SPring-8 BL44XU, a synchrotron radiation beamline for biological macromolecular assemblies, operated by the Institute for Protein Research, Osaka University

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The use of X-ray crystallography for structure determination of large macromolecular assemblies can encounter many difficulties. In addition to the difficulties associated with crystallization, the unit cells of crystals built from macromolecular assemblies can be very large and additionally the crystals are often extremely fragile. These problems cause difficulties with the collection of diffraction data related to extremely weak diffraction power, narrow spacing between diffraction spots, and extreme X-ray radiation sensitivity of the crystal. These features particular to crystals of biological macromolecular assemblies often pose a severe limitation to the collection of high-quality diffraction data. Synchrotron radiation is an indispensable resource for overcoming these limitations associated with data collection from such biological macromolecular crystals possessing large unit cell and poor diffraction properties.

The synchrotron radiation beamline BL44XU at SPring-8 is specially designed for X-ray crystallography of crystals built from biological macromolecular assemblies (Higashiura et al. 2016). The optimized BL44XU beamline has been managed by the Institute for Protein Research (IPR) of Osaka University since 1999. The IPR BL44XU beamline is capable of high-precision diffraction data measurement from biological macromolecule crystals

due to a combination of the high brilliance and highly parallelized synchrotron radiation and high-performance detector possessing a large measurement area. The light source of BL44XU is a SPring-8 standard type in-vacuum undulator. The X-rays are monochromatized by a liquid-nitrogen-cooling silicon double-crystal monochromator and focused (or collimated) using rhodium-coated horizontal and vertical mirrors (TOYAMA Co. Ltd., Japan) (Fig. 1). Wavelength range 0.7 Å to 1.95 Å is available. A pinhole system is used to define various beam shapes and sizes to support diverse crystal types and measurement conditions. The photon flux on a sample position of this beamline is about 4×10^{12} photons/s after passage through the 50-μm pinhole at a wavelength of 0.9 Å. The goniometer is controlled by a high-speed air-bearing goniometer with a small sphere-of-confusion ($< 1 \mu\text{m}$) (Fig. 2). A multi-axis goniometer, SmarGon (SmarAct GmbH, Germany), will be installed in this coming year (2019). The SmarGon will work as virtual 4-circle diffractometer with ω , κ , and ϕ -axes, providing freedom of crystal orientation and enabling various experiments, such as simultaneous data collection of Bijvoet pairs, sample rotation-assisted escape from diffraction spot overlap, and reduction of the blind region in the reciprocal space. A state-of-the-art large area photon counting-type pixel array detector, the Eiger X 16 M (Dectris, Switzerland), was installed in 2018 and is mounted on the bench with a wide crystal-to-detector distance of 115–1200 mm with a vertical offset of 0–150 mm and 2θ angle of 0–15 degrees. This detector is capable of collecting up to 3.7 Å resolution data from a crystal with a unit cell length of 2000 Å. In addition, the beamstop can be moved 16 to 99 mm from the sample position to the downstream direction. Ultra-low-resolution data ($> 400 \text{ Å}$) can be collected by

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Fig. 1 Schematic of the beamline layout of the optics hutch and the experimental hutch

changing the position of the beamstop. Ultra-high-resolution data (<0.6 Å) also can be collected with this system. The sample crystal can be cooled to 90 K by nitrogen gas or 30 K by helium gas using a cryo-stream system (Cryo Industries of America, Inc., USA). The beamline operation software BSS (Beamline Scheduling Software) and sample auto-changer SPACE system (SPRING-8 Precise Automatic Cryo-sample Exchanger) are installed to unify user operation throughout protein crystallography beamlines in the SPRING-8. Sample storage using SPACE enables the operator to set 8 Unipucks (Molecular Dimensions, UK) and crystals can be unmounted and mounted in less than 20 s.

As an example of the advantages associated with the IPR BL44XU specialist beamline, here we describe its usage to determine the structure to 3.5 Å resolution of the 10-MDa large ribonucleoprotein, vault (isolated from rat liver). The

unit cell dimension was $a = 702.246$ Å, $b = 383.796$ Å, $c = 598.480$ Å, and $\beta = 124.69^\circ$ with $C2$ space group (Tanaka et al. 2009) (Fig. 3).

Structure of the *Rice Dwarf Virus* (RDV) was previously determined at 3.5 Å resolution using the diffraction data collected at the Photon Factory, KEK, Japan (Nakagawa et al. 2003). The unit cell dimensions of RDV are some of the largest deposited in the Protein Data Bank; $a = 770.5$ Å, $b = 796.2$ Å, and $c = 812.2$ Å with $I222$ space group. Up to around 2.3 Å resolution diffraction spots are observed using a FWHM beam size around 0.05×0.05 mm² and an exposure time of a few seconds. The low-resolution reflection with (0 2 0), which corresponds to around 400 Å resolution, could be observed with the beamstop placed at 99 mm from the sample position and the wavelength of 1.9 Å. For high-resolution data collection, a dataset of bovine H-protein was collected at BL44XU and the structure was refined at 0.87 Å resolution. As a result,

Fig. 2 Environment at the sample position

Fig. 3 Ribbon representation of a Vault from rat liver. The particle is related by 2-fold fold symmetry which shares the crystallographic 2-fold axis

about 40% of the electron density of the hydrogen atoms and many multiple conformations were visualized (Higashiura et al. 2013).

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Compliance with ethical standards

Conflict of interest Eiki Yamashita declares that he has no conflict of interest. Atsushi Nakagawa declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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