BL41XU
Macromolecular Crystallography I

1. Introduction

BL41XU is a public macromolecular crystallography (MX) beamline with 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 used mainly for the structural determination of challenging targets such as membrane proteins and macromolecular complexes using a high-flux beam of $2.3 \times 10^{12} – 1.1 \times 10^{13}$ 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, began 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 FY2022.

2. Development for TR crystallography

The combination of a high-flux microbeam and a fast-reading detector at the MX beamline allows for MX data collection with exposure times in the millisecond range, in combination with serial synchrotron crystallography (SSX). This capability enables time-resolved experiments at the millisecond scale. Drawing from our experience with SSX, we are actively developing two types of TR-SSX experiments at BL41XU.

One approach involves TR-SSX with a high-viscosity injector (HVI) developed at SACLA. Following the completion of HVI installation last year, we conducted static experiments to establish a protocol for its utilization at SPring-8. Microcrystals of standard samples mixed in cellulose or grease were ejected from HVI, and diffraction images were continuously collected by the EIGER 16M detector at a frame rate of 100 Hz. The 'hit' images, where diffraction spots were observed, were selected using KAMO software, which is employed in the ZOO system to identify crystal positions based on the number of diffraction spots in an image. We collected diffraction data up to a resolution of 2 Å, and the structure was determined with R_{work} of 18.34% and R_{free} of 21.03% without significant radiation damage. These results demonstrate that the data obtained through this method possesses sufficient quality for protein structure studies.

The other approach involves fixed-target SSX

using a silicon chip with 10,000 tapered holes designed to trap protein crystals. By sequentially moving the tapered holes onto the X-ray beam, we can collect data from the microcrystals trapped within each hole. Precise control of the fast-moving stage for translating the silicon chip in sync with the detector readout is crucial in this experiment. To achieve this, we have been developing a control system utilizing PMAC, which enables accurate timing control at sub-millisecond levels. The software for moving the chip was completed this year, and its installation into the data collection system of BL41XU is scheduled for the next fiscal year.

3. Development for RT crystallography

Collecting diffraction data from crystals in a crystallization plate, which is called *in situ* data collection, is suitable for quality checks of crystals obtained in the crystallization screening under a vast variety of conditions, because it eliminates troublesome tasks such as cryo-protection and harvesting crystals with a cryoloop. To perform RT crystallography, we have installed an *in situ* data collection system at BL41XU,

When the beamline is operated in the *in situ* diffraction measurement mode, the sample changer SPACE-II is evacuated and a newly designated goniometer for *in situ* data collection is installed facing the goniometer for conventional data collection (Fig. 1). The goniometer has a gripper to hold a crystallization plate compatible with SBS standard low-height plates. It also has a rotational axis with a stroke of 30° for rotation data collection. Operations required for the *in situ* data collection, such as positioning of the crystal on the X-ray beam path, can be performed by using the beamline control software BSS. The data collected by small wedge method can be processed by the KAMO system to obtain comlete data.

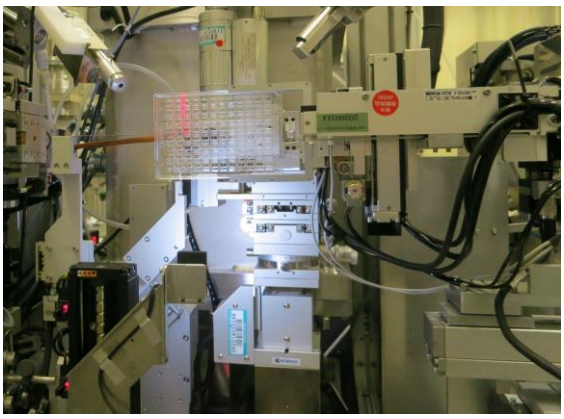

Fig. 1. *In situ* data collection diffraction measurement system.

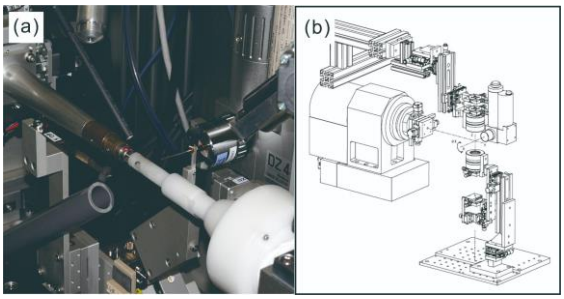

Fig. 2. Apparatuses used in RT measurements. (a) Temperature and humidity control system. (b) Microspectroscopy system.

To perform RT crystallography by the humid-air and glue-coating (HAG) method [2,3] at BL41XU, a temperature and humidity control system was installed in EH2 and its operation was checked [Fig. 2(a)]. In addition, the microspectroscopy system being developed at BL26B1 was designed to be installed in EH2 of BL41XU [Fig. 2(b)]. The installation and operation will be checked in 2023A.

These apparatuses are scheduled to be provided to users in FY2023.

4. Development of automatic data collection

Automated measurements at BL41XU were

performed at 20 Hz for 2D diffraction scans to determine the crystal position in the loop. To further increase the scan speed, the timing control of the shutter and goniometer was adjusted. Therefore, the 2D diffraction scan could be performed at 50 Hz. After the robot automatically places the sample pin on the goniometer, the sample pin is stretched and the crystal position in the loop shifts during the temperature increase from 100 K to room temperature. Therefore, a waiting time is required for the sample pin elongation to subside. Therefore, a system operated at BL45XU to blow dry air at the base of the pin was installed to reduce the waiting time for the temperature to rise. As a result, the waiting time for temperature to rise was improved from 60 s to 10 s for SPINE-type sample pins. These improvements have made it possible to perform automated measurements in the same measurement time as that at BL45XU.

To further improve data accuracy, a new collimator system was designed to reduce X-ray scattering from the air. The inner diameter of the collimator will be changed from 0.8 mm to 0.4 mm to prevent scattering from the air in front of the sample [Fig. 3(a)]. In the new design, the collimator evacuates diagonally upstream relative to the X-ray beam, which can also free up space around the sample position to ease manual sample exchange in TR and RT crystallography experiments [Fig. 3(b)]. This system is scheduled to be installed in 2023A.

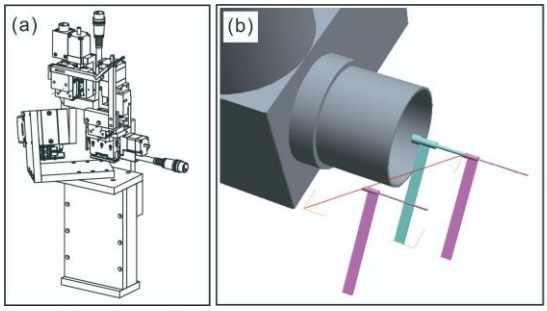

Fig. 3. New collimator system. (a) It was designed to reduce X-ray scattering from the air. (b) Design of the collimator evacuation direction.

5. Public use of CryoTEM

Two CryoTEMs are available for PX-BL users to investigate 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.

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