BL41XU
Macromolecular Crystallography I

1. Introduction

BL41XU is a public macromolecular crystallography (MX) beamline using an undulator as a light source, and has been contributing to various structural biology studies since 1997. It provides two operation modes: the normal mode (NM) and the 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 specification 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.

Meanwhile, public use of two cryogenic transmission electron microscopes (CryoTEM), EM01CT and EM02CT, started in 2021B as ancillary facilities of BL41XU. CryoTEM is especially powerful in structural studies on membrane proteins and large macromolecular complexes, which are usually difficult to crystallize. Therefore, CryoTEM provides additional opportunities for structural biology studies at the SPring-8 campus by compensating MX. Here, we report our activities in FY2023.

2. Upgrade of X-ray optics and installation of new detector

EH2 conducts automated measurements and structural dynamics studies. The beam size is changed by defocusing the X-ray focal point by moving the diffractometer upstream or downstream. In this method, the minimum distance to the detector changes depending on the beam size used. In order to always use the shortest distance of the detector, the beam-size-changing system adopts a method of changing the slit size of the virtual light source and the angle of the KB mirror, which was introduced at BL45XU. As a result, the beam size can be changed from 5 μm × 5 μm (H × V) to 50 μm × 100 μm (H × V), and the shortest distance to the detector can always be 120 mm.

To enable faster measurements, the FE slit aperture size was changed from 0.3 mm × 0.3 mm to 0.5 mm × 0.5 mm, and the flux was increased by 2.5 times. Furthermore, Eiger2XE 16M was installed in January 2024 (Fig. 1).

Fig. 1. Eiger2XE 16M.

A new collimator system was installed to reduce X-ray scattering by the air and to enable sample observation during measurement with the collimator inserted (Fig. 2). The design with the collimator evacuation direction being oblique upstream to the X-ray can also free up space around the sample position to ease manual sample exchange for TR and RT crystallography experiments (Fig. 2).

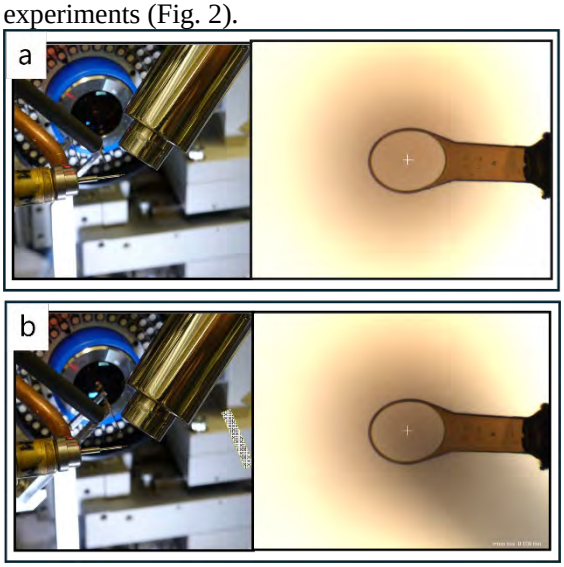

Fig. 2. New collimator system. (a) Collimator evacuation position (left). Image of a sample loop observed with a coaxial camera (right). (b) Collimator insertion position (left). Image of a sample loop observed with a

coaxial camera(right).

3. Development for TR crystallography

Time-resolved structure analysis of proteins enables a deep understanding of reaction mechanisms along reaction pathways. We have been setting up an environment for time-resolved data collection using serial crystallography, aiming at a time resolution of milliseconds or longer at BL41XU.

For this purpose, we have introduced the high-viscosity cartridge-type injector (HVC), which was developed at SACLA for serial femtosecond crystallography [2]. Additionally, we have installed both nanosecond wavelength-variable lasers and continuous-wave lasers, along with laser optics to illuminate the laser on the sample stream extruded from the HVC, thereby triggering the reaction. We have also established real-time monitoring of the hit rate and indexing rate, as well as timing control systems to synchronize laser illumination and detector readout.

We are also preparing a time-resolved experiment using the fixed-target serial method. In this approach, small crystals trapped in a sample chip with a lattice of tapered holes are moved on the X-ray beam path using high-speed 2D translational stages. Prototype sample holders with ten thousand holes have been manufactured, and the installation of a timing control system to synchronize the detector readout with the rapid movement of the sample chip has been completed. Static diffraction data measurement using standard samples is now underway.

In addition to the above two approaches, we have established an environment to cryo-trap intermediate states of light-responsive proteins after initiating reaction by laser illumination in the sample preparation room (Fig. 4).

4. Development for RT crystallography

In order to perform RT crystallography using the humid air and glue coating (HAG) method [3, 4] at BL41XU, a temperature and humidity control system was installed in the EH2 and operated from 2023B. The microspectroscopy system developed at BL26B1 was installed in EH2. Microspectroscopy instruments are available for both RT with HAG and the cryo condition (Fig. 3).

5. Public use of CryoTEM

Four CryoTEMs are available for PX-BL users to investigate their sample properties such as monodispersity or conformational heterogeneity. By using these CryoTEMs, users can screen their samples and prepare good grids for high-resolution structure determination. As the sample is not necessarily crystallized, solution structures with small conformational variations are analyzed, classified, and refined to a resolution of up to 2 Å. This technique has been found to be beneficial for those who have had difficulty in crystallizing their target of interest.

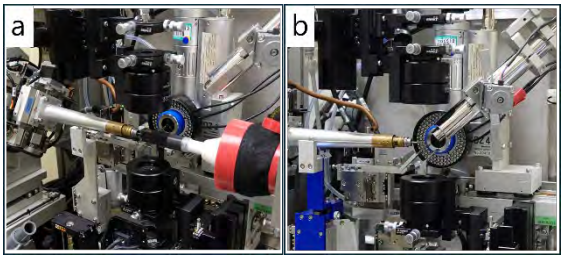

Fig. 3. Microspectroscopy system. (a) Combination with a temperature and humidity control system. (b) Combination with cryostream.

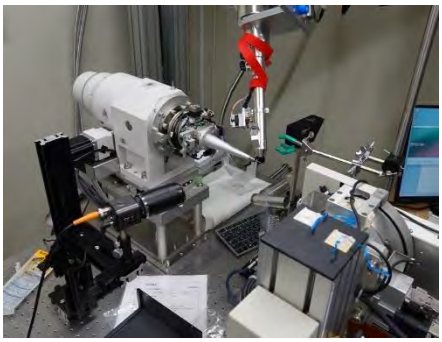

Fig. 4. Laser irradiation and crystal freezing system for cold trapping of intermediate states

BABA Seiki[*1], HASEGAWA Kazuya[*1], MURAKAMI Hironori[*1], MASUNAGA Takuya[*1], FUKUI Tomoki[*2], IRIE Takaki[*2], SHIGEMATSU Hideki[*1,3], and KUMASAKA Takashi [*1]
[*1]Structural Biology Division, JASRI
[*2]Engineering Support Group, JASRI
[*3]SPring-8 Center, RIKEN

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