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Development of microfluidic devices for *in situ* diffraction data acquisition

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1 Introduction

The traditional method for structure determination of macromolecular crystals at synchrotrons is to grow crystals *in vitro*, then transport them to the synchrotron and collect X-ray diffraction data. This process is time-consuming and labor-intensive. Microfluidic devices offer a potential solution to this problem. Microfluidic devices, such as small, enclosed chips made with X-ray transparent membranes/polymers, enable the *in situ* growth and screening of protein crystals directly at the synchrotron. This eliminates the need to transport crystals, saving time and eliminating the risk of unnecessary and potentially damaging crystal handling. In this project, we will develop microfluidic devices for *in situ* diffraction data collection. We will test various formats of microfluidic devices and optimize the protocols for growing and screening protein crystals in these devices. We will also investigate the use of microfluidic devices for the structure determination of protein-ligand complexes. The development of microfluidic devices for *in situ* diffraction data collection has the potential to revolutionize the field of protein crystallography. These devices could make structure determination faster, more efficient, and more accessible to researchers around the world.

2 Experiments

The protein crystals were cultivated directly inside a microfluidic chip, utilizing a Lysozyme solution. Incubation at room temperature for 2 hours yielded crystals with a maximum dimension of 200 µm. Optimization of the crystallization conditions ensured that the crystal sizes were compatible with both the beam size and the dimensions of the microfluidic chip channels [1]. One notable advantage of microfluidic chips is their ability to maintain the crystals in a hydrated environment throughout the entire course of the experiments. The chip was securely affixed to a 3D-printed scaffold, which was subsequently mounted on the goniometer head for precise sample centering. Crystal positions were registered using the UGUI user interface, and data collection commenced directly from the software. To prevent contact between the chip and the beamline hardware, the chip's oscillation range was restricted to -30º to 30º, resulting in partial data sets of 60º each.

In situ X-ray diffraction experiments were conducted at the AR-NW12A end station of KEK [2], employing an X-ray beam with a wavelength of 0.75 Å, focused on the sample position. The X-ray beam was adjusted to dimensions of 200 x 130 µm² to accommodate the crystal size. Fine-slicing data sets were obtained using a Pilatus3 S2M detector (Dectris, GmbH), acquiring 6,000 images per data set with an oscillation range of 0.1º and an exposure time of 0.1 sec *per* image.

The integrated software package *XDS* [3] was employed for image integration, while the *CCP4* software suites [4] were utilized for solving and refining the crystal structures.

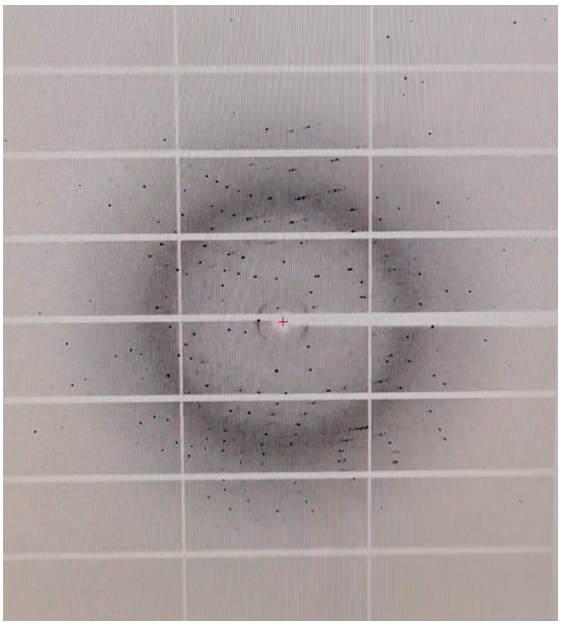

Figure 1: Clean diffraction pattern of lysozyme protein crystals grown in the microfluidic chip. Representative diffraction pattern obtained from protein crystals grown inside the microfluidic chip. The diffraction pattern displays well-defined Bragg peaks and a minimal scattering background, indicating the high quality and order of the crystals and the low-background capacity of the microfluidic chip.

3 Results and Discussion

In this study, *in situ* X-ray diffraction experiments were performed on protein crystals grown inside a microfluidic chip. The crystals exhibited diffraction patterns with excellent quality, allowing for data collection up to a resolution of 1.8 Å. The diffraction images showed well-defined Bragg peaks and a high signal-to-noise ratio, indicating the high quality and order of the crystals.

Notably, the scattering background originating from the microfluidic chip was found to be minimal, enabling clear visualization and accurate measurement of the diffraction

intensities. This is particularly advantageous, as a low scattering background reduces noise and enhances the accuracy of data analysis.

Furthermore, the handling of the microfluidic chip during the experiment was straightforward and convenient. The chip was securely mounted on the goniometer head, and the oscillation range was carefully controlled to prevent any collisions with the beamline hardware. These factors contributed to the overall ease of use and experimental efficiency.

Significantly, there were no evident indications of radiation damage observed during the data collection process under the aforementioned experimental conditions. The crystals maintained their diffraction quality throughout the entire data collection, indicating their stability and resilience to X-ray exposure. This is a significant finding, as radiation damage can limit the accuracy and reliability of structural determination.

Overall, the results demonstrate the successful application of the microfluidic chip for *in situ* X-ray diffraction experiments. The high-resolution data obtained, the minimal scattering background from the chip, the ease of chip handling, and the absence of radiation damage provide a solid foundation for further structural studies and analyses. The combination of these favorable features makes the microfluidic chip a promising tool for future investigations in the field of structural biology.

Acknowledgment

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(a) (b)

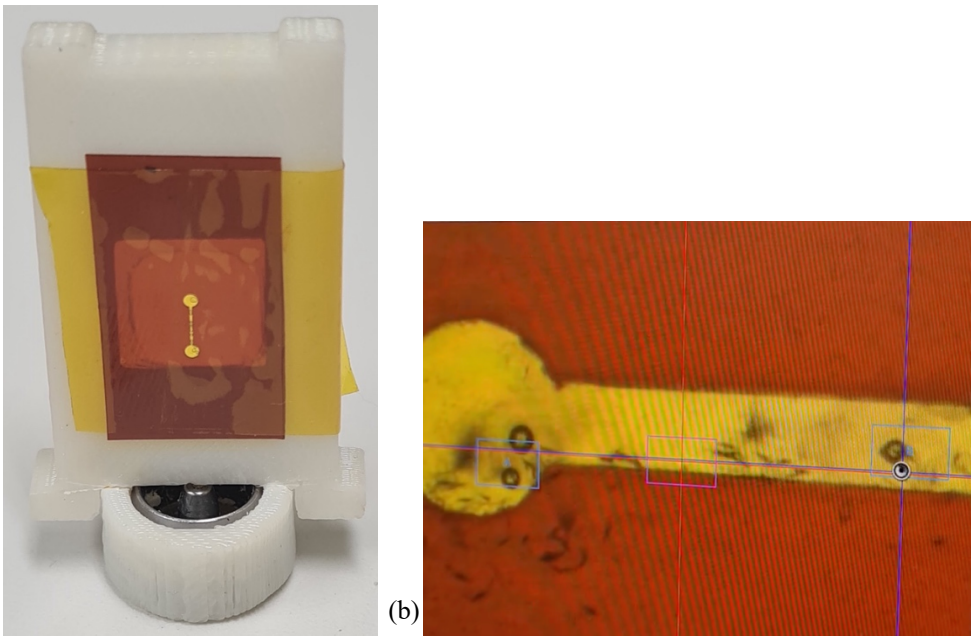

Figure 2: Microfluidic chip and crystal visualization. (a) 3D-printed scaffold holding the microfluidic chip during in situ X-ray diffraction experiments. The scaffold provides stability and precise positioning of the chip within the experimental setup. **(b)** Close-up view of the microfluidic chip revealing the protein crystals grown inside. The chip design allows for controlled crystal growth and enables direct observation of the crystals during data collection. The positions for data collection can be saved directly within the UGUI user interface (*blue rectangles* on the picture).

References

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