BL32XU
(RIKEN Targeted Proteins)

BL32XU, which is the RIKEN target protein beamline, is dedicated to high-resolution diffraction data collection from protein microcrystals under our R&D. Since FY2015, we have been developing a fully automated data collection system dedicated to protein crystallography named ZOO at SPring-8 in Japan [1, 2]. This system supports all experimental schemes in goniometer-based data collection from protein crystals. Because ZOO has achieved un-attended data collection, remote users can acquire high- resolution datasets using SPring-8 just by sending crystals.

The ZOO system was initially developed at the microfocus beamline BL32XU at SPring-8 by combining a brilliant microfocus X-ray beam, pixel array detector, and automated sample changer. The system consists of several programs such as one for automatic loop centering, two-dimensional raster scanning, defining crystal size and positions, and estimating radiation damage. An automated data processing system immediately processes the collected datasets using a PC cluster [1].

The ZOO system controls data quality well by automated crystal selection and managing radiation damage. Enhanced experimental efficiency and data quality accelerate the accumulation of better datasets. Merging many datasets improves the signal-to-noise ratio required for higher resolution structure determinations. The synergy provided by the ZOO system has accelerated high-resolution structural analysis of challenging samples [3-5]. The ZOO system can collect 200–300 full datasets within 24 h from crystals larger than 50 μm (one-crystal/one-loop). This is useful for fragment-based drug design by examining a large number of crystals in a complex with small ligands.

To support such projects, we started a scrap-and-swap beamline BL45XU at SPring-8 at the end of FY2018. The SAXS activity of the beamline was finally switched to high-throughput protein crystallography. Beamline operations will be resumed in May 2019. The beamline is dedicated to automated data collection from protein crystals with sizes ranging 10–500 μm with a flexible beam size. All types of crystals (*e.g.,* LCP crystals for multiple small-wedge data collection and soluble protein crystals for normal rotation or helical data collection) are welcome. Users do not need to come to Japan. They can simply send their crystals and a data backup disk to SPring-8. After data collection with ZOO, the samples are returned along with the collected datasets and processed diffraction data.

The ZOO system has greatly enhanced the experimental efficiency in goniometer-based data collection. We are now developing efficient crystal harvesting to enhance the throughput to harvest protein crystals from crystallization plates. Crystallization via the lipid mesophase method presents some technical challenges. In the lipid mesophase method, crystallization is often performed using the glass sandwich method. Literally, it is a special plate that maintains the crystallization conditions by sandwiching a spacer / double-sided tape with a hole between two pieces of glass. Crystals come out in the holed part of the spacer. After checking with a microscope, the glass in that part is scratched and broken to scoop the crystal. This process requires mature technical skills.

Occasionally inexperienced experimenters cannot identify problems occurring in diffraction datasets. Problems may result from the crystal scooping process or from the intrinsic diffracting power of the crystals.

Another difficulty in harvesting crystals is limited time. To maintain the crystallization condition, the harvesting time should be less than 15 s after breaking the glass plate. After 15 s, various conditions change, degrading the crystal quality. Depending on the skill level of the experimenter, only 20–30% of the crystals obtained by crystallization can be used for diffraction experiments. (The degraded crystals are inevitably discarded before freezing.)

In FY2018, we undertook a joint research project with Kyoto University to solve this problem. We studied a method to collect diffraction data without removing crystals crystallized by the lipid mesophase method from the plate. A Diffrax plate manufactured by Molecular Dimensions can be used as a substitute for glass to crystallize with two films possessing a high water barrier property and a low X-ray scattering power. When crystallization is finished, the film is cut with scissors, frozen as-is, and used for diffraction experiments. The holder attached to this plate clips the film. However, the film itself sways during the measurement, hindering the collection of a better diffraction.

To stabilize the position of the cut film during measurements, we developed a new film holder. Now, all crystals produced can be frozen and used for measurements without removing the crystals from the film. Actually, this method can measure samples that are five times more numbers than the previous method.

We recently developed an automatic measurement program for this large-area holder, which is about 6 mm in diameter and is cut from plastic plates. First, the robot arm was modified so that the sample exchanger robot could grab this holder. Since the large-area holder must be adjusted at the center of the X-ray, an optical microscope with a large field of view is required. For this reason, we introduced an optical microscope with a large field of view, allowing accurate positioning of the holder at the center of the X-ray. A high-magnification camera is also introduced to adjust the orientation of the holder perpendicular to the X-ray. The camera views the holder from the top in order to align the holder at the focal spot of the X-ray. Consequently, we successfully automated data collection from the crystal after aligning the large holder to the X-ray center, adjusting the posture, and specifying the crystal position by a two-dimensional raster scan. In the future, we plan to focus on automation from the crystallization plate to freezing the measuring holder.

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