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RIKEN Structural Genomics II

1. Introduction

RIKEN Structural Genomics Beamline II consists of SPring-8 standard bending magnet beamline components and an end station dedicated to high-throughput protein crystallography [1]. Asymmetric diffraction crystals have been adopted for the double-crystal monochromator (asymmetric angle of 6.33°) to increase the total photon flux of the monochromatic beam. Also, the user can select optional focusing with capillary lens optics (Hamamatsu J12432) upstream of the sample to enhance the flux density of the incident X-ray beam at the sample [2].

Diffraction data can be automatically collected from a vast amount of cryo-cooled protein crystals through the user interface BSS with the high-framerate CCD detector MX225HS (RAYONIX), utilizing the twin-armed auto-sample exchanger SPACE installed at the end station [3,4]. Two types of remote access for users are supported: mail-in data collection in which a web database system, D-Cha, supports sample and experimental information input/output on a web browser [5] and the remote control of beamline equipment via a dedicated interface program, SP8Remote, which allows users to directly log in to the beamline control system under a restricted safety interlock system [6].

2. Recent activities

The development of new sample-handling devices and further improvement of data collection throughput are being continuously conducted to contribute to research projects such as ligand screening for drug discovery. To improve the throughput of X-ray crystallography, including the sample preparation process, a new microfluidic device that allows the injection of crystal suspensions and the trapping crystals aligned in pit patterns inside the fluidic channel without the need to handle each crystal is being developed. Ligand–protein complex crystals are formed by replacing the content buffer with a ligand solution. The device is made of a transparent material, polydimethylsiloxane (PDMS), and is designed to be mounted on the goniometer to collect diffraction data at room temperature [7]. Aiming at application to large-scale ligand screening, a device integrated with multiple channels, enabling 32 compound conditions to be accommodated on a holder whose outer dimensions are of compatible size with the SBS crystallization plate, was developed (Fig. 1). Sample injection and replacement are automated with a commercially available autopipetting robot, ASSIST PLUS (INTEGRA Biosciences). At the end station of the beamline, a goniometer for a crystallization plate is installed for data collection. We have conducted a test experiment of ligand screening with an automatic sample injection system followed by sequential dataset collection at the beamline and determined the structure of ligand and trypsin complexes.

By the end of the 2023A operation period, the beamline terminated user operation and was used for research and development for adopting a new beamline control system. In 2023A, 20% of the total

beam time was assigned to public users, and 10% was assigned to Basis for Supporting Innovative Drug Discovery and Life Science Research by AMED (BINDS) project users.

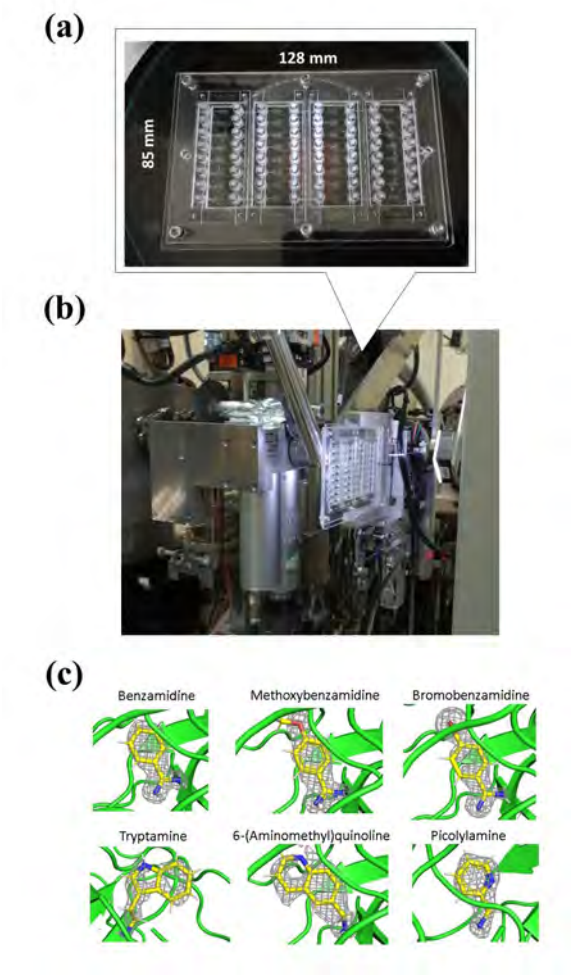

Fig. 1. Development of the new microfluidic device. (a) A 32-ch integrated microfluidic device fixed on a holder designed to be of an SBS plate-compatible size. (b) Goniometer for the integrated device at the end station. (c) Examples of trypsin–ligand complex structures were determined using an automatic sample injection and sequential data collection system. PanDDA event maps at the 3σ level. Ligand concentration was 10 mM.

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