# Osaka University Beamline (BL44XU)

**Tomitake TSUKIHARA**
**Eiki YAMASHITA**
**Min YAO**

## 1. Introduction

There exist various biological macromoleular assemblies consisting of proteins, nucleic acids, sugars, lipids and other substances in living cells. These molecular assemblies have key roles in such biological reaction systems as protein synthesis, chromosomes, RNA synthesis, DNA synthesis, photosynthesis, respiration, membrane transport, cell adhesion and cell signaling. About 5,000 protein structures are now known since the first crystal structure determination of myoglobin [1]. On the other hand the number of structures of the molecular assemblies determined by X-ray analysis is no more than 40 including viruses. This is because of difficulties in preparation, crystallization, X-ray diffraction experiment and crystal structural analysis of these very large molecular assemblies with molecular weights ranging from 105 to 107 Da. Crystals of molecular assemblies including membrane protein complexes are increasing in number due to extensive efforts of biochemists. Accurate measurement of X-ray diffraction intensities enables the multiple isomorphous replacement method to be applied to phase determination of crystals of the molecular assemblies [2]. We are constructing a beamline for biological macromolecular assemblies at BL44XU to collect high resolution diffraction data of crystals with a large unit cell. We intend to collect diffraction data at the beamline presently at 3.0 A resolution for a unit cell of 1000 x 1000 x 1000 A and ultimately at 2.5 A resolution for that of 2000 x 2000 x 2000 A.

## 2. Outlines of the beam line

A side view of the Osaka University Beamline for biological macromolecular assemblies at BL44XU is shown in Figure 1. X-rays with energy of about 12 KeV chosen from an undulator designed according to the standard specifications of the SPring-8 is monochromatized by a double crystal monochromater. The expected energy resolution is (DE/E) 2x10-4. The X-rays with beam divergence less than 100 mrad are focused with a double-mirror system and a collimator. Beam sizes at the sample position are 0.1 to 0.02 mm in both of vertical and horizontal directions.

## 3. Diffractometer

We have not yet determined the specifications of diffractometer. A rotation axis of the crystal is not aligned before X-ray diffraction data are collected at this beamline. A two-axis goniometer will be used in order to obtain high accuracy of the rotation centers with a positional error less than 0.05 mm. The minimum spot-spot distance is estimated to be 0.483 mm for a unit cell of 1000 x 1000 x 1000 A under following experimental conditions: wave length, 1.0 A; crystal-detector distance, 500 mm, diameter of detector, 300 mm and resolution, 3.0 A. We are looking for a detector with a high positional resolution.

The diffractometer will be equipped with a spectrometer to observe absorption spectrum of the crystal on the goniometer during the X-ray experiment. The simultaneous observation of X-ray diffraction and absorption spectrum is experimentally important to elucidate various reaction mechanisms from X-ray structures of proteins.

## 4. Data collection and processing

Intensity data are acquired by oscillation method without any alignment of the rotation axis before the intensity measurement. Systematic errors in the observed intensity data are reduced by merging data sets obtained for different orientations of crystals. Anomalous dispersion effect of Df"=2 was detected for the crystal with asymmetric unit molecular weight of 200,000 Da by this method [3].

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## References

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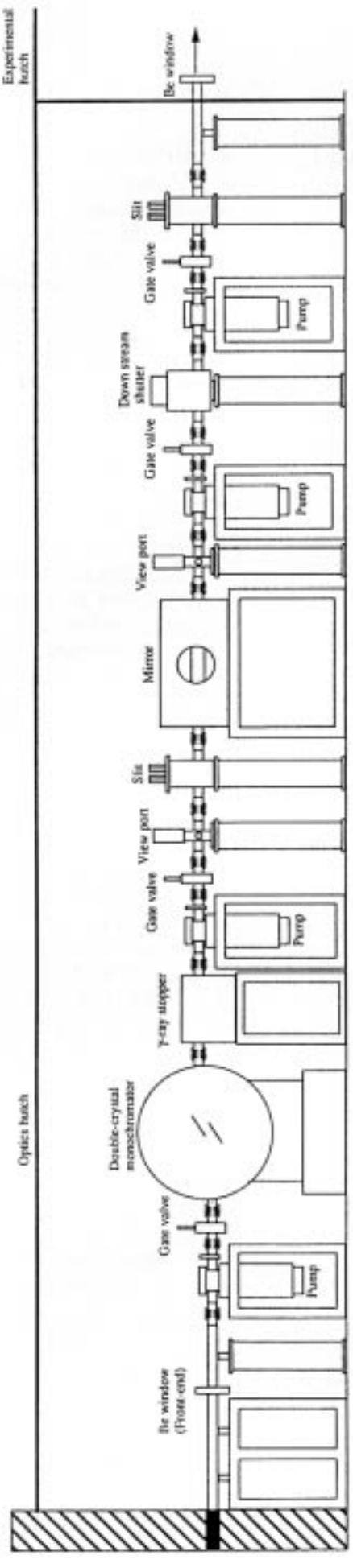


Fig.1 Schematic view of Optics Hutch of Osaka University Beamline for Biological Macromolecular Assemblies