BL44XU
(Macromolecular Assemblies)

1. Introduction

BL44XU at SPring-8, which is called the beamline for macromolecular assemblies, is designed for high-precision diffraction data measurements from large biological macromolecular assemblies. Since 1999, it has been managed by the Institute for Protein Research (IPR) of Osaka University. This beamline was initially constructed with financial support of the Research for Future Program by the Japan Scientific Promotion Society, the Japan Science and Technology Corporation (currently Japan Science and Technology Agency: JST), and the Ministry of Education (currently, Ministry of Education, Culture, Sports, Science and Technology: MEXT). Since then, the beamline has been upgraded by the financial support from the Institute for Protein Research 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 in Drug Discovery and Life Science Research (Platform for Drug Discovery, Informatics, and Structural Life Science) by MEXT, 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 Japan Aerospace Exploration Agency (JAXA), and Grants-in-Aid 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 due to its large unit cell. Therefore, data diffraction collection requires high brilliance and paralleled synchrotron radiation as well as a high-performance large-area detector. The light source of this beamline is a SPring-8 standard type in-vacuum undulator with 140 periods. The beamline consists of an optics section, goniometer section, and detector section (Fig. 1).

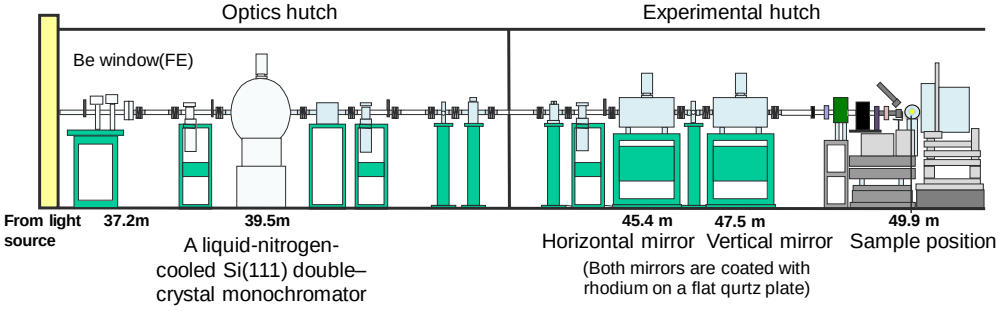

Fig. 1. Beamline components.

3. Optics section

X-rays are monochromatized by a liquid nitrogen–cooled Si double-crystal monochromator and focused (and/or collimated) 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 along with various measurement conditions. The photon flux on a sample position is about 4×10^{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. To adapt to different crystal sizes/shapes and experimental conditions, 12 different pinholes are available (Fig. 2). The exit slit placed just after the pinhole can reduce 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 high-precision goniometer with a μ-axis, direct beam stopper with an *x*-direction translation stage, LED light, co-axial telescope, and cryo-stream cooler (Fig. 3). Very low–resolution data below 400 Å resolution can be collected with this beamline (Fig. 4). The goniometer is controlled by a high-speed air-bearing goniostat with a small sphere-of-confusion (< 1 μm) (Kohzu Precision). A crystal spindle axis can be inclined from 0° to 10° by the μ-angle axis, which is perpendicular to the horizontal plane to provide more freedom from the crystal geometry and reduce the blind region in reciprocal space. A 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, USA).

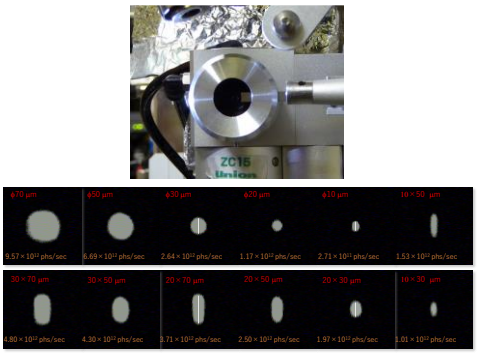

Fig. 2. (upper) Pinhole system. (lower) Shapes and beam intensities after different pinholes.

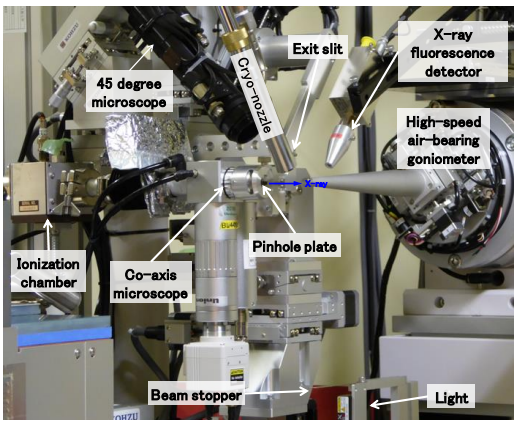

Fig. 3. Goniometer section

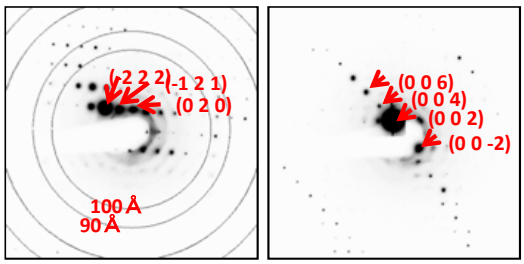

Fig. 4. Ultralow-resolution data from a Rice Dwarf Virus crystal (*I*222, *a*=768.9, *b*=794.7, *c*=810.4 Å) (λ=1.9 Å).

5. Detector section

A high-efficiency two-dimensional X-ray photon counter, EIGER X 16M (DECTRIS), which was installed in 2018, is mounted on the bench with a wide crystal-to-detector distance of 115–800 mm, vertical offset of 0–150 mm, and 2θ angle of 0–15°. The combination of this high-speed detector and an automatic sample changer (described below) provides beamline users with high-throughput measurements (Fig. 5).

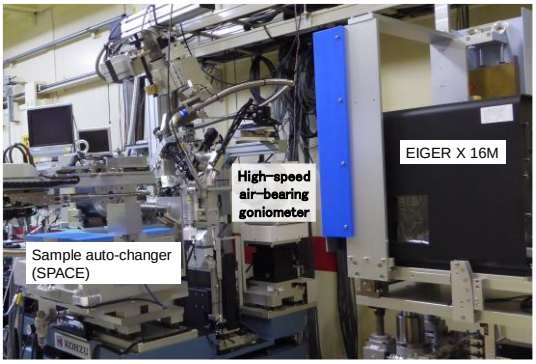

Fig. 5. Detector section with a sample changer.

6. Sample changer and operation software

The beamline operation software BSS (Beamline Scheduling Software)[1] and a sample auto-changer SPACE (SPring-8 Precise Automatic Cryo-sample Exchanger)[2], whose mount arm was upgraded to a double-mount arm in 2018, are installed to unify user operations for all protein crystallography beamlines at SPring-8. Eight Uni-Pucks can be set in the SPACE 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 50% of the beamtime was allocated to researchers outside of the IPR. In 2018, a total number of more than 800 researchers used the beamline. The IPR supported travel expenses for researchers outside of Japan under the International Collaboration Program by IPR. In addition, about 10% of the beamtime was shared with Taiwan users under an agreement with the National Synchrotron Radiation Research Center (NSRRC) of Taiwan (Fig. 6).

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

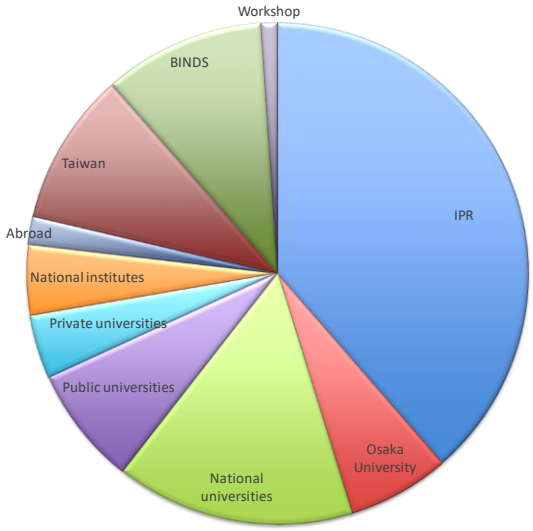

Fig. 6. Beamtime allocation in FY2018

8. Platform project to support drug discovery and life science research (Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS))

As a member of the Structure Analysis Unit of the BINDS project, we are upgrading the beamline and supporting data collection of large unit-cell-crystals and allocating about 10% of the

total machine time 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°. The beamline collected 3.5 Å resolution diffraction data, and its atomic structure was successfully solved [3].

Various important structures were determined using the data collected at BL44XU: the B12-dependent isomerases (eliminating) diol dehydratase and ethanolamine ammonialyase complexed with adenosylcobalamin [4]; SmgGDS, which has dual functions in cells and regulates small GTPases as both a guanine nucleotide exchange factor (GEF) for the Rho family and a molecular chaperone for small GTPases [5]; ubiquitin by genetic fusion to the highly porous honeycomb lattice of R1EN [6]; p62/SQSTM1 autophagy adapter [7]; and efflux transporter AcrB [8] (Fig. 7).

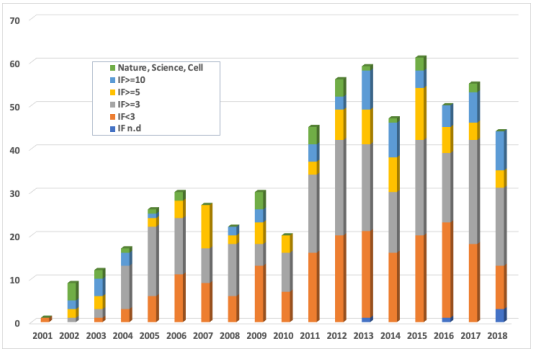

Fig. 7. Publications of research from BL44XU (IF: Impact Factor).

10. International collaborations

International collaborations and academic exchanges between the NSRRC and the IPR promote scientific activities under a 2017 agreement between the two organizations.

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