BL41XU
(Structural Biology I)

BL41XU is a public macromolecular crystallography (MX) beamline that uses an undulator as a light source. The beamline is mainly employed for 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). Since the beam size can be changed from 5 μm (H) × 5 μm (V) to 20 μm × 45 μm, it can handle samples whose sizes range from a few to several hundred μm. Another characteristic feature is that BL41XU can collect data using high-energy X-rays from 20 keV to 35 keV. Here we report our activities in FY2018.

1. Installation of Eiger 16M

Eiger 16M was installed at BL41XU for the normal operation mode (NM), which covers a wavelength range of 0.7–1.9 Å. Compared to the previous detector, Pilatus3 6M, Eiger 16M has a much shorter dead time of 3 μs, which enables more rapid data collection. The smaller pixel size of 75 μm × 75 μm provides more accurate data collection from large unit cell crystals.

Fig. 1. Eiger16M installed at experimental hutch 2.

2. Operation of a new diffractometer for the high-energy mode

The high-energy mode (HM) is useful for ultrahigh-resolution data collection beyond 0.8 Å. It also allows for positional determination of anomalous nuclei whose absorption edges are located at these energy ranges. So far we had used the old diffractometer which had been used for NM before the upgrade of BL41XU in 2014. To improve the HM setup and achieve more accurate data collection, a new diffractometer designed for HM was installed in FY2018 (Fig. 2 (a)).

After the installation and offline alignment of the diffractometer, commissioning was performed in 2018A. In the new diffractometer, parasitic scattering, which previously was a problem, was completely removed by the 0.5-mm pinhole placed 40-mm upper stream from the sample (Fig. 2 (b)). Therefore, the lowest resolution was extended down to 25 Å even at a wavelength of 0.35 Å.

One feature of the new diffractometer is the installation of a translation stage for the X-ray compound refractive lens (CRL). We confirmed that the beam size can be changed from 10 μm to 50 μm by a 260-mm translation of CRL along the beam path. The performance of the new diffractometer was verified using a standard sample crystal of lysozyme in the *P*1 form. We successfully obtained diffraction data up to 0.6-Å resolution with good statistics, $\langle I/\sigma_I \rangle$ of 2.3 and $CC_{1/2}$ of 0.757 at the highest resolution shell. The new diffractometer is

now available to users.

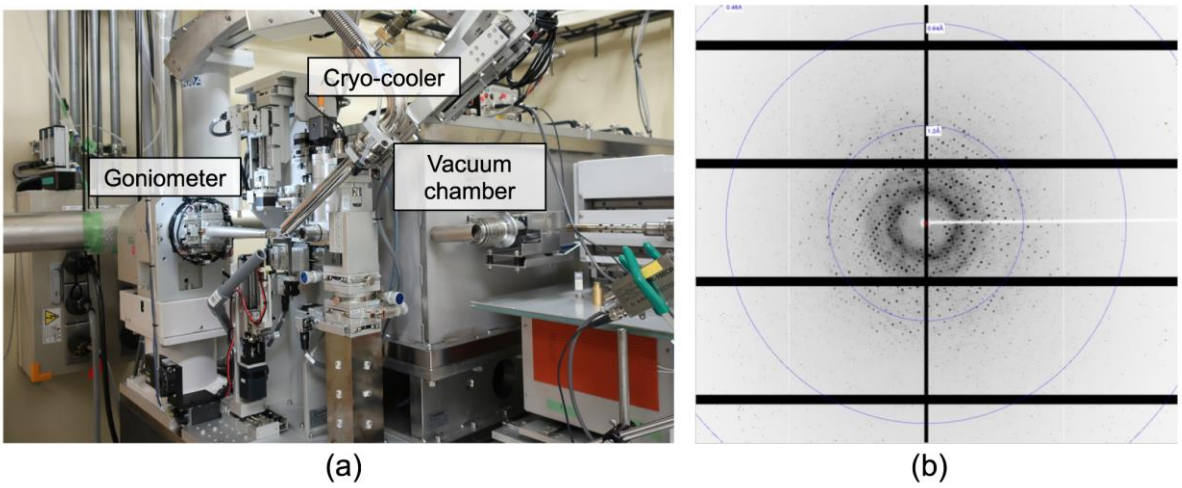

Fig. 2. (a) Diffractometer for the high energy mode and (b) diffraction pattern of *P*1 lysozyme crystal.

3. Development of HAG RT-SSX

In the past two decades, most MX data were collected at cryogenic temperature (CT) to mitigate the influence of radiation damage. However, structures at room temperature (RT) can reveal structural features that cannot be observed at CT. The challenge for RT data collection is progression of radiation damage at 70 times lower absorbed dose compared to CT [1]. Therefore, RT data collection of microcrystals is quite difficult at synchrotron beamlines. To cope with this problem, we combined serial synchrotron rotation crystallography (SS-ROX) [2] with humid air and a glue-coating (HAG) technique [3], and demonstrated its feasibility using a standard sample.

Microcrystals of lysozyme (3 μm in size) were scooped by a 1-mm round-shaped Kapton loop after spreading polyvinyl alcohol over the loop. These were mounted on the goniometer. Diffraction data were collected by a two-dimensional raster scan under humidity-controlled air. After merging 7,700 indexed images, we obtained data with up to 2.3 Å resolution. The refine structure had R_{work} of 17.0% and R_{free} of 25.6%, with a good quality electron density map. Our results show that the HAG is applicable to RT data collection of microcrystals.

One problem with SS-ROX experiments at BL41XU was that a real-time data reduction system had yet to be implemented. Real-time calculation of statistics such as hit rate, index rate, completeness, and achieved resolution are advantageous, because it is directly related to beamtime and simple consumption. Therefore, we installed multi-core computers to process data in real time.

4. Development of a sample cassette storage system

Installation of SPACE-II at BL41XU reduced the sample exchange time to 16 s, contributing to efficient use of beamtime. Another issue for SPACE-II was the limited number of samples that could be stored simultaneously. To resolve this issue, we designed and manufactured a sample cassette storage system, which can store up to 42 sample

cassettes (Fig. 3). Cassettes are transferred from the storage system to SPACE-II via a multi-axis robot. This will be especially useful for our new undulator MX beamline BL45XU, where almost all data will be collected automatically.

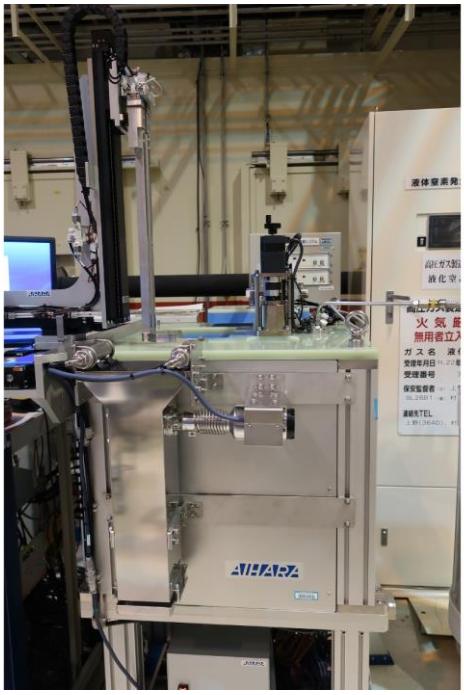

Fig. 3. Sample cassette storage system.

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