

## Microsymposium

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#### *Intact structure determination of a highly radiation sensitive protein at SACLA*

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X-ray irradiation on a protein crystal can cause some subtle structural modification on the protein structure even if the radiation dose is much smaller than a dose used for a common crystal structure determination. In some case such structural modification increases ambiguity of structural inspection, and eventually could be an obstacle on the elucidation of structure basis of protein function. Bovine heart cytochrome c oxidase (CcO) is one of such proteins having some problem caused by the radiation damage. The proton pumping of CcO is coupled with O<sub>2</sub> reduction at the O<sub>2</sub> reduction site, thus accurate structure determination of bound ligand as well as CcO itself is very important. Whereas accurate structure determination was impeded by the immediate photolysis of the peroxide ligand upon X-ray irradiation even at a cryogenic temperature[1]. We developed a goniometer based data collection system for the femtosecond crystallography at SACLA (SPring-8 Angstrom Compact free-electron LAser). The femtosecond crystallography is expected to have an advantage in high-resolution and radiation damage free structure determination of very large protein by combined usage of large crystal and femtosecond intense X-ray pulse. In this presentation we are going to show the result of the femtosecond crystallography on the crystal of CcO having large unit cell dimensions. The close inspection of the electron density map calculated at 1.9 Å resolution showed the femtosecond crystallography worked fine for the high resolution and radiation damage free crystal structure determination of CcO.

[1] H. Aoyama, K. Muramoto, K. Shinzawa-Itoh et al., *Proc Natl Acad USA*, 2009, 106, 2165-2169

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