

## 01-Instrumentation and Experimental Techniques (X-rays, Neutrons, Electrons)

MS-01.02.02 DATA ACQUISITION AND PROCESSING FACILITIES AVAILABLE AT SERC DARESBURY LABORATORY. By P.F.Lindley\*, SERC Daresbury Laboratory, Warrington, Cheshire WA4 4AD, UK.

Synchrotron radiation provides a source of high intensity, highly collimated, wavelength tunable X-radiation which is becoming increasingly important in studies of structure-function relationships in biological macromolecules. At a fixed wavelength, the high intensity and low beam divergence enable diffraction patterns from crystals with large unit cells to be resolved. The use of shorter wavelengths can lead to a reduction in radiation damage as well as minimising systematic errors due to absorption. Weakly diffracting samples and/or small crystals often give useful data not obtainable from conventional sources. The wavelength tunability can also be used to optimise anomalous dispersion effects and lead to phase determination through multi-wavelength methods. Alternatively, the high intensity of the radiation over a large part of the X-ray spectrum is well-suited to the Laue technique. The Laue technique can be applied not only to protein crystals where the interest is in rapid data collection, but also to extremely small crystals of the order of tens of microns long.

At the Synchrotron Radiation Source, SERC Daresbury Laboratory, two stations provide "state of the art" facilities for protein crystallography. Both stations derive their radiation from a 3-pole wiggler magnet operating at 5 tesla. Station 9.5 is a dual purpose station designed for focussed Laue experiments and rapidly tunable monochromatic applications including multi-wavelength anomalous dispersion measurements. The principal optical components are a platinum coated fused quartz toroidal mirror and water cooled channel cut Si(111) double crystal monochromator. The mirror has an acceptance aperture of 1.2 mrad. horizontally and 0.1 mrad. vertically giving a white beam focal spot size of 1.3 x 0.4 mm some 32 m from the source. The monochromator can be interposed at about 30 m from the source for monochromatic work and gives a band pass  $\Delta\lambda/\lambda = 0.00015$ . The station is equipped with a Mar-Research image plate detector with the option of an Arndt-Wonacott camera and X-ray film for Laue work if appropriate.

Station 9.6 is mainly used as a fixed wavelength station. The optical components are a platinum coated fused cylindrically curved quartz mirror which provides 1:1 vertical focussing and a bent triangular Si(111) monochromator giving an 8:1 horizontal defocussing at 0.895 Å with a band pass,  $\Delta\lambda/\lambda = 0.0004$ . The size of the focal spot at the specimen is 0.5 x 0.3 mm in the horizontal and vertical directions respectively. The station can be equipped with an Enraf-Nonius Fast TV detector system or an image plate device.

Most data processing mainly involves MOSFLM as modified by Dr. A. Leslie (Cambridge) and the CCP4 suite of programmes.

MS-01.02.03

IMAGE PLATE DATA COLLECTION IN HAMBURG

By Z. Dauter, EMBL, c/o DESY, Notkestr. 85, 2000 Hamburg 52, Germany

After a relatively long shut-down, when the DORIS storage ring was rebuilt, it has come back into operation as a dedicated source of synchrotron light. At present there are three active protein crystallography beam-lines, two

belonging to EMBL and one owned by the Max Planck Institute. All of them are equipped with Image Plate scanners, either originally developed at EMBL or commercial MAR devices.

The scanners used in Hamburg have a fixed image plate, which after every exposure is read-out on-line in a spiral manner and the diffraction pattern stored on computer disk after appropriate corrections and transformation to cartesian pixels. The images are then ready to be processed by any of the existing data processing packages (Mosflm, Denzo, Xds). The read-out and erasure cycle takes about 2 min. for the 180 mm plate and 5 min. for the larger, 300 mm plate.

The quality of X-ray data produced by the scanners will be illustrated by a few examples. On one side, the image plate proved to be very useful for virus data collection, where its sensitivity at short, below 1 Å, wavelengths, makes it possible to obtain more data from one crystal and achieve higher completeness. On the other hand, using the data collected with Mo K $\alpha$  radiation, the structure of the very small molecule, potassium tartrate, refined to an R value of 1.35 %, favourably competing with the quality achievable on a diffractometer. Moreover, in many cases the accuracy of the anomalous signal recorded by the system allowed the solution of the protein structures with only one heavy atom derivative.

MS-01.02.04 WEISSENBERG CAMERA FOR MACRO-MOLECULES WITH IMAGING PLATE DATA COLLECTION SYSTEM AT PF, PRESENT STATUS AND FUTURE PLAN  
By N. SAKABE<sup>†</sup>, K. SAKABE<sup>†</sup>, T. HIGASHI<sup>††</sup>, A. NAKAGAWA, N. WATANABE & S. ADACHI<sup>†††</sup>, Photon Factory, National Laboratory for High Energy Physics, Tsukuba 305, Japan, <sup>†</sup>Dept. of Chemistry, Faculty of Science, Nagoya Univ., Chikusa, Nagoya 464, Japan, <sup>††</sup>Rigaku Corporation, Matsubara, Akishima, Tokyo 196, Japan, <sup>†††</sup>The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako, Saitama, 351-01, Japan.

The Photon Factory is operating at 2.5 GeV and 300 mA. There are two data collection systems for macromolecular crystallography at BL6A2 and BL18B, consisting of Weissenberg camera, imaging plate, image reader (BA100 & BAS2000), data reduction program "WEIS" and autoindexing program.

The optical system of BL6A2 is equipped with bent plane fused quartz mirror and triangular bent asymmetric cut Si(111) monochromator and that of BL18B is equipped with 1 m long fused quartz bent cylindrical Pt coated mirror with 1:1 focusing and a double-crystal monochromator which can be changed either using Si(111) or Ge(111) without opening the mirror house.

The main basic ideas of Weissenberg camera for macromolecular crystallography are: 1) Film cassette should be designed cylindrical to get higher resolution data nicely, 2) The radius (r) of film cassette must be the larger the better because back ground noise will decrease approximately proportional to  $1/r^2$ ; on the other hand, Bragg reflections do not

01-Instrumentation and Experimental Techniques (X-rays,  
Neutrons, Electrons)

13

be affected so much by r. 3) More data will be recorded on a film, when the Weissenberg geometry will be applied, 4) Bijvoet pairs are recorded with high accuracy on the same film as simultaneous reflections. 5) Almost all X-ray pass should be evacuated or filled with helium gas to reduce the scattering and absorption by air.

The fundamental requirements for the detection of X-ray diffraction from protein crystals are: 1) high detective quantum efficiency, 2) wide dynamic range, 3) linearity of response, 4) high spatial resolution, 5) large detective area, 6) uniformity of response, 7) high counting rate capability and 8) low background noise.

A X-ray integrated-type area detector, called an imaging plate (IP) is one of the most suitable detectors for the data collection of X-ray diffraction from protein crystals. The combination with the camera, IP and SR X-ray becomes a very powerful (N. Sakabe, Nucl. Instr. and Meth., 1991, A303, 448).

The camera type 3 was designed for PF users at BL6A2 station. Normally, one data set around an axis is recorded on 10-30 IP sheets with consuming only one or two crystals and 5-10 sets of data are collected in 24 hours.

The size of IP which we are using is 400x200mm. Strong demanding of the large size of IP, we have designed 800x400mm IP reader which will be installed this April detached room at BL18B (N. Watanabe et al, poster in this meeting).

The application of this data collection system and future data collection system will be discussed.

MS-01.02.05 PRESENT AND FUTURE PROSPECTS FOR MACROMOLECULAR CRYSTALLOGRAPHY AT THE CORNELL HIGH ENERGY SYNCHROTRON SOURCE (CHESS). Steven E. Ealick, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853

The Cornell High Energy Synchrotron Source (CHESS) consists of five beamlines and 11 experimental stations which receive radiation from the Cornell Electron Positron Storage Ring (CESR). Beamlines A, B and C receive radiation from circulating electrons while beamlines D and F receive positron radiation. Macromolecular crystallography is currently supported on four experimental stations. Station F-1 is a doubly focused, tuneable (6-14 keV) station that receives half the radiation from a 25-pole wiggler. This popular station is used primarily for monochromatic data collection on samples that have large unit cells, small size, weak diffraction or radiation sensitivity. Station F-1 is part of a BL-3 containment facility that can be used for X-ray diffraction studies of bio-hazardous samples. For the past ten years, station A-1 has been a doubly focused, fixed wavelength station (8.0 keV) that receives radiation from a 6-pole wiggler. Station A-1 has been used for routine

monochromatic data collection on a variety of crystallographic systems. In January of 1993, station A-1 was dismantled to make way for a new tuneable station that will be comparable to F-1. Station B-2 receives white radiation from a bending magnet and has been used for Laue protein crystallography and micro-diffraction. Station F-2 receives the other half of the radiation from the F line wiggler and is the most recent station at CHESS to be used for macromolecular crystallography. The F-2 optics consist of a double crystal monochromator with fixed offset and a vertically focusing mirror. Sagittal bending of the second monochromator crystal provides focusing in the horizontal direction. The station can be rapidly tuned over the range 6-40 keV with an energy resolution of a few eV. The station has been used for multiple wavelength anomalous diffraction (MAD) phasing experiments and general purpose diffraction on non-biological samples. The high critical energy of the F line wiggler (24 keV) makes station F-2 an ideal station for high energy diffraction studies. High energy diffraction is potentially useful for maximizing signal-to-noise, reducing radiation damage and reducing absorption effects. Future plans at CHESS include an upgrade of the A line by insertion of a 25-pole wiggler and rebuilding of a tuneable experimental station A-1. In addition, current plans call for a gradual increase of the storage ring current from 100 to 500 mA during the next two years. Finally, the long term capabilities of CHESS will be determined by the outcome of a pending B-factory proposal. If approved, the storage ring will be reconstructed as two separate rings one operating at 8 GeV and 1 A and the other operating at 4 GeV and 2 A. The corresponding CHESS laboratory will consist of eight beamlines mostly based on insertion devices.

MS-01.02.06 PROCESSING OF DATA FROM LARGE UNIT CELL VIRUS CRYSTALS. By \*M. S. Smyth, J. G. Tate and D. I. Stuart. Laboratory of Molecular Biophysics, University of Oxford, U. K.

We have crystallized three picornaviruses: bovine enterovirus (BEV), a benign virus of interest as a vaccine vector; coxsackievirus A9 (CAV9), causative agent of a range of human disorders, and human rhinovirus type 2 (HRV2), a common cold virus. The crystal morphologies are suitable for X-ray analysis, and each diffracts to beyond 3Å resolution. The unit cell dimensions and space groups are summarized in the table.

Virus	a	b	c	$\beta$	Sp. group
BEV	388Å	390Å	360Å	113°	P2 <sub>1</sub>
CAV	495Å	495Å	695Å	90°	P4 <sub>n</sub> 22
HRV	382Å	352Å	681Å	90°	P2 <sub>n</sub> 2 <sub>n</sub> 2 <sub>n</sub>

The collection of such data necessitates the limitation of resolution to 3.5Å in the cases of the CAV9 and the HRV2. Nevertheless, we have collected sufficient data from each of the virus crystals