BL45XU
Macromolecular Crystallography II

1. Introduction

One of the important requirements of macromolecular crystallography (MX) beamlines is to provide high-throughput diffraction data collection. BL45XU has performed high-throughput diffraction experiments of protein crystals with automated measurements using the ZOO system [1]. The development of peripheral technologies associated with automatic measurement, such as the development of an automatic processing pipeline for diffraction data and the simplification of information communication with users, is also underway. The optics layout consists of a double-crystal monochromator of SPring-8 standards, a horizontal focusing mirror, a virtual source slit, and a Kirkpatrick–Baez mirror [2]. The available energy range is 6.5 to 16 keV. The beam size at the sample position can be changed from 5 μm (H) × 5 μm (V) to 50 μm (H) × 50 μm (V) with a photon flux of 5.70 × 10^{12}–1.75 × 10^{13} photons/s at 12.4 keV.

2. Mixed mode in Automated measurement

The automatic measurement has been operated in three modes of the ZOO system: the single scheme to measure without translation, the helical scheme to measure with translation using the crystal volume, and the multi scheme to measure multiple crystals in a small-wedge. When mounting a sample, multiple crystals of various sizes may be mounted, and the above three modes may not be sufficient. The mixed scheme consisting of multiple small-wedge and helical schemes was introduced in the ZOO system to set optimal measurement conditions based on the analysis of the heat-map file of the scores with the goniometer coordinates created using the SHIKA program [3].

3. Development of UniPuck exchange system for continuous automatic measurement

We are now commissioning a UniPuck exchange system (puck stocker) in order to automatically install UniPuck in the sample changer SPACE [4] (Fig. 1). We have already confirmed that it is possible to transport packs from the large-volume stocker to the sample exchange robot SPACE while keeping them at a cryo temperature. We began test operation using user samples and have succeeded in continuous operation for 45 hours. In addition, to ensure safe operation during continuous operation, a sensor that detects the puck has been added so that

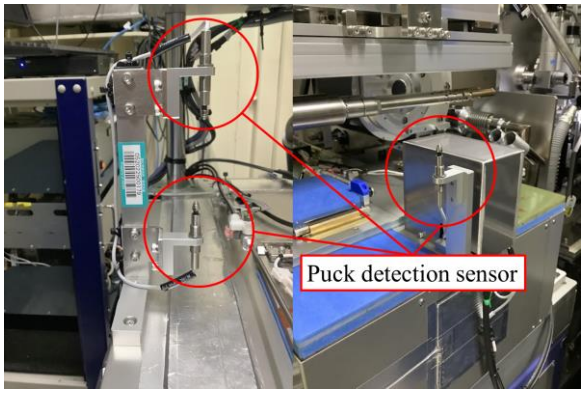

Fig. 1. Puck detection sensor.

the sample is kept at a cryo temperature even when the robot stops due to an error (Fig. 1).

4. Data Download server for Automated measurement users

The automated measurement allows users to obtain diffraction data of samples without coming to the SPring-8 site. The diffraction data are sent to the users on a storage medium such as a hard disk. Users have wanted to be able to check data immediately after measurement, so we set up the data download server from outside the site. The download server is based on the Nextcloud system. After the data measured at the beamline are stored in the large storage space shared by the Structural Biology Group, only the necessary data are transferred to the download server using Python scripts. These data can be downloaded for a certain period of time.

5. Beamtime Announced system for Automated measurement users

The automated measurement requires that samples and measurement condition sheets be sent in prior to the measurement, and the user is notified of the deadline. Analysis data and statistics of analysis results are also notified as a preliminary report. Until now, the user has had to copy the standardized text using email software, change the wording to suit the user, and then send the text. Since this process was time-consuming, we developed a web application (Fig. 2) using Django (web application framework implemented in Python: https://www.djangoproject.com/) that automates the change of the standard text, enables the sending of email, and allows staff to share the contents of e-mails.

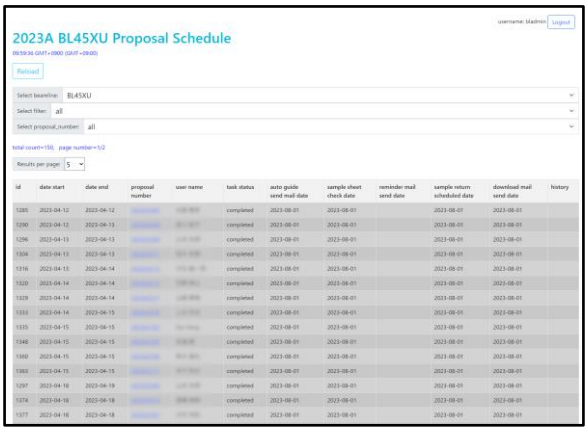

Fig. 2. Web application image.

6. Beamtime schedule management web site for Automated measurement

In introducing automated measurement, we have been operating a system at BL45XU in which a certain number of users are allocated within a certain period of time, and each beamtime can be freely adjusted by staff within the period. However, this system was difficult to manage centrally owing to the high fluidity of the beamtime. Initially, the system was managed manually by writing on a whiteboard, but it was difficult to share information among the staff because changes had to be discussed at the beamline. By setting up an on-premise Kanban software server (Fig. 3, Wekan: https://wekan.github.io/) and allowing each user to freely edit each beamtime on the browser, we

succeeded in creating an environment in which fluid beamtime could be shared among staff members wherever they were.

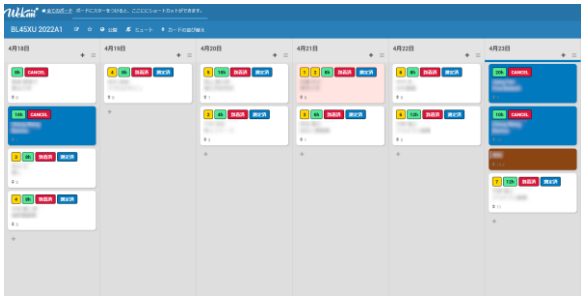

Fig. 3. Web image of Kanban software.

7. Fragment Screening Pipeline

As the field of protein crystallography continues to advance in efficiency, novel screening methods have been employed to identify molecules that bind to target proteins within crystal structures using a library of low-molecular-weight compounds encompassing diverse structural characteristics. The primary objective of this endeavor is the rational design of new compounds with enhanced affinity for specific drug target proteins. One such method, which utilizes relatively small compounds, known as "fragments", with molecular weights of 300 Da or less, is commonly referred to as fragment screening. This approach is particularly promising because it allows for the rapid and rational development of drug-like compounds on the basis of the binding interactions of these fragments with the target proteins.

The process of fragment screening necessitates the utilization of X-ray crystallography for analyzing target protein crystals that incorporate hundreds of distinct fragments. In FY2022, we made substantial progress by establishing a comprehensive compound screening pipeline, closely integrated with automated systems for sample preparation. This encompassed critical steps such as crystallization, compound introduction, data collection, and structural analysis. A specialized facility was successfully installed within the outer room of BL45XU, enabling the efficient preparation of crystal samples for subsequent measurements (Fig. 4). This facility played a pivotal role in supporting our screening efforts.

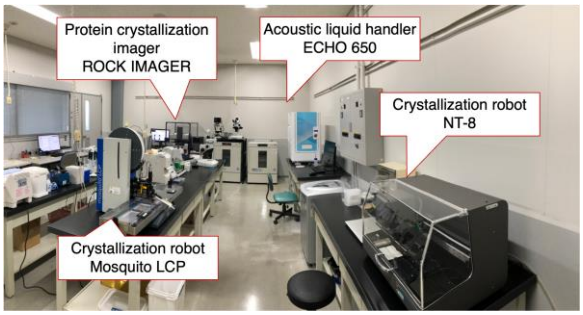

Fig. 4. Crystal preparation facility close to BL45XU.

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