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

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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.