Biology

Recent progress in time-resolved crystallography at SACLA

M. Suga (Okayama Univ.)
"Structure of oxygen evolving photosystem II and possible mechanism for the O=O bond formation."

A. Shimada (Gifu Univ.)
"Time-resolved XFEL crystal structure analysis reveals the mechanism of blockage of pumping proton back leak prerequisite for high effective proton pump by Cytochrome c Oxidase"

E. Nango (RIKEN)
"Toward understanding of enzyme reaction mechanism: serial crystallography using mixing injectors"

In SFX:

- Small crystals
- Ambient structure
- Avoidable radiation damage
- Dynamics: Time-resolved study

Opinion from Biology

Style of Research and Experiment in Biology

- Need to focus on sample preparation and biochemical assay
- Many different methods for structural analysis—X-ray, Electron Microscopy, NMR, AFM…

→ What is necessary to create fantastic results from SACLA?

1. Good experimental environment that facilitates measurement on challenging samples

2. IT infrastructure for data analysis

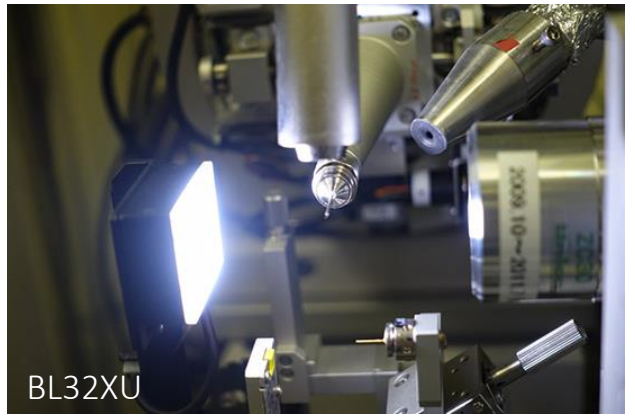
3. Toward single particle analysis: overcoming limitation due to crystallization

Opinion from Biology

1． Good experimental environment that facilitates measurement on challenging samples

- User-friendly device and automated experimental setup

TR-SFX experiments require many operations.
HPLC, He gas flow, pump laser, XFEL…
It is not trivial to perform it.



- Supportive service of data collection

Most of researchers from biology are unfamiliar with measurement techniques (including crystallization, data collection and data analysis) using XFEL.
The supportive service encourages all users from biology.

Opinion from Biology

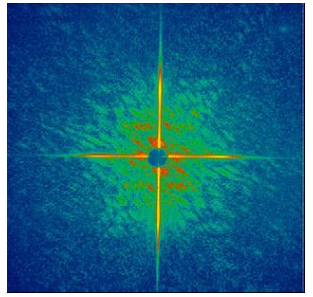
2. IT infrastructure for data analysis

- Supplying data storage connected to the SACLA HPC

We, the SFX consortium, need to share and analyze processed data from SACLA among many international collaborators.
The current storage is too small and deleted within a short-time period, which hinders completion of our work.
So, we would like to request enough data storage (150-200 TB) which can be accessed at any time, without time-lag.

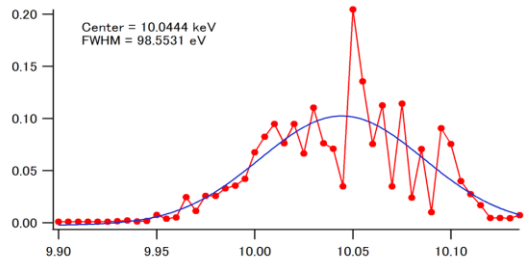
3. Toward single particle analysis: overcoming limitation due to crystallization

- Why do we still need to crystalize a sample?



We anticipated that we are finally free from necessity of crystallization when XFEL emerged. The necessity of crystallization limits scientific expansion.

In future, Intensity of XFEL should be increased dramatically to get atomic structure from a single biomolecule.



補足資料

Requests for SACLA

① For determination of the high-resolution crystal structure, upgrading the flux of XFEL is necessary.

Although XFEL enables us to determine a damage-free crystal structure, the photon flux of one pulse of XFEL is too low to determine the crystal structure at sub-angstrom resolution.

② In SACLA, since there are few methods to initialize a catalytic reaction synchronously by protein in a crystal, most proteins are hardly used for time-resolved analysis using XFEL to determine the intermediate structures in enzymatic reaction.

Indeed, most of reported time-resolved analyses using SACLA use the proteins whose reaction are induced by light irradiation. Using a caged compound released a substrate for an enzyme involved in catalytic cycles by light irradiation is one of the useful methods to raise the universal usage of XFEL time-resolved analysis. However, it is hard to construct the desirable caged compounds.

Requests for SACLA from Okayama

1. Ultra high brilliance of XFEL.
2. Sample delivery system for SFX with smaller consumption of the sample.
3. High repetition rate of XFEL (or simply more beamtime).
4. Labor-saving, user friendly data collection system like synchrotron radiation. Otherwise we need more non-technical support for data collection.

1. Good experimental environment that facilitates measurement on challenging samples

- Higher intensity of XFEL, higher repetition rate

- User-friendly setup

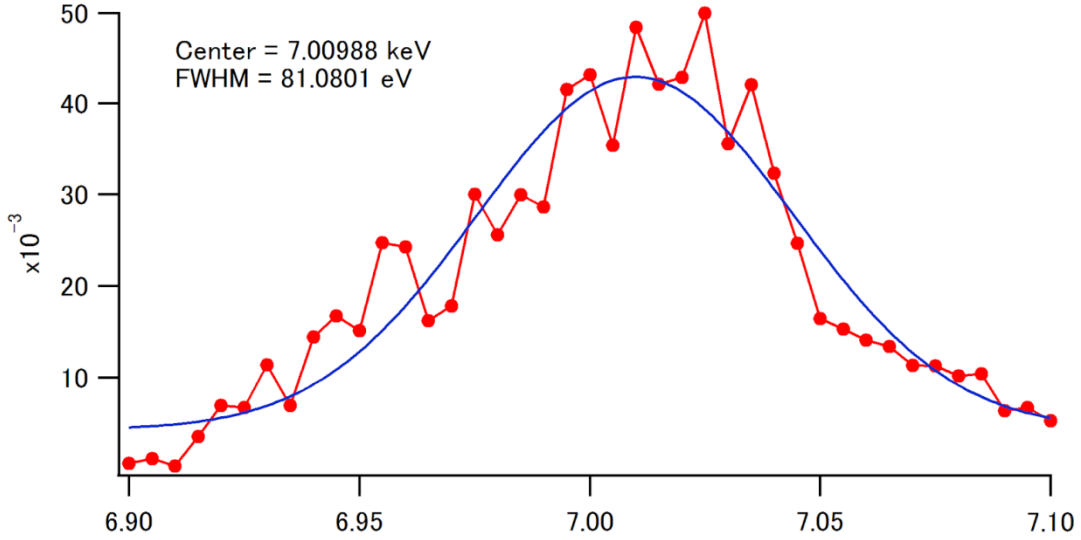
- Development of devices and techniques for SFX
  - Smaller consumption
  - Versatile method

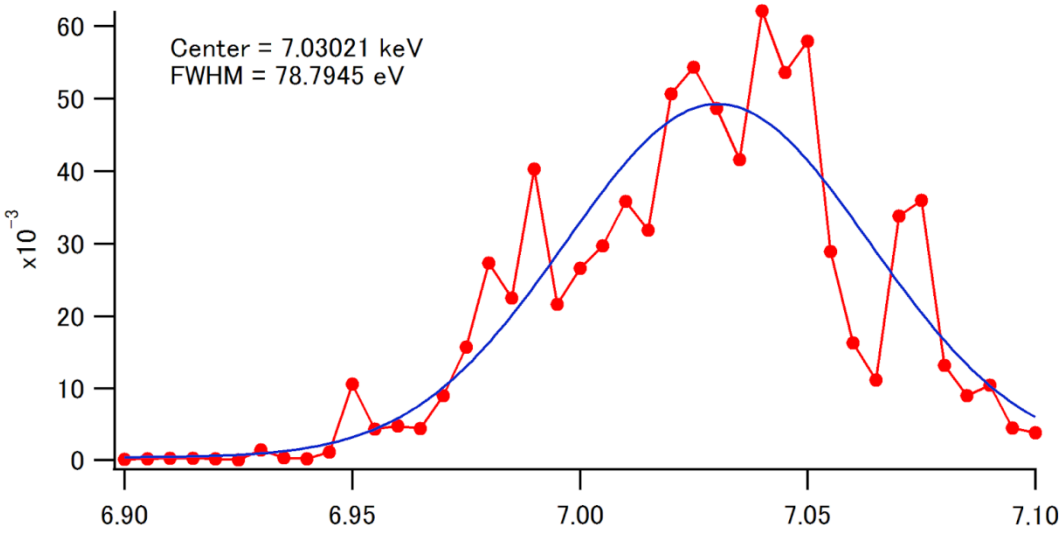
- Support

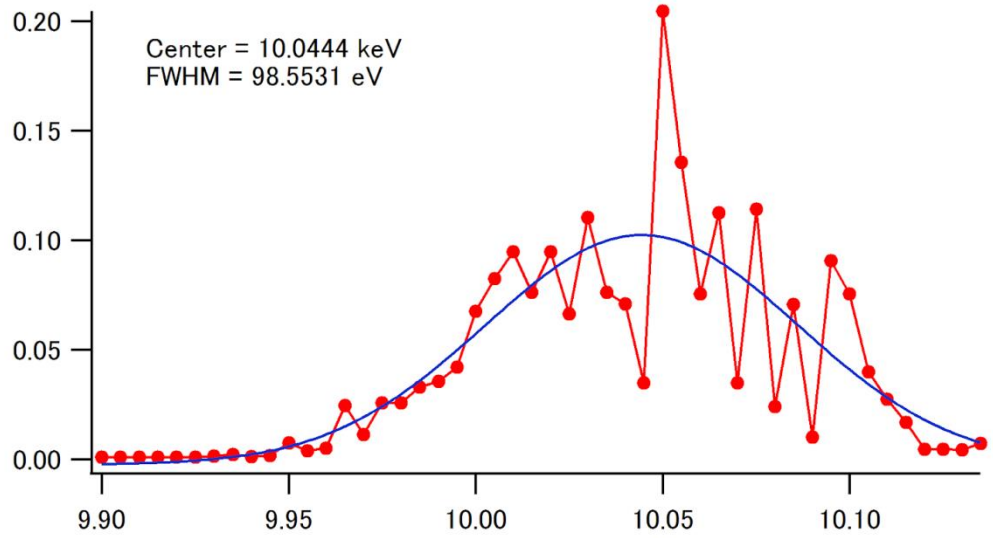
Influence by wide Bandwidth

Wide bandwidth causes broader diffraction spots

→ it influences refinement of the detector geometry (bad rmsd)







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