Experimental platform for serial femtosecond crystallography at SACLA

Serial femtosecond crystallography (SFX) with an X-ray free electron laser (XFEL) provides damage-free diffraction patterns from tiny crystals with micrometer sizes [1]. In this method, a stream of crystals interacts with an XFEL pulse, a duration of which is on the order of femtoseconds. Radiation damage to the sample can be circumvented because diffraction events can be terminated within a timescale much shorter than those of damage processes. Typically 10^4 or more diffraction patterns are collected from randomly oriented crystals to provide the statistical reliability required for building a structure model.

A key process in SFX experiments is the delivery of crystals. Micrometer-size crystals are dispersed in a fluid carrier and delivered to the interaction point using a fluid injector. Various types of injectors have been developed so far [2]. A liquid-jet injector with a gas dynamic virtual nozzle (GDVN) produces a micrometer-size stream of a crystal suspension. The electro spinning technique is also employed to deliver crystal suspensions at low flow rates on the order of 10^{-1} μl·min^{-1}. To reduce sample consumption, viscous-fluid injectors have been developed to produce a slow flow of crystals dispersed in a highly viscous carrier such as an LCP matrix or a grease-matrix carrier.

In the early SFX experiments, the interaction point was in a vacuum environment, which is advantageous for reducing background signals. One technical issue in the case of crystal delivery into vacuum is that the carrier fluid can freeze easily. A frozen carrier would inhibit an injector from stable operation, and more seriously, it would produce strong X-ray signals that may cause damage to a detector.

At SACLA, we have developed an ambient-pressure system for SFX experiments, Diverse Application Platform for Hard X-ray Diffraction in SACLA (DAPHNIS) [3]. The freezing issue can be avoided under the ambient-pressure operation, which also helps to maintain an appropriate temperature and humidity around the sample. Because of these advantages, DAPHNIS is applicable to a variety of samples beyond protein crystals, including live organisms, solutions, and powders dispersed in liquids.

Figure 1 shows a photograph of DAPHNIS, which basically consists of a sample chamber, injectors, and a multiport charge-coupled-device (MPCCD) detector with eight sensor modules [4]. This system is connected to a micrometer focusing system on a hard X-ray beamline of SACLA. Samples are delivered to the X-ray focal point using an injector, which is mounted on a motorized manipulator. The X-ray beam is blocked by a beam stopper in front of the detector. The distance between the detector sensor and sample is adjustable in the range between 50 and 100 mm. The highest nominal resolution is 1.5 Å (2.5 Å) at a distance of 50 mm (100 mm) and an X-ray wavelength of 1.24 Å.

Two types of liquid-jet injectors are available for delivering soluble-protein crystals. One with a GDVN produces a thin liquid beam from a nozzle with an inner diameter (ID) of 50–150 μm. Figure 2(a) shows a schematic drawing of the injector and a microscopic image of the nozzle tip. A helium gas stream through the outer capillary squeezes the sample beam to a diameter of 4–40 μm. The beam size is varied via the flow rate of the liquid and the stagnation pressure of the helium. In the case of a 10 μm stream from a 150-μm-ID nozzle, for example, a typical flow rate is 0.3 ml·min^{-1}. The other kind of injector is used for circulating a sample suspension with a peristaltic pump (Fig. 2(b)). The liquid-beam diameter is nearly the same as that of the nozzle aperture (100 or 200 μm). The flow rate is typically 1.5 ml·min^{-1} (2.5 ml·min^{-1}) with a 100 μm (200 μm) nozzle.

For crystals in highly viscous carriers, a syringe-pump injector is employed [5]. A carrier medium with crystals is extruded from a syringe needle at a low flow rate, which can be as low as ~0.1 μl·min^{-1}. The standard ID of the needle is 110 μm. Because of the low flow rate, the protein consumption is less than 1 mg in most cases. The flow rate can be further reduced by using a thinner needle, for example, ~0.03 μl·min^{-1} with a 50-μm-ID needle.

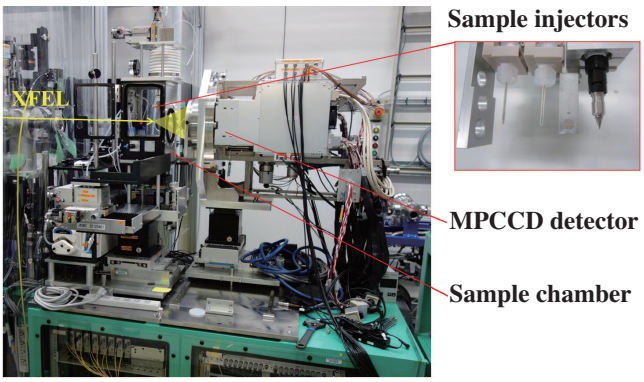

Fig. 1. DAPHNIS system with major components: sample chamber, injectors, and MPCCD detector with eight sensor modules.

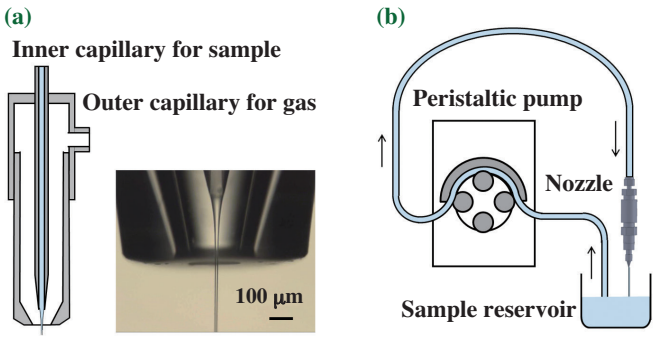

Fig. 2. Liquid-jet injectors. **(a)** Schematic drawing of a liquid-jet injector with a GDVN and a photograph of the nozzle tip. A helium gas stream is provided through the outer capillary to focus a liquid beam ejected from the inner capillary. **(b)** Schematic drawing of a liquid-jet injector with a sample circulator.

The DAPHNIS system was applied to SFX measurements of the model protein lysozyme. The XFEL beam at **BL3** of SACLA had a center photon energy of 10 keV with a bandwidth of 5×10^{-3} (FWHM; full width at half maximum) and an average pulse energy of 110 μJ (7×10^{10} photons/pulse) at the sample position. The repetition rate was 20 Hz. The focal size of the 10 keV beam was 1.5 μm (FWHM) in both the horizontal and vertical directions. The sample chamber was filled with helium gas with a partial pressure of >0.9 atm.

Lysozyme crystals with sizes of about 1 μm were dispersed in an aqueous buffer solution comprising 10% (w/v) sodium chloride and 1.0 M sodium acetate (pH 3.0). The number density of the crystals was ~10^9 ml^{-1}. The crystal suspension was delivered using a liquid-jet injector with a GDVN having a 150 μm ID. The injector provided a 10-μm-diameter sample beam with a flow rate of 0.3 ml min^{-1}. The sample-to-detector distance was ~50 mm. Diffraction patterns were recorded with the MPCCD detector in a shot-by-shot manner.

In one measurement series, 45084 shots were applied to the sample in ~40 min. The 1 μm crystals of lysozyme provided clear diffraction patterns (Fig. 3(a)), 3226 of which were able to be processed for indexing Bragg spots. From the indexed images, 99.8% completeness was achieved at resolutions of 30.0–2.40 Å. An electron density map was successfully refined at a resolution of 2.4 Å (Fig. 3(b)). Higher-resolution data can be obtained by using larger crystals. For example, 7–10 μm crystals provided a structure model with a 2.0 Å resolution [5].

In conclusion, DAPHNIS has been successfully applied to protein SFX at SACLA. Even 1 μm crystals of lysozyme provided a clear electron density map with a 2.4 Å resolution in a measurement time of ~40 min. This result indicates that DAPHNIS can be used for the fast structure analysis of proteins for which large crystals are difficult to obtain. The ambient-pressure operation is useful for preventing fluid samples from freezing. The simple and compact structure of DAPHNIS facilitates future upgrades, for example, it can be easily modified to investigate ultrafast dynamics by a pump-and-probe technique. It is also adaptive to new sample-delivery methods such as pulsed droplet injection as well as the fast scanning of fixed crystal targets.

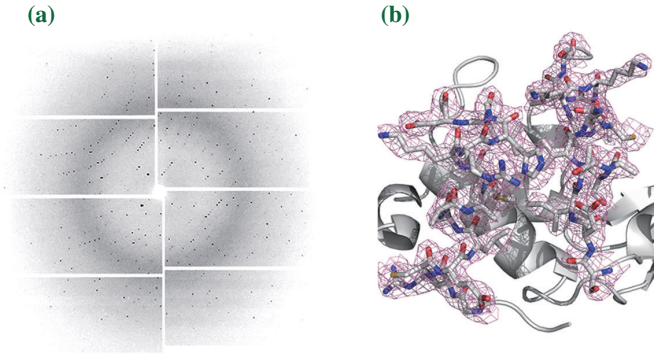

Fig. 3. Results of the SFX measurement of lysozyme. **(a)** One of the diffraction patterns of the 1 μm lysozyme crystals. **(b)** Close-up view of the lysozyme structure with an electron-density map contoured at the 1.0σ level. This figure was drawn with the PyMol program (http://www.pymol.org).

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