstructions by adjusting the positions and orientations of domains, and experimental parameters defining phase contrast and magnification.

A function has been derived through which it is possible to calculate from an atomic model, the appearance of an electron density map at any arbitrary experimental resolution [Chapman (1995) Acta Crystallogr. **A51**: 69-80]. This is the basis of a stereochemically restrained least squares refinement protocol that has been applied to 3 virus x-ray crystallographic structures. The method has been adapted to lower resolution EM through the approximation of electron scattering factors by a 4-term exponential series. Following attenuation by an isotropic approximation to the contrast transfer function, the electron density contribution of each atom is calculated by analytic Fourier transformation. The sum of these contributions is compared to the EM reconstruction at each point. The method is being tested on the cryo-EM images of a virus-Fab complex, but will also be applicable to other EM techniques such as tomography.

Other

PS01.11.01 AN EFFICIENT AND CONVENIENT METHOD FOR RECORDING LOW TEMPERATURE X-RAY DIFFRACTION PHOTOGRAPH. S. A. Chawdhury, Suvechcha 130/2, East Subidbazar, Sylhet 3100, Bangladesh

An experimental technique has been developed for taking x-ray diffraction photograph within the range of gaseous nitrogen temperature. This technique has not only made possible the study of single crystals of interesting substances which are liquids or gases at room temperature and the phase change of certain compounds but also made possible a convenient means of increasing the quantity and improving the quality of intensity data. A metal dewar of special design, the outer tube of which is of stainless steel and the inner tube is of German silver, was constructed. It was then fitted to the specially constructed liquid nitrogen container and connected to the goniometer. A steady temperature anywhere from room temperature down to -185°C or so can be obtained.

PS01.11.02 THE *Fddd* DIFFRACTOMETER: HARDWARE INNOVATIONS AND A STUDY OF $[Zn(H_2O)_6][C_6H_2(COOH)_2(COO)_2]$. R.C.B. Copley,[a] C.W. Lehmann,[a] J.A.K. Howard,[a] K. Wade,[a] G. Walker,[a] J.M. Archer,[b] and K.N. Trueblood.[c] [a]Dept. of Chemistry, University of Durham, Durham DH1 3LE, UK; [b]Institute Laue Langevin, BP 156X, F-38042 Grenoble, France; [c]Dept. of Chemistry and Biochemistry, UCLA, CA, 90024, USA.

The *Fddd* four-circle diffractometer has been developed to collect X-ray diffraction experiments at temperatures down to 9K and here we describe some hardware innovations and a study at five different temperatures on the compound $[Zn(H_2O)_6][C_6H_2(COOH)_2(COO)_2]$ (**1**). The diffractometer consists of: (i) a Siemens molybdenum rotating anode generator; (ii) Huber circles with offset chi; (iii) a Siemens Fast Scintillation Detector; and (iv) an APD '202' Displex cryogenic refrigerator.

The belt-driven rotating anode gives X-ray fluxes far superior to those obtained with a conventional X-ray tube. X-ray alignment requires precise movements of the 300kg circles and this is achieved using air pads attached to the base of the goniometer. When activated with compressed air, the pads 'float' above the polished surface of an aluminium tabletop and allow precise movements of the circles.

The steel braided gas lines between the Displex and the helium compressor are supported by a counter balance system. The stress on these lines has been reduced by attaching them to the Displex via rotating joints and by passing them through a metal ring 50cm above the Displex. The ring is supported by a framework attached to the chi circle. A compact vacuum gauge has been mounted through one of the four ports on the top of the cryostat and gives interesting information on the vacuums obtained within the Displex during an experiment. Crystals are mounted on 'sharpened' 0.3mm graphite pencil leads and a new sample mount has been designed.

X-ray diffraction data for **1** have been collected at 296, 210, 120, 50 and 9K. Full analysis of the ADPs at the different temperatures demonstrates the high resolution capabilities of the *Fddd* diffractometer.