Macromolecular Assemblies (BL44XU)

1. Introduction

There exist various biological macromolecular assemblies consisting of proteins, nucleic acids, sugars, lipids, and other substances in living cells. These molecular assemblies play key roles in many biological reaction systems, like protein synthesis, chromosomes, RNA synthesis, DNA synthesis, photosynthesis, respiration, membrane transport, cell adhesion, and cell signaling. More than 10,000 protein structures are now known since the first crystal structure determination of hemoglobin [1] and myoglobin [2]. On the other hand, only a few structures have been determined by X-ray crystallography. This is because of the difficulties faced in the preparation, crystallization, X-ray diffraction measurement, and crystal structural analysis of large molecular assemblies. We are constructing a beamline for biological macromolecular assemblies at BL44XU to collect high resolution and high quality diffraction data of crystals with a large unit cell.

2. Beamline and Optics

The design of the beamline is for structural studies of biological macromolecular assemblies. A side view of the beamline at BL44XU is shown in Fig.1. X-rays from an in-vacuum undulator are monochromatized by a rotating-inclining double crystal monochromator in the optical hutch. The monochromatized X-ray beam introduced into the experimental hutch is reflected by a Rh-coatred mirror. The mirror can be used for focusing or collimating purpose as well as for cutting off higher order harmonics. The total photon flux at the sample position is on the order of 10^{13} photons/sec.

The approximate beam size of the full width at half maximum (FWHM) at the sample position is 1.0(W) × 0.7(H) mm^2 without focusing. The photon flux after the 100-micron collimator is on the order of 10^{10}-10^{11} photons/sec. The X-ray beam can be focused by a horizontal mirror if a higher brilliance beam is needed.

3. Diffractometers

Two individual single rotation axis (horizontal and vertical) goniometers are mounted on a goniometer, and either of them can be used according to the user requirements. Either an array CCD detector PX210 (Oxford Instruments, UK), which was originally designed and developed by the detector design group of Argonne Structural Biology Center [3], or an imaging plate system DIP2040 (MAC Science, Japan), can be mounted on the camera base. The sample to detector distance can be changed from 100 mm to 1,000 mm. The camera base for the CCD detector can be lifted up or brought down between + 15mm and -5 mm and can be tilt 0 - 45 degrees to collect higher angle data. The readout time of the PX210 detector is about 2 seconds, which is an appropriate readout time for exposure of several seconds.

The high speed shutter and precision goniometers are controlled by a PC running Linux.

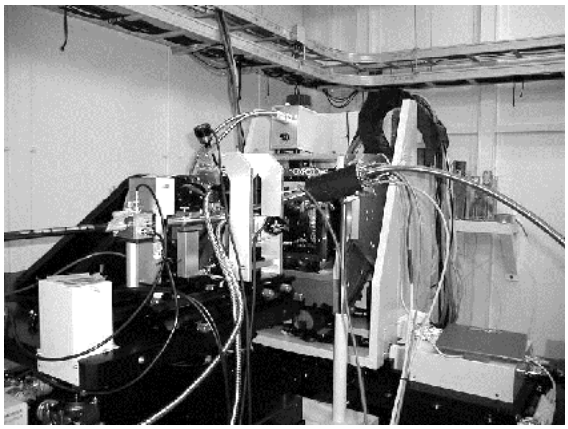

Fig. 2. Photograph of goniomater and CCD detector installed in the experimental hutch at BL44XU.

4. Peripheral Devices

A nitrogen gas stream cooler (Niki Glass, Japan) will be used for data collection at cryo temperatures (~100 K). This system will produce liquid nitrogen for cooling purposes from the air, and will not need liquid nitrogen supplements.

Several workstations with a large disk storage capacity, which are connected to control computers of detectors with a 10BaseT Ethernet, can be used for

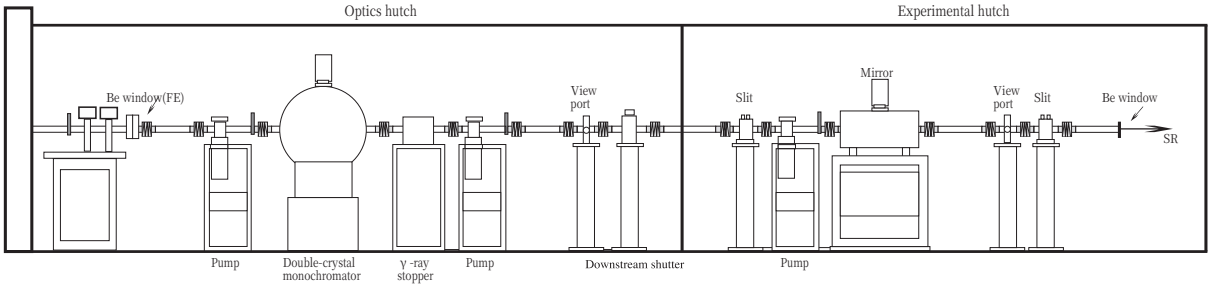

Fig. 1. Schematic view of Optics Hutch of BL44XU.

data processing purpose.

5. Data Collection and Processing

At present, d*TREK (MSC, USA) is being used to process CCD data. MOSFLM [4] (and DPS [5]) and/or HKL [6] possible other choices as data processing software.

6. Current Status of the Beamline

The optics and detector system were evaluated during the commissioning period (7th and 8th shifts in 1999). The first exposure from the beamline was successfully taken from a cytochrome *c* oxidase crystal [7]. During that period, diffraction data was obtained for cytochrome *c* oxidase and lysozyme crystals were taken at that period. Figure 3 shows original (un-corrected) images of hen-egg white lysozyme and of cytochrome *c* oxidase taken at the beamline. The exposure time for the diffraction image of the lysozyme crystal was only one second for one degree rotation using a 0.1 × 0.1mm^2 collimator; the intensities of most of the reflections below 3 Å resolution were overloaded. We came to realize some intrinsic and extrinsic problems of the beamline components and the detector system during the commissioning period, and we found that we still need to overcome these problems during the summer shutdown period for public use. However, we believe that the performance of the beamline is good enough for the collection of diffraction data from biological macromolecular assemblies.

Atsushi NAKAGAWA
Eiki YAMASHITA
Tomitake TSUKIHARA
(Osaka University)

References

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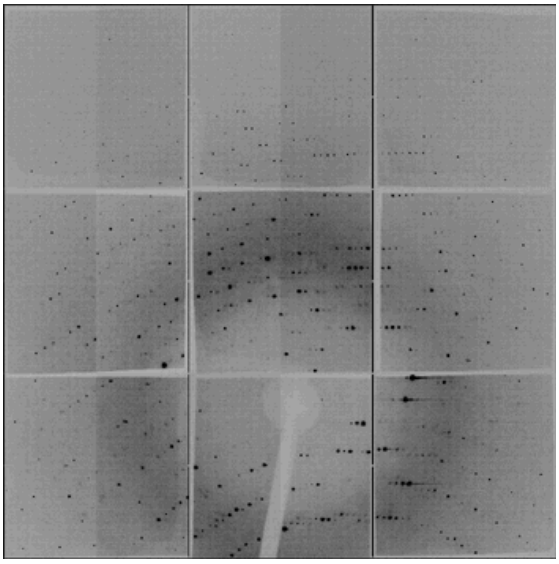

Fig. 3a. Diffraction image of lysozyme crystal taken at the BL44XU (distortion and non-uniformity un-corrected).

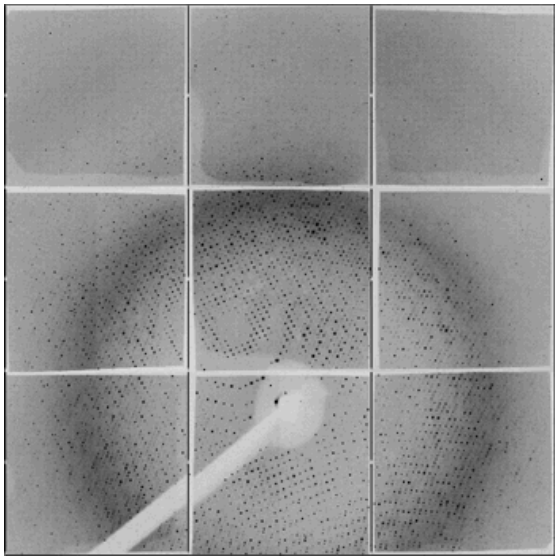

Fig. 3b. Diffraction image of cytochrome *c* oxidase taken at the BL44XU (distortion and non-uniformity uncorrected)