BL41XU
Structural Biology I

1. Introduction

BL41XU is a public macromolecular crystallography (MX) beamline using an undulator as a light source, and it has been contributing to various structural biology studies since 1997. It provides two operation modes: normal mode (NM) and high-energy mode (HM). NM is set up in experimental hutch 2 (EH2), and the X-ray energy range is 6.5–17.7 keV. It has been mainly used for the structural determination of challenging targets such as membrane proteins and macromolecular complexes using a high-flux beam of 2.3×10^{12}–1.1×10^{13} (photons/s at 12.4 keV). HM allows data collection using X-rays of 20–35 keV in experimental hutch 1 (EH1), which provides unique opportunities, such as ultrahigh-resolution data collection.

After BL45XU started operation in 2019, which has almost the same beam specifications as BL41XU and specializes in automatic data collection using the ZOO system [1], we decided to develop BL41XU as a beamline that can also be used for structural dynamics studies, i.e., time-resolved (TR) crystallography and room-temperature (RT) crystallography.

Moreover, the public use of two cryogenic transmission electron microscopes (CryoTEMs), EM01CT and EM02CT, as ancillary facilities of MX beamlines started in 2021B. CryoTEMs are especially powerful in structural studies on membrane proteins and large macromolecular complexes, which are usually difficult to crystallize. Therefore, CryoTEMs provide additional opportunities for structural biology studies at the SPring-8 campus by compensating MX. Here, we report our activities in FY2021.

2. Development of serial synchrotron crystallography

Serial synchrotron crystallography (SSX) is a data collection technique that uses thousands of microcrystals delivered on the X-ray beam path for data collection. We are installing two types of SSX device for the TR experiment. One is the injector-based SSX device using the fluidic sample injection device developed at SACLA [2]. To use this device, a sample stage for this apparatus has been installed on the diffractometer together with a suction device that stabilizes sample flow (Fig. 1).

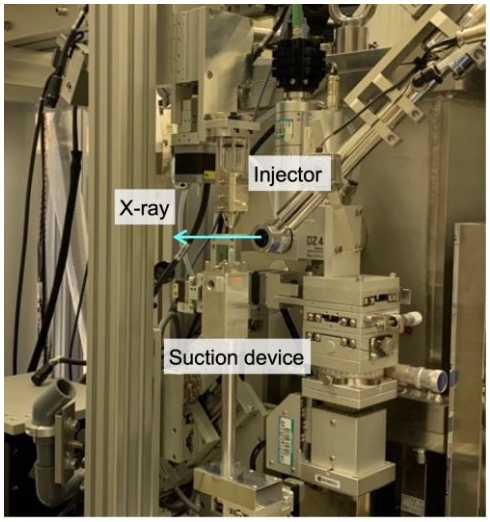

Fig. 1. High-viscosity injector installed at EH2.

The other type of SSX device is the fixed-target SSX, in which microcrystals trapped on a solid sample holder are conveyed to the beam path by fast-moving stages. We designed silicon sample holders with 10,000 tapered holes for this purpose, and fast-moving translational stages were installed

on the diffractometer. For the synchronization of the sample holder movement with the detector readout, the high-precision motion controller PMAC was introduced, and the incorporation of PMAC into the beamline control system is ongoing.

3. Implementation of trigger for time-resolved experiment

A pumping laser is one of the most popular reaction triggers for crystallography. A nanosecond tunable wavelength laser, NT230-30 (EKSPLA), was installed in 2020. This year, laser optics for NT230-30 has been set up in EH2. The optics consists of mirrors, prisms, and focusing lenses. A motor-driven ND filter with gradually changing thickness along the lateral direction was equipped on the light path to adjust the laser energy. A laser shutter to precisely control exposure by synchronizing with laser oscillation was also installed. The optics thus prepared was used in the user operation for the frozen crystal attached to the goniometer. The installation of optics for SSX is ongoing.

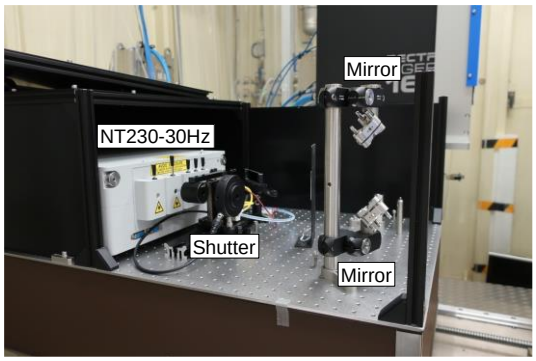

Fig. 2. Laser optics for NT230-30.

Another reaction trigger we are preparing is an injection device of substrate solutions on the microcrystals trapped on the solid sample holder. Inkjet devices for this purpose were installed this year.

4. Side room for sample preparation

A tentative experimental room was built downstream of EH2. This room was used for (1) the flush freezing of protein crystals to trap the intermediate state, (2) sample preparation for the TR experiment, (3) offline micro-spectroscopy experiment, and (4) offline laser optic test. For the flush freezing, a cryocooler, goniometer, and sample changer robot were installed to collect frozen crystals into UniPuck. An interlock system for safely using a laser was also implemented in this room.

5. Start of public use of CryoTEMs

Both EM01CT and EM02CT are applicable for confirming the condition of a sample that is difficult to crystallize and for determining the solution structure. We have established a protocol to train new users to operate CryoTEMs by themselves through a regular training course and user support during their machine time. EM02CT is assigned as a sample screening machine and mainly used for the training course and for confirming the sample condition because of its acceleration voltage and detector specification. On the other hand, EM01CT is used for the high-resolution solution structure

Fig. 3. EM01CT (left) and EM02CT (right).

determination of a sample whose condition has been confirmed. The first result was published in June 2022 [3].

Hasegawa Kazuya*[1], Murakami Hironori*[1], Masunaga Takuya*[1], Fukui Tomoki*[2], Shigematsu Hideki*[1,3], and Kumasaka Takashi*[1]

*[1] Protein Crystal Analysis Division, JASRI

*[2] Engineering Support Group, JASRI

*[3] SPring-8 Center, RIKEN

References:

[1] Hirata, K. Yamashita, K. Ueno, G. Kawano, Y. Hasegawa, K. Kumasaka, T. & Yamamoto, M. (2019). *Acta Cryst*. D**75**, 138–150.

[2] Shimazu, Y. et al. (2019). *J. Appl. Cryst.* **52**, 1280–1288.

[3] Tanaka, S. et al. (2022). *J. Med. Chem.* **65**, 11, 7843–7853.