Serial femtosecond rotation crystallography: an opportunity for high-resolution crystal structure determination free from radiation damage

Radiation damage from X-ray irradiation has been a longstanding problem in protein crystallography. X-ray crystallographic analysis is very powerful method to determine the high-resolution atomic coordinates of proteins. The resultant atomic coordinates are very informative to understand the structural basis of protein function. Nevertheless, in some cases a high-resolution crystal structure does not necessarily reflect the nature of a protein due to the radiation damage. However, radiation damage of proteins is probably unavoidable as long as conventional light sources such as synchrotron (SR) are used because the normal exposure time to acquire a diffraction image (e.g., several seconds) is long enough for X-ray dependent formation of reactive molecules and successive attacks on protein molecule in a crystal. Despite the temperature dependency of the diffusion process for a reactive molecule, a cryogenic temperature is insufficient to prevent radiation damage during data collection. A crystallographic method that is free of radiation damage and provides a high spatial resolution has been desired.

X-ray free electron laser (XFEL) opens the door for the structural biology based on high-resolution crystal structure analysis free of radiation damage. The concept to prevent radiation damage is femtosecond data collection. An X-ray pulse of SACLA contains middle of 10^{10} photons for a pulse duration less than 10 femtoseconds, which makes it possible to acquire a diffraction image 100 times faster than the X-ray dependent formation of reactive molecules. Actually, the total pulse flux is almost equal to the flux available by a one second exposure time at beamline **BL32XU** as an advanced microbeam beamline for protein crystallography.

To utilize the beam feature of SACLA in high-resolution protein crystal structure analysis without radiation damage, a new diffractometer was installed at beamline **BL3** EH3 of SACLA [1] (Fig. 1). The concept of the camera with a goniometer is combined usage of a brilliant femtosecond X-ray pulse and large crystals compatible with conventional protein crystallography using SR in size. The large diffraction volume in a large crystal strengthens the diffraction intensity of a Bragg spot, resulting in an improved diffraction resolution. In addition, we can utilize the knowhow that is accumulated in the long history of protein crystallography to grow high quality large crystals. To date, we have successfully determined the high-resolution crystal structure of very large macromolecular complexes without radiation damage: bovine heart cytochrome *c* oxidase at a 1.90-Å resolution [1] and cyanobacteria photosystem II at a 1.95-Å resolution [2]. Those are outstanding resolutions compared to the resolution of the structure determination of proteins with similar molecular weights by serial femtosecond crystallography using micrometer crystals.

Data collection from large crystals is performed by a step scan in which the diffraction intensity of a Bragg spot with a certain spread is sampled by few

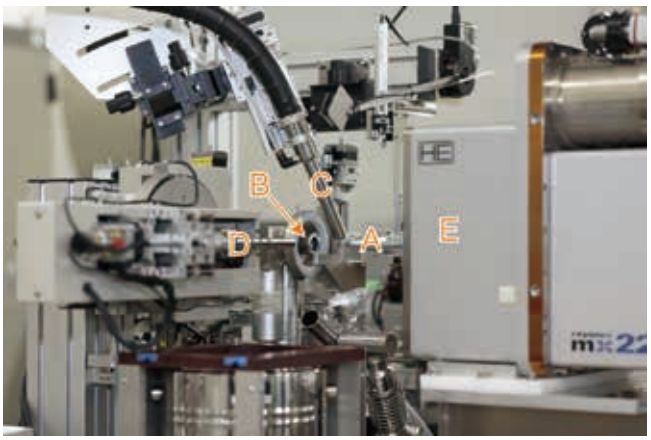

Fig. 1. Close up of the sample position of the diffractometer installed at EH3/BL3 of SACLA. **(A)** Goniometer to mount a large crystal, **(B)** X-ray coaxial camera for crystal alignment. X-ray pulse passes through a pinhole at the center of the objective lens. **(C)** Cryostat. **(D)** Crystal exchange robot SPACE. **(E)** CCD X-ray detector.

still diffraction images that are separated by a step angle of δθ (Fig. 2(a)). The data collection sequence is as follows. Acquire a still diffraction image at a crystal rotation angle of θ. Rotate the crystal by a step angle of δθ. Acquire the next diffraction image at a crystal rotation angle of θ + δθ. An appropriate step angle is an angle smaller than one-third of the crystal mosaicity. A large step angle increases the unobserved diffraction intensities, resulting in decay of the quality of diffraction intensity data. The preliminary experiment performed at BL32XU shows that the refined structures are comparable between the data collected by the step angle of half of crystal mosaicity and the data collected by the conventional oscillation method [1].

As shown above, in this method, a series of still diffraction images are collected from a single crystal. The X-ray pulse breaks the portion of crystal irradiated after producing a diffraction image without radiation damage [3]. Thus, an appropriate distance between adjacent paths of X-ray pulses in a crystal guarantees radiation damage-free diffraction intensity data (Fig. 2(b)). In actual measurements at SACLA, an irradiation of X-ray pulse decreased the order of crystal lattice in the region surrounding the beam path with approximately 11 μm radius [1].

The 50-μm separation of the path of the X-ray pulses in a crystal is effective to avoid radiation damage not only on the large scale but also on the small scale such as structural change in the protein. In the case of the structure determination of bovine heart cytochrome *c* oxidase bound a peroxide ligand, the X-ray dependent photolysis of the peroxide ligand is not observed, although it has been a longstanding problem in SR experiments [1,4]. The bond length between the peroxide oxygen atoms is refined to be 1.55 Å, which is acceptable for a bond length of peroxide ligand. In contrast, the bond length has not been shortened below 1.7 Å with SR data due to contamination of the photolysis product of the peroxide ligand. In addition, the peak height of the electron density next to the side chain of the tyrosine residue in the ligand binding site, which increases concomitantly with the photolysis of the peroxide ligand, is kept as low as the basal level observed in the cytochrome *c* oxidase whose peroxide ligand is substituted by a cyanide ion.

This method, which can be called as serial femtosecond rotation crystallography, provides a practical way to determine the high resolution crystal structure of huge biological macromolecules without radiation damage.

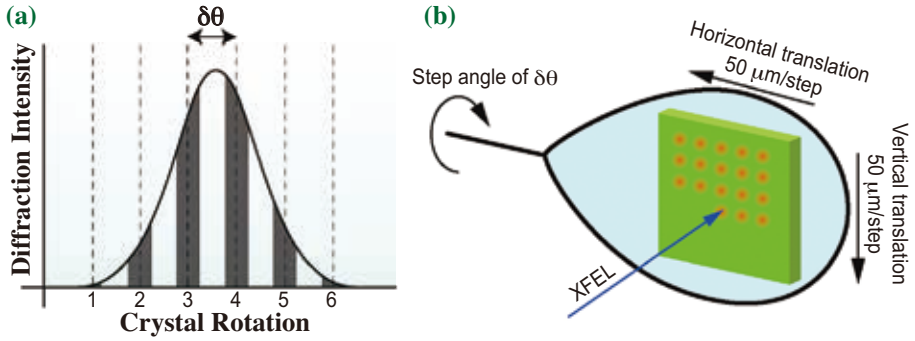

Fig. 2. Schematic drawing of the data collection procedure. **(a)** Diffraction intensity of a Bragg spot is sampled by a set of discrete still diffraction images. Total diffraction intensity is estimated based on the observed partial intensities colored in gray. **(b)** Data collection by crystal translation and rotation. Crystal is translated by 50 μm to acquire a diffraction image from the portion of the crystal that is unaffected by previous X-ray irradiations. In addition, the crystal is rotated by a step angle of δθ to sample the diffraction intensity of a Bragg spot with a certain spread of a few diffraction images.

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References

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