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Development of a new microfluidic device aiming at ligand screening with protein crystallography

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In pharmaceutical research and development as typified by structure based drug design (SBDD) which requires comprehensive structural analysis of complex of targeted macromolecule and chemical compounds, there are growing expectation for further improvement of throughput in X-ray crystallography including the sample preparation process. In the structural analysis of protein-compound complexes with X-ray crystallography, samples are typically prepared by the soaking method in which protein crystals are immersed in a solution containing the compound. However, time-consuming manipulation of samples under a microscope to pick up sample crystals one by one is an obstacle to large-scale and high-throughput analysis.

In this study, we have developed a new microfluidic device which allows to inject crystal suspension into the fluidic channels, and trap crystals aligned in pit arrays inside it, expecting to improve the efficiency by saving labor-intensive sample preparation. By replacing the content liquid with ligand solution, ligand-protein complex crystals are formed (Fig. 1a). The device is made of transparent silicone material (PDMS), and is readily mounted on the goniometer to collect X-ray diffraction data at room temperature. We have demonstrated the feasibility in ligand screening experiment using trypsin crystals with a single channel test device at RIKEN Structural Genomics Beamline II, SPring-8 [1]. We have confirmed that ligand screening with room temperature crystallography with merged diffraction datasets with multiple crystals were available with this method.

Currently aiming at the application for larger scale ligand screening, the design of the device is being optimized to increase the efficiency of crystal trapping. Also integration of the multiple channels into a device, which allows 32 compound conditions equipped on a holder compatible size with SBS crystallization plate (Fig. 1b, Fig. 1c). Notably, sample injection and replacement are automated with a commercially available auto-pipetting robot ASSIST PLUS (INTEGRA Biosciences). Also the upgrading of beamline equipment such as a goniometer for crystallization plate and control system for efficient data collection from the microfluidics are in progress. The room temperature crystallography with the new microfluidic device combined with automatic data processing and analysis is expected to be an option for large-scale ligand screening pipeline at SPring-8.

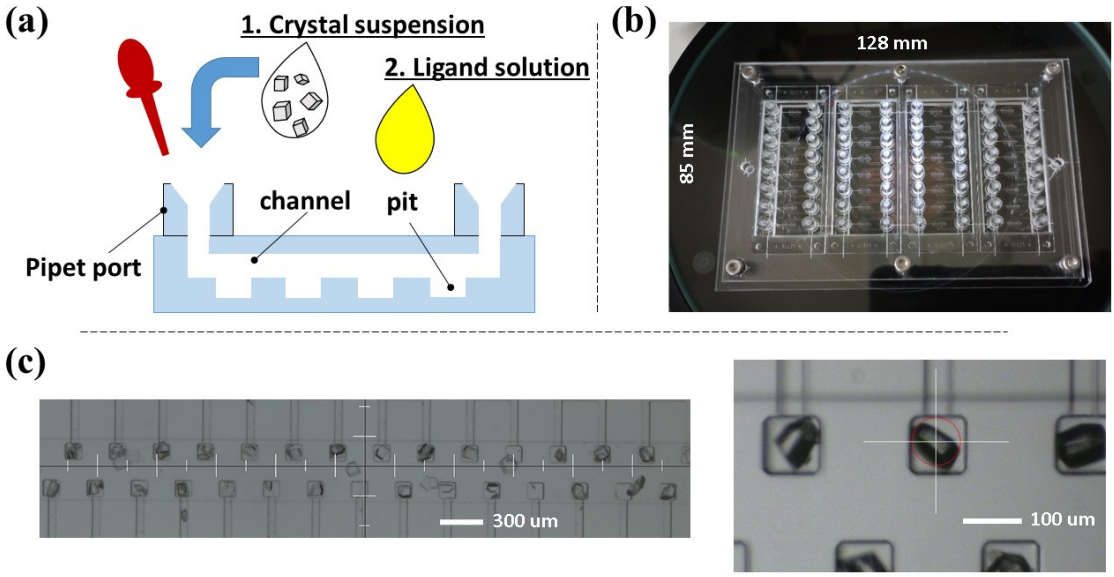

Figure 1. (a) Schematics of the new microfluidic device, (b) a 32-channel device fixed on a holder designed compatible size with a SBS crystallization plate, and (c) magnified channel and pit photographs in which lysozyme crystals are trapped.

[1] Maeki, M., Ito, S., Takeda, R., Ueno, G., Ishida, A., Tani, H., Yamamoto, M, and Tokeshi, M. (2020). *Chem. Sci.* **11**, 9072.