Fixed target serial crystallography at SACLA

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The crystallographic analyses of many challenging and scientifically important proteins are still difficult due to their weakly diffracting crystals. Radiation damage free crystal structure with high-resolution is important for the elucidation of the protein functions. The active sites of metalloproteins are especially susceptible to radiation damage due to changes in the oxidation states of catalytically important ions. In fact even at a cryogenic temperature, radiation damages of the active site of bovine heart Cytochrome *c* oxidase (C*c*O) and the oxygen evolving complex of cyanobacterial Photosystem II (PSII) were reported.

We developed a method that is powerful not only in radiation damage free structure determination but also in high-resolution structure determination even if the molecular weight of the target molecule is very big. The characteristics of this method is collection of a series of diffraction images along with crystal rotation from fresh parts of fixed crystals. To improve diffraction resolution, large crystals, as opposed to micro-crystals popular in serial femtosecond crystallography (SFX), were used. Recently we successfully determined the radiation damage free high-resolution structure of C*c*O (Hirata, K. *et al.* (2014) *Nature Methods* **11**, 734-736) and PSII (Suga, M. *et al.* (2015) *Nature* **517**, 99-103) using X-ray Free Electron Laser (XFEL) pulses. Both C*c*O and PSII are supramolecular complexes containing metal ions as catalysts of their redox reactions. XFEL pulse provides a diffraction image before the protein is destroyed by the extensive X-rays. As actions for next advancement of the method, equipment for time-resolved X-ray crystal structure analysis have been devised (Sakaguchi, M. *et al*. (2016), *J Synchrotron Radiat* **23**: 334-338). Regarding faster data collection, development of goniometer and associated tools, an overall review of data processing procedure are ongoing and reached to image readout at 10 Hz so far.

In this presentation we will present the fixed target data collection system developed at SACLA (SPring-8 Angstrom Compact free-electron LAser) and the damage free and time-resolved crystal structures of C*c*O and PSII.