BL32XU
RIKEN Targeted Proteins

1. Introduction

BL32XU is the RIKEN targeted protein beamline at SPring-8, dedicated to collecting high-resolution diffraction data from protein microcrystals. Since its launch, the beamline has specialized in providing structural biology researchers with unique opportunities to study challenging proteins, including membrane proteins and protein complexes.

Since FY2015, a fully automated data-collection system, ZOO [1,2], has been developed and continuously upgraded at BL32XU. ZOO enables unattended high-throughput diffraction experiments using a goniometer, allowing users to obtain high-resolution datasets remotely simply by shipping their cryocooled crystal samples. This system has become the backbone of protein crystallography operations at BL32XU, especially during and after the COVID-19 pandemic, when remote experiments became essential.

As part of the AMED/BINDS program, BL32XU has played a key role in supporting structural biology for drug discovery, with a strong track record in targeting challenging proteins, such as GPCRs. Beyond BINDS, the beamline continues to serve a wide community of academic users through the public beamtime framework.

In parallel with routine operation, BL32XU has also been engaged in research and development to advance data collection and analysis methodologies. These efforts include the following:

- establishing high-throughput pipelines for microcrystal structure determination,
- developing methods to explore structural polymorphism and dynamics from large-scale datasets, and
- implementing robot-assisted crystal handling systems such as HACHI and ARI to overcome bottlenecks in sample preparation.

Together, these developments make BL32XU one of the leading synchrotron facilities worldwide for automated, high-throughput, and high-resolution macromolecular crystallography.

2. Operation

In FY2024, ~60% of the beamtime at BL32XU was allocated to users of the AMED/BINDS project and ~20% to public use. The remaining time was utilized for in-house research and development.
Automated measurements using the ZOO system have become fully established, allowing users to send cryocooled crystals to the beamline. The entire process, from data collection to preliminary processing, is carried out automatically, and results are returned to the users in a stable and efficient manner.

3. Research and Development

An international collaborative team, including RIKEN, Institute of Science Tokyo, and Kochi University, analyzed the structure of a hydrothermal vent at a depth of 5,700 m in the Mariana Trench. The team discovered nanoscale channels that selectively transport ions, revealing that the hydrothermal vent itself generates electricity. This mechanism, which relies on ion concentration gradients, is identical to the principle by which living organisms—including humans—produce energy within cells. The finding provides critical clues to the origin of life on Earth [3].

A collaborative research group from Institute of Science Tokyo, Hokkaido University, Kyoto Prefectural University, and RIKEN developed a novel approach to determining the structures of intrinsically disordered proteins (IDPs). Using the cell-free protein crystallization (CFPC) method, the team fused IDPs with polyhedrin protein crystals that serve as a stable scaffold. This "puzzle-like" assembly allowed the structural determination of IDPs that were previously intractable. The approach significantly reduced the experimental scale (from 3 L to 30 μL) and time (from 3 months to within 3 days). Furthermore, the system was combined with a publicly available web-based structural analysis platform, enabling rapid molecular design. Applying this method, the team succeeded in determining the α-helical structure of a critical region of the IDP c-Myc, closely resembling its natural conformation. This achievement paves the way for drug design based on previously inaccessible protein structures and for high-throughput data-driven drug discovery [4].

At BL32XU, a new methodology that integrates hundreds to thousands of small-wedge diffraction datasets to achieve substantial improvements in both the resolution and accuracy of electron density maps was developed. Increasing the number of observations clarified fine structural details, enabling high-resolution structure determinations that were previously unattainable. To ensure efficiency, machine learning was applied to group datasets based on crystal quality and structural similarity, enabling the selection of more targeted data with fewer steps. The method is broadly applicable to various protein samples, including membrane proteins, and is expected to contribute widely in areas from fundamental research to pharmaceutical applications [5].

4. WebDB ECHA and Beamline Implementation

A web-based experimental database (ECHA), currently under development at RIKEN, has been introduced to BL32XU. In FY2024, ZOO was upgraded to automatically register experimental conditions provided by users through Excel files into the ECHA system, allowing fully automated measurements. A pilot function enabling users to download test measurement results via the web was also introduced. Looking ahead, the integration of ZOO and ECHA will allow users to register experimental conditions remotely, trigger automated data collection, receive notifications upon completion, and view results through a web browser.

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