BL44XU
Macromolecular Assemblies

1. Introduction

BL44XU is the beamline for macromolecular assemblies [1,2]. It is designed for high-precision diffraction data measurements from large biological macromolecular assemblies. It has been managed by the Institute for Protein Research (IPR) of Osaka University since 1999. This beamline was initially constructed with financial support from Japan Society for the Promotion of Science (Research for the Future Program), Japan Science and Technology Corporation (currently Japan Science and Technology Agency: JST), and the Ministry of Education (currently the Ministry of Education, Culture, Sports, Science and Technology: MEXT). Since then, the beamline has been upgraded with financial support from the IPR of Osaka University, the National Project on Protein Structural and Functional Analyses by MEXT, the Targeted Proteins Research Program by MEXT, the Platform Project for Supporting Drug Discovery and Life Science Research (Platform for Drug Discovery, Informatics and Structural Life Science: PDIS) by MEXT and Japan Agency for Medical Research and Development (AMED), the Platform Project for Supporting Drug Discovery and Life Science Research (Basis for Supporting Innovative Drug Discovery and Life Science Research: BINDS) by AMED, the JAXA-GCF project 'High-Quality Protein Crystallization Project on the Protein Structure and Function Analysis for Application' by the Japan Aerospace Exploration Agency (JAXA), and Grants-in-Aids for Scientific Research by MEXT.

2. Overview of the beamline

X-ray diffraction from a crystal with a biological macromolecular assembly is generally weak and closely spaced owing to its large unit cell. Therefore, diffraction data collection requires high-brilliance paralleled synchrotron radiation as well as a high-performance large-area detector. The light source of this beamline is a SPring-8 standard in-vacuum undulator with 140 periods. The beamline consists of an optics section, a goniometer section, and a detector section (Fig. 1).

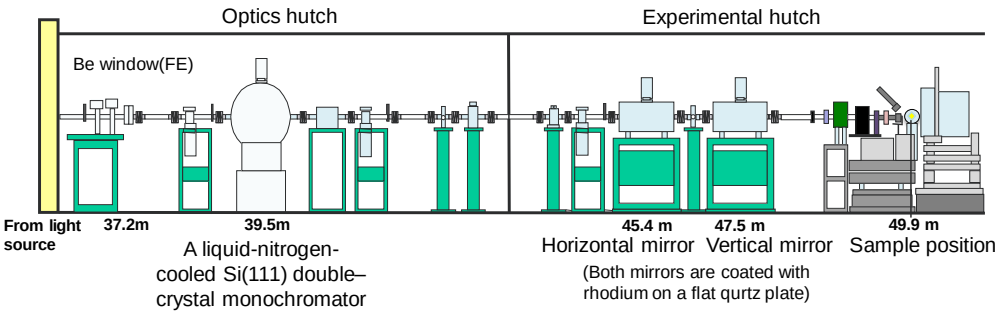

Fig. 1. Beamline components.

3. Optics section

X-rays are monochromatized by a liquid-nitrogen-cooled Si double-crystal monochromator and focused by rhodium-coated horizontal and vertical mirrors. Various beam shapes and sizes are defined using a pinhole system to support diverse crystal shapes and sizes as well as diverse measurement conditions. The photon flux on a sample position is about 5.1 × 10^{12} photons/s after a 50-μm pinhole at a 0.9-Å wavelength. The high-speed shutter can be opened/closed in 1 ms. For use in experiments with different crystal sizes/shapes and experimental conditions, 12 different pinholes are available (Fig. 2). The exit slit placed just after the pinhole can reduce the background noise caused by parasitic scattering and air scattering. Users can change the wavelength between 0.7 Å and 1.9 Å without the assistance of the beamline staff.

4. Goniometer section

The goniometer section consists of a multi-axis goniometer, a direct beam stopper with an *x*-direction translation stage, an LED light source, a coaxial telescope, and a Cryocool system (Fig. 3). This beamline can collect ultralow-resolution data below 400 Å (Fig. 4). The goniometer is controlled by a high-precision multi-axis goniometer,

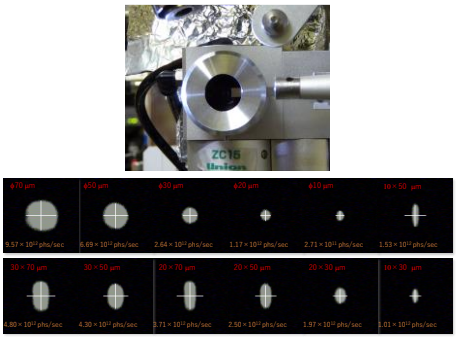

Fig. 2. (upper) Pinhole system. (lower) Shapes and beam intensities obtained when using different pinholes.

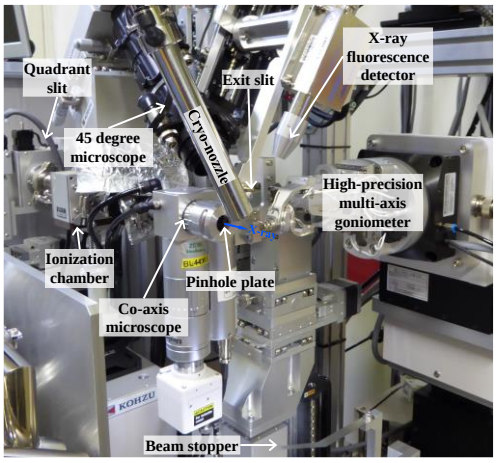

Fig. 3. Goniometer section.

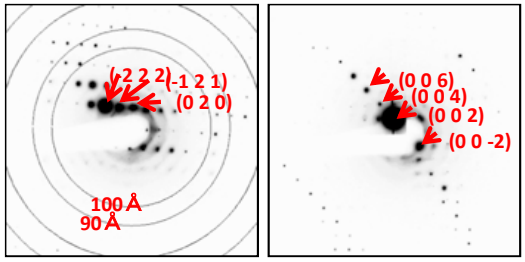

Fig. 4. Ultralow-resolution data from a rice dwarf virus crystal (*I*222, *a*=768.9, *b*=794.7, *c*=810.4 Å) (λ=1.9 Å).

Table 1. Specifications of SmarGon

Axis	ω (#6)	χ (#3/#4)	ϕ (#5)
Travel range (°)	Unlimited	0 – 35	Unlimited
Sphere of confusion (μm)	< 1.0	< 7.0	< 10
Resolution of motion (μ°)	< 100	< 20	< 20
Velocity (°/s)	165	10	80

Axis	X (#3/#4)	Y (#1)	Z (#2)
Travel range (mm)	± 2.0	± 2.0	± 2.0
Resolution of motion (nm)	< 5.0	< 5.0	< 5.0
Velocity (mm/s)	~ 10	~ 10	~ 10

SmarGon (SmarAct GmbH, Germany), which emulates a four-circle goniometer (Table 1) and provides more freedom in the crystal geometry. A crystal can be cooled to 90 K by using nitrogen gas or 30 K by using helium gas with a Cryocool system (Cryo Industries of America, USA).

5. Detector section

A high-efficiency two-dimensional X-ray photon counter, EIGER X 16M (DECTRIS, Switzerland), which was installed in FY2018, is mounted on the bench with a large crystal-to-detector distance of 132–1200 mm, a vertical offset of 0–150 mm, and a 2θ angle of 0–15°. The combination of this high-speed detector and an auto sample changer provides beamline users with high-throughput measurements (Fig. 5).

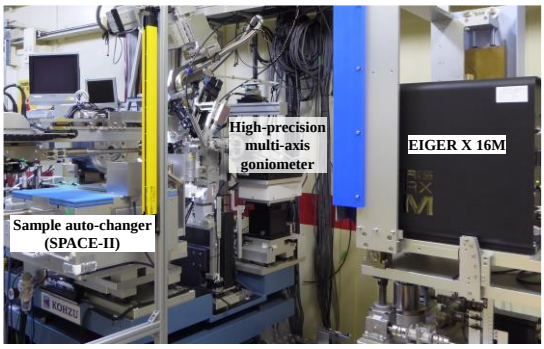

Fig. 5. Detector section with an auto sample changer.

6. Auto sample changer and operation software

The beamline operation software BSS (beamline scheduling software) [3] and the auto sample changer SPACE-II [4] are installed to unify user operations for all protein crystallography beamlines at SPring-8. Eight Uni-Pucks can be set in the SPACE-II sample storage, allowing users to efficiently use 12 h of beam time.

7. Joint usage

As the Joint Usage/Research Center for Proteins, the IPR accepts domestic and international researchers who work on macromolecular crystallography. About 40% of the beam time was allocated to researchers outside of the IPR. In FY2020, about 300 researchers used the beamline. Mail-in data collection service was started to support users during the COVID-19 pandemic. The IPR supported travel expenses for researchers outside of Japan under the International Collaboration Program. In addition, about 10% of the beam time was shared with Taiwan users under an agreement with the National Synchrotron Radiation Research Center (NSRRC) of Taiwan (Fig. 6).

Each year, proposals for experiments at the beamline under the Joint Usage program of IPR are received in early December. In addition, urgent proposals are accepted at any time.

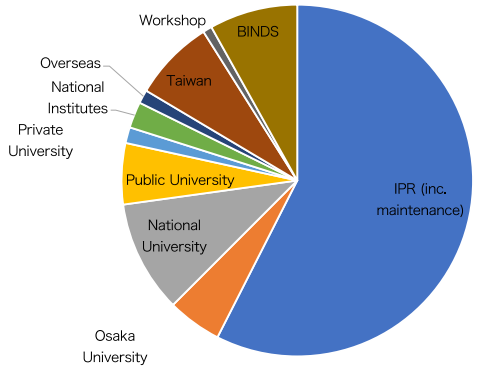

Fig. 6. Beamtime allocation in FY2020.

8. Platform project to support drug discovery and life science research (BINDS)

As part of the Structure Analysis Unit of the BINDS project, the beamline and supporting data collection

of large unit-cell crystals are being upgraded, and about 10% of the total machine time is allocated to the project.

9. Output from the beamline

Vault is a large ribonucleoprotein particle with a molecular mass of about 13 MDa. Its crystal belongs to the space group *C*2 with unit cell dimensions of a = 707.2, b = 383.8, c = 598.5 Å, and β = 124.7°. 3.5-Å-resolution diffraction data was collected on this beamline, and its atomic structure was successfully solved [5].

Various important structures were determined using the data collected at BL44XU: the S_8-meSRK–S_8-SP11 complex [6], the PMab-1 Fv-clasp/MAP complex [7], peptidyl tryptophan dihydroxylase [8], and methanol-PQQ-bound methanol dehydrogenase [9] (Fig. 7).

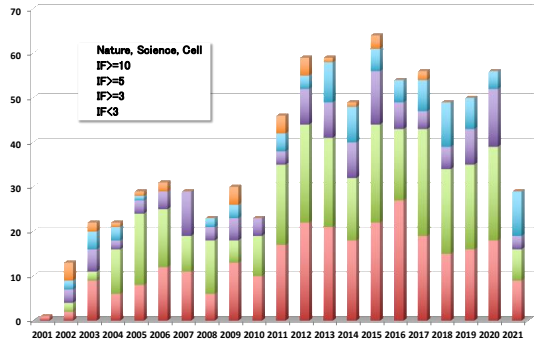

Fig. 7. Publications of research from BL44XU (IF: impact factor).

10. International collaborations

International collaborations and academic exchanges between the NSRRC and the IPR have promoted scientific activities under the agreement between the two organizations since 2007.

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