BL32XU
RIKEN Targeted Proteins

1. Introduction

BL32XU is the RIKEN target protein beamline specialized for collecting high-resolution diffraction data from protein microcrystals. Since FY2015, RIKEN has been developing a fully automated data-collection system called "ZOO" at BL32XU, specifically designed for protein crystallography [1]. ZOO enables fully automated data collection in all experimental schemes that use a goniometer. Therefore, remote users can obtain high-resolution datasets using SPring-8 simply by sending crystal samples. Since FY2017, as part of the Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS) program, BL32XU has supported numerous structure determinations of challenging proteins, such as membrane proteins [2,3] (e.g., GPCR).

2. Recent activities

Because of the global COVID-19 outbreak and the resulting restrictions on on-site experiments, users no longer need to come to the site; automated data collection using ZOO has become the primary measurement method. With ZOO's automated data-collection system, users need only to send cryo-cooled crystal samples to SPring-8, and the measurements, including raw data acquisition and data processing, will be automatically completed. Sixty percent of BL32XU's machine time was allocated to the BINDS project, and almost all of that time was used for automated measurements.

The main research, development, and outcomes at BL32XU this fiscal year are as follows:

(1) High-throughput accumulation of high-resolution structures,

(2) Development of structural analysis techniques for understanding structural dynamics, and

(3) Development of an automatic crystal harvest system for large-scale data collection.

For (1), the accumulation of high-resolution structures remains an ongoing challenge in the field of structural biology. It is a permanent task for BL32XU, and we have been conducting research, development, and operations to achieve this. We continued the development of a high-throughput data collection system utilizing ZOO. In collaboration with Professor Brian Kobilka of Stanford University, we reported the results of a typical membrane protein crystal structure analysis using small-wedge synchrotron crystallography (SWSX), which is a strength of ZOO [4].

For (2), a new paradigm has emerged in crystal structure analysis owing to the significant improvement in data collection efficiency. Developments in (2) involves finding and classifying polymorphic structures within a large number of datasets and performing high-resolution structural analysis of each. To implement this, we utilized the already-developed automated data processing system, KAMO [5]. We investigated a method for classifying groups with different structures using hierarchical clustering based on intensity correlations among diffraction data (referred to as HCCC). In standard trypsin samples, we prepared two types of crystal, the apo form and the ligand-bound form, and collected diffraction data from each. By mixing the diffraction data on

the computer, we examined whether they could be classified back into their original forms using HCCC. As a result, even though the structural difference between the apo form and ligand-bound form was as small as 1%, HCCC successfully classified them. We continued to examine this method using more practical samples and published a paper demonstrating that this method is useful for identifying structural polymorphisms within large datasets [6].

For (3), as the performance of the beamline has improved and automated data collection has accelerated, the efficiency of diffraction data collection has improved significantly. As a result, the bottleneck in structure analysis is shifting to the processes of crystallization and transporting crystals to the beamline. We have initiated the development of a robot to automate crystal sample preparation. We have already developed a semi-automatic crystal cryo-cooling system called HACHI, which uses a six-axis collaborative robot called Universal Robot (UR3e). We are currently developing a robot called ARI, which punches out and mounts sample holders from film sandwich plates, cryo-cools the samples, and stores them in a universal sample container called Unipuck. The crystal plates use DiffraX plates from Molecular Dimensions, and the crystals in the 5-mm-diameter wells are punched out and attached to a dedicated holder. This freezing method utilizes the wells and allows full use of crystallized proteins obtained through batch methods for measurement. We aim to combine ARI and HACHI to automatically cryo-cool sample crystals and store them in Unipucks.

Moving forward, we will continue to carefully meet ongoing and new challenges, conduct research and development, and consider the use of new light sources.

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