BL41XU
Macromolecular Crystallography I

1. Introduction

BL41XU is a public macromolecular crystallography (MX) beamline that uses an undulator as its light source. Since 1997, it has been contributing to various structural biology studies. The beamline provides two operation modes: the normal mode and the high-energy mode. The normal mode (NM) is set up in experimental hutch 2 (EH2), with an X-ray energy range of 6.5–17.7 keV. NM is primarily used to determine the structure of challenging targets such as membrane proteins and macromolecular complexes, using a high-flux beam of 5.5×10^{12}–2.9×10^{13} photons/s at 12.4 keV. The high-energy mode (HM) enables data collection using X-rays of 20–35 keV in experimental hutch 1 (EH1) and provides unique opportunities, such as ultrahigh-resolution data collection [1].

After the alternative public MX beamline, BL45XU, which has nearly identical specifications to BL41XU and specializes in automatic data collection using the ZOO system [2], began operating in 2019, we decided to adapt BL41XU for structural dynamics studies including time-resolved (TR) crystallography and room-temperature (RT) crystallography. Here, we report our activities in FY2024.

2. Development for high-frame-rate and high-precision event acquisition system

At BL41XU, we are preparing to conduct accurate time-resolved experiments by introducing high-precision axis control using the PMAC motion controller.

We developed an event acquisition system that achieves highly accurate synchronous control (Fig. 1). The system can log RF, detector trigger, and shutter ON/OFF signals. It can also synchronously validate digital signals with an accuracy of 1 μs and analog signals with an accuracy of 1 ms. Furthermore, the system logs hundreds of devices and can efficiently manage the status of beamline devices.

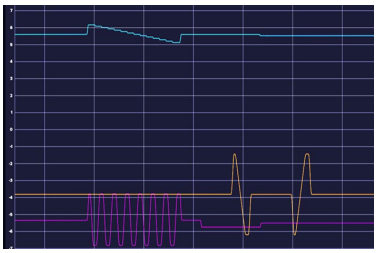

Fig. 1. Event acquisition system. It collects and displays the operation of the X-axis (orange), Y-axis (magenta), and Z-axis (cyan) of the goniometer during 2D scanning using X-rays.

3. Evaluation of X-ray chopper system

We propose to upgrade BL41XU to SPring-8-II to enable serial crystallography using a pink beam. This upgrade requires a chopper to enable sub-microsecond exposure, thus avoiding radiation damage to the sample from continuous X-ray exposure. In preparation for the pink beam, we have worked to establish an environment in which the chopper is synchronized with the accelerator's radio frequency (RF) signal.

Last year, the RF signal line was extended to the BL41XU experimental hutch. This year, we have prepared the necessary items, such as amplifiers, a signal distributor, and frequency dividers, to utilize the signal. We plan to use a

chopper developed at SPring-8 [3], but the manufacture of its control board has been discontinued, which makes maintenance and production impossible. With the help of Dr. Kudo from the JASRI Research DX Division, however, we were able to create a new control board and successfully verify its offline operation.

We have also fabricated a stand to install the chopper upstream of the diffractometer in EH2. Next year, we plan to conduct online tests of the chopper.

4. Development for TR crystallography

The time-resolved structure analysis of proteins provides a deep understanding of reaction mechanisms along reaction pathways. We have set up an environment for time-resolved data collection using serial crystallography, aiming for time resolutions of milliseconds or longer.

4.1. Injector system for time-resolved experiments

To facilitate time-resolved structural analysis, we have constructed a measurement system that uses a high-viscosity cartridge injector (HVC), which was developed at SACLA [4]. To this system, we newly introduced a sample catcher to stabilize the sample stream (Fig. 2). This device has a rotating cylinder that assists in stabilizing the flow by reeling in the sample ejected from the injector to the X-ray irradiation position. Additionally, we installed a laser excitation system that synchronizes continuous wave laser irradiation with the detector readout and detects the intensity of the laser illuminating the sample. Evaluation tests confirmed the performance of these systems, making it possible to conduct time-resolved experiments using laser excitation.

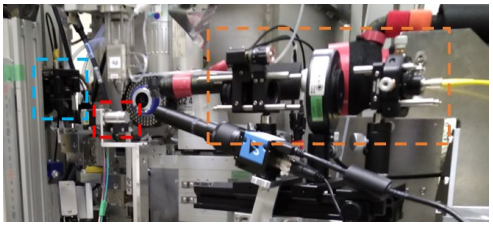

Fig. 2. Overview of the injector system with laser optics. Red-dash square: sample catcher. Orange-dash square: CW laser optics. Blue-dash square: laser detector.

4.2. Fixed-target serial system

We are 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 along the X-ray beam path using high-speed 2D translational stages. We are developing a method to initiate reactions by spraying substrate solutions onto protein crystal positions within this chip using an inkjet system. To prevent the dehydration of samples, we enclosed the sample holder with a transparent film and implemented a temperature and humidity control system that allows diffraction data measurement while air is blown (Fig. 3a). In an offline test, we evaluated the control parameters for dispensing droplets with the inkjet. We also developed and installed a micro-drop observation system comprising a combination of a video camera and lighting to confirm that the droplets land on the

sample plate. For the online environment, we fabricated fixtures to mount the inkjet, observation camera, and lighting at the diffractometer of BL41XU (Fig. 3b). Furthermore, we developed software for data processing. This software allows for the real-time plotting of the hit rate after measurement begins (Fig. 3c) and displays the hit and indexing rates for each measurement in a web browser (Fig. 3d).

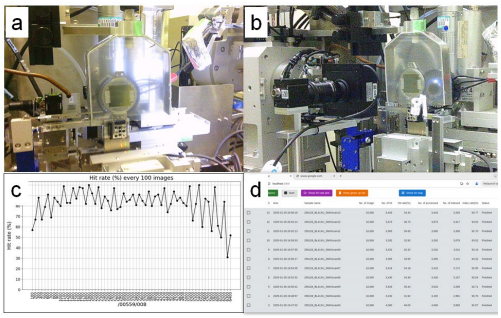

Fig. 3. Fixed-target serial system. (a) Sample holder with a transparent film and a temperature and humidity control system. (b) Fixture for installing an inkjet, an observation camera, and lighting. (c) Hit rate plot. (d) Hit rate and indexing rate results.

5. Crystallization plate diffractometer for RT crystallography

A compact-type crystallization plate diffractometer with X, Y, and Z axes on the ω axis was designed and evaluated (Fig. 4a). Initial test operations confirmed that the translational accuracy of the Y-axis and eccentricity accuracy of the ω-axis were insufficient. Therefore, to improve the eccentricity of the ω-axis and to increase the accuracy of Y-axis movement, the Y-axis was updated with a highly rigid stage and the layout under the ω-axis was modified (Fig. 4b). The improved diffractometer will be available to users in 2025 after evaluation testing.

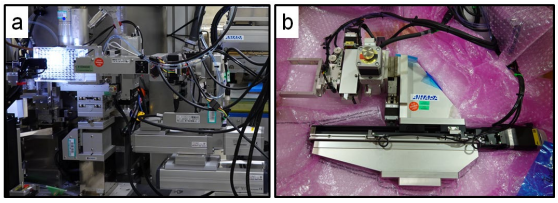

Fig. 4. Crystallization plate diffractometers. (a) Diffractometer of initial design. (b) Improved diffractometer.

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References:

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