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, 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 [2,3] (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 at the beamlines during FY2020 was the experimental restriction due to the global outbreak of COVID-19. As in the previous fiscal year, the automated data acquisition system developed for structural biology beamlines such as BL32XU played a vital role in FY2021. With ZOO's automated data collection system, users only need to send frozen crystal samples to SPring-8, and the measurement is completed. While 60% of the machine time as an activity of the beamline BL32XU was provided to the BINDS project, more than 90% of the machine time of the project was carried out by automated measurements.

The filling development items for BL32XU in this fiscal year continued from the previous year:

1. high-throughput accumulation of high-resolution structures,
2. development of data collection techniques from complex crystal samples, and
3. development of structural analysis techniques for understanding structural dynamics.

In addition to the above, (4) a collection of high-resolution diffraction data from ultrasmall crystals was also taken up as a development item because of the availability of a high-flux microbeam. For (1), we continued to develop a system that works well with ZOO to achieve higher throughput data collection. In the public beamlines, we are developing an automatic puck-exchanging robot that automatically transfers sample containers (Uni-pucks) to the sample-changing robot SPACE to reduce staff work time for measurements and perform automated measurements for more extended periods. The sample exchange robot SPACE can be equipped with 8 Uni-pucks, and ZOO's automated measurement system can automate approximately 16 hours of measurement. Until now, when the measurement of up to 8 Uni-pucks was completed, the next 8 Uni-pucks had to be manually loaded by staff. As mentioned above, the maximum duration of automated measurement was about 16 hours. The automated puck-exchanging robot is equipped with a dewar that can store 42 Uni-pucks in liquid nitrogen, allowing the replacement of the Uni-pucks to be set in SPACE. By a simple calculation, this allows the robot to

continue automatic measurement for more than 80 hours without interrupting staff work. JASRI has taken the lead in the significant development of the hardware part, and a prototype of the device has been completed using a cooperative robot manufactured by FANUC. In parallel with the development of the hardware, since the linkage with the automatic measurement system ZOO is essential, the operational sequence was covered, and the development of the linkage program was implemented. It was confirmed that automatic measurement proceeds sequentially if a file containing the Uni-puck is to be used for measurement and the corresponding measurement conditions is set at the start of the measurement. We plan to conduct careful and continuous operation checks and debugging to start user operations in the next fiscal year. Related to the high-throughput data analysis, we also published a paper on optimal measurement conditions for efficient phase determination using an automated measurement system [4].

In (3), we advanced the NABE system and its internal functions, which we began to develop last year. Although the collection of a large number of data sets was realized by improving the efficiency of measurement, it is necessary for humans to confirm the structural information of these data sets manually. Last year, we implemented a function to perform the initial structural analysis of the collected data sets by the molecular replacement method using PDB coordinates of known structures and a function to automatically perform initial phase determination, making it possible to perform comprehensive structural analysis more efficiently. In this fiscal year, we developed a new analytical method focusing on polymorphic structure analysis in crystals. Specifically, we investigated a method of classifying a large amount of diffraction intensity data into groups with different structures by analyzing them by hierarchical clustering. Using standard protein samples, we immersed different inhibitors and substrates in them, and diffraction data were collected from each crystal. Using the diffraction data obtained, we examined whether the diffraction data derived from different structures could be classified into the original structure by hierarchical clustering by mixing two or three types of data. Hierarchical clustering currently uses one-dimensional parameters: the variance of the diagonal distance of the crystal lattice parameter and the correlation coefficient between diffraction intensities. Hierarchical clustering was performed on the basis of these values, structural analysis and refinement were carried out on the classified data sets, and the final electron density maps were used to investigate the pros and cons of structural classification by hierarchical clustering. As a result, it was found that hierarchical clustering based on intensity correlation coefficients between data sets can be used to classify a surprisingly small amount of structural information at present. Specifically, the original protein structure and the compounded structure can be classified very clearly, and two different compounds bound to the same site can also be classified. This test data suggests that the presence of polymorphic structures in the crystal can be classified by the statistical treatment of diffraction datasets. In the future, we plan to brush up on this analysis method and establish it as a method of elucidating the mechanism of protein action by actively inducing changes in the crystal structure.

Regarding (4), we succeeded in collecting diffraction data from submicron-sized crystals this year. Our collaborators developed the 'cell-free protein crystallization method,' which is a technology used to obtain protein crystals directly from cell-free reaction mixtures in which proteins are produced without "purification," a feature that markedly simplifies the purification and crystallization processes compared with the conventional cell-based protein synthesis. The crystal sizes obtained with the methods often corresponded to sub-microns confirmed by SEM. The automated SSROX measurement using the high-flux microbeam with beam size of 1 μm square available at BL32XU has obtained a high-resolution structure from crystals of ~580 nm size obtained by the technique. In the future, we plan to challenge the ultrasmall crystal structure analysis of various samples and continuously investigate the limited size of protein crystals that can be measured on synchrotron radiation beamlines.

Hirata Kunio[*1], Kawano Yoshiaki[*1], Matsuura Hiroaki[*2], and Sakai Naoki[*2]

[*1] SR Life Science Instrumentation Team, Life Science Research Infrastructure Group, Advanced Photon Technology Division, RIKEN SPring-8 Center

[*2] Life Science Research Infrastructure Group, Advanced Photon Technology Division, RIKEN SPring-8 Center

References:

[1] Hirata, K. et al. (2013). *J. Phys.: Conf. Ser.* **425**, 012002.

[2] Maeda, S. et al. (2021). *Sci. Adv.* **7**, 24.

[3] Taguchi, Y. et al. (2021). *Commun. Biol.* **4**, 941.