BL32XU
RIKEN Targeted Proteins

1. Introduction

BL32XU is the RIKEN targeted protein beamline dedicated to high-resolution diffraction data collection from protein microcrystals. Since FY2015, we have been developing a fully automated data-collection system dedicated to protein crystallography, which is named ZOO, at BL32XU [1]. ZOO covers all existing experimental schemes in goniometer-based data collection from protein crystals. Furthermore, it has achieved unattended data collection. Hence, remote users can acquire high-resolution datasets using SPring-8 just by sending crystal samples. BL32XU has supported numerous structure determinations of challenging proteins such as membrane proteins [1–3] (e.g., GPCR) as part of the BINDS (Basis for Supporting Innovative Drug Discovery and Life Science Research) program since FY2017.

2. Recent activities

The most significant change that occurred at the beamlines during FY2020 was the restriction of experiments caused by the global COVID-19 pandemic. First, severe restrictions were placed on user access at the SPring-8 site, and there are many beamlines where normal measurements are no longer possible. In such a situation, the automatic data acquisition system we developed for structural biology beamlines such as BL32XU played a very important role. With the automatic data acquisition system, users should only send a frozen crystal sample to SPring-8 to complete the measurement itself. In other words, there is no need to come to SPring-8 because unattended measurements are possible. ZOO has contributed to the crystallographic analysis of several COVID-19-related proteins in the urgent proposal frame prepared for COVID-19. ZOO has also contributed significantly to several COVID-19-related protein crystallography projects.

At BL32XU, we have set the following themes for sustainable upgrading related to data collection and analysis technologies: (1) the high-throughput accumulation of high-resolution structures, (2) the development of technology for data collection from difficult crystal samples, and (3) the development of structural analysis technology for understanding structural dynamics.

In relation to (1), we have started to develop a system that is well integrated with ZOO to achieve even higher throughput data collection. In particular, in FY2020, we developed HACHI, an automated crystal cooling system using collaborative robots. A so-called ‘cobot’ is an industrial robot with a 6-axis arm that can work safely in coexistence with humans and can be made to automatically perform a series of tasks by inputting a motion sequence. In the HACHI system we developed, the robot quickly freezes, in liquid nitrogen, the sample crystals received from the user and then automatically stores the sample in the specified ID in the sample container.

In the area of upgrading related to (2), we continued to improve the intelligence of ZOO during automatic data collection from the previous year. Specifically, we upgraded the HITO component implemented in ZOO, which automatically selects measurement schemes and

brushes up the procedures to be followed for each measurement scheme. Upgrades from last year include the ability to perform the measurement carefully and quickly so that it is more accurate and closer to human behavior, and the sophistication of the determination of the coordinates of both ends of the crystal when the HITO component collects a "partial helical dataset".

A development related to (3) is the NABE system, which we started developing as a pipeline to automatically perform structural analysis on the data collected by ZOO. For example, we implemented a function to perform the initial structural analysis of the collected dataset by the molecular replacement method using the PDB coordinates of known structures, and also implemented a function to automatically perform initial phase determination. In particular, since the measurement efficiency of the recent automatic data collection systems has improved explosively, the amount of data that can be acquired continues to increase as well. Therefore, it is becoming increasingly difficult to perform structural analysis and select the most suitable data for corresponding projects on the basis of the electron density map and crystallographic statistics. The NABE system not only performs the structural analysis automatically, but also has the ability to create animated files showing the structure and electron density map in the ROI in 3D and display them on a web browser. This functionality allows the structural analyst to easily select data that may be used for structural analysis in accordance with the purpose via the web browser.

In the automatic data processing system, KAMO, many crystals (or data sets) are often merged and used for structural analysis. In this case, the grouping of datasets by hierarchical clustering may be performed using the cell parameters or the correlation of diffraction intensities as an index. The isomorphism of each crystal is used as an index to create a group of datasets to be merged. In this case, the data obtained from different combinations of datasets have different degrees of isomorphism. In other words, even if the same sample crystal is prepared, there is a possibility that different structures may be mixed, but hierarchical clustering is able to distinguish them.

We started R&D on the possibility of applying the NABE system to the polymorphism analysis of crystal structures by combining the automatic structure analysis and comprehensive ROI display functions described above with the data merging of many patterns by hierarchical clustering.

At present, it has been suggested that crystals of standard protein samples can be immersed in several compounds, automatic data collection can be performed with ZOO, and ROI electron density maps can be examined with the NABE system, which may allow the classification of several compounds by hierarchical clustering. By continuing the case study, it is feasible to induce the reaction inside the crystal and obtain the structure of the intermediate with high resolution by hierarchical clustering based on diffraction intensity correlation. We believe that such an analysis method can probably be applied to the dynamic analysis of protein crystal structures using XFEL as well as to the analysis of single particle structures using cryo-electron microscopy.

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