2 Structural Biology Research Center

2-1 Overview

The Structural Biology Research Center (SBRC) was established in May 2000 at the Photon Factory (PF) located in the Institute of Material Structure Science (IMSS). The main tasks of the Center are to provide user support for X-ray synchrotron radiation studies of bio-macromolecules, encourage advanced technical development, and boost in-house structural biology research. The Center has approximately 40 members, including one professor (Dr. T. Senda), six associate professors (Drs. R. Kato, N. Igarashi, M. Kawasaki, N. Shimizu, N. Matsugaki, and F. Yumoto), and three assistant professors (Drs. Y. Yamada, N. Adachi, and M. Senda) as core members (Fig. 1). About half of the SBRC members are engaged in beamline operation and development. X-ray crystallography activities are carried out under the leadership of Drs. Matsugaki and Yamada. Drs. Shimizu and Igarashi are responsible for small-angle X-ray scattering (SAXS). In 2013, Dr. L. M. Chavas, who had contributed to the PX beamlines of the PF, moved to CFEL of DESY as a project leader.

The structural biology building was built in April 2001 (430 m^2), and its area was later almost doubled to 765 m^2 (Fig. 2). About half of the SBRC members are involved in structural biology research in this building. All necessary structural biology experiments can be carried out in this laboratory. It has cell culture systems (bacteria, insect, and mammalian cells), liquid chromatography systems, a fully automated crystallization system, and equipment for physicochemical analyses such as Biacore, dynamic light scattering, mass-spectroscopy and SEC-MALS. In-house biological research is carried out under the leadership of Drs. Senda, Kato, Kawasaki, and Adachi.

The SBRC has constructed five beamlines for protein crystallography: BL-1A, BL-5A, BL-17A, PF-AR NW12, and PF-AR NE3A. BL-5A was constructed using "Special Coordination Funds for Promoting Science and Technology" (FY2001–FY2003) from the Japan Science and Technology (JST) Agency. During the "Development of System and Technology for Advanced Measurement and Analysis" project, we developed a microbeam beamline, BL-17A. BL-1A was constructed in the "Targeted Proteins Research Program" by MEXT/JST (FY2007–FY2011), and was designed for sulfur-SAD experiments (see below).

Under the PF Program Advisory Committee (PAC) system, the SBRC accepts many researchers from outside KEK who wish to collect diffraction data for their own macromolecular crystals. The number of academic proposals and users has reached 100–120/year in recent years. As a result of advances in structure-based drug design, pharmaceutical companies require a large amount of beam time. Many Japanese companies have been using the bio-macromolecular crystallography beamlines of KEK-PF. The Tsukuba Consortium, which is composed of seven companies (four from another Pharmaceutical Consortium for Structure Analysis and three other companies), uses our beamlines. Among them, Astellas Pharma, Inc. financed the construction of the beamline AR-NE3A for their research.

Figure 1: Members of the Structural Biology Research Center (SBRC).

Figure 2: Structural Biology Research Center.

2-2 Leading the National Project for Structural Life Science – PDIS Starting from FY2012

The SBRC plays a key role in a national project for structural life science, the Platform for Drug Discovery, Informatics, and Structural Life Science (PDIS). The PDIS, which was launched with the support of MEXT in FY2012, is an open platform providing comprehensive support for life-science researchers. The support includes protein production, bioinformatics, 3D-structure analysis, compound-library screening, etc. The PDIS is composed of three platforms: platforms of structural life science, drug discovery, and informatics. The SBRC is the head of the platform of structural life science. We plan to develop beamlines for protein crystallography and bio-SAXS and will provide services for researchers in biology fields to accelerate studies in structural life science. The SBRC also provides a high-throughput crystallization service using an automated crystallization and observation robot (PXS). In PDIS, the SBRC has accepted approximately 25 projects, and supports research ranging from crystallization to structure determination by X-ray crystallography; five crystal structures were determined in 2013. In addition, the SBRC has studied the sulfur-SAD method using low-energy X-rays, and has already succeeded in determining the crystal structures of some proteins by this method. In addition to the sulfur-SAD, BL-1A can be used to identify calcium ions in proteins. Prof. Miki's group in Kyoto University used BL-1A to identify calcium ions in the supramolecular complex between the photosynthetic reaction center and the light-harvesting antenna core [1]. They collected diffraction data of the supramolecular complex using two different wavelengths, 2.70A and 3.15A, and clearly showed the calcium ions bound to the supramolecular complex (Fig. 3).

2-3 Research Progresses under Several External Grants

In addition to the PDIS, the SBRC has obtained several external grants, such as CREST and PRESTO from JST, "Key strategic research for the use of X-ray free-electron lasers" from MEXT, "Photon and Quantum Basic Research Coordinated Development Program" from MEXT, and KAKEN-HI from MEXT/JSPS. One of the main projects of SBRC is to study the structural biology of chromatin related protein complexes. We have succeeded in purifying several protein complexes involved in chromatin transcription from budding yeast. Physicochemical analyses of these complexes are in progress for their structural study. This project is supported by PRESTO and KAKEN-HI. The second project is a study of the structural biology of the CagA oncoprotein derived from *Helicobacter pylori*, which causes some stomach diseases including stomach cancer. While the crystal structure of the N-terminal region of CagA has already been determined [2], the C-terminal region of CagA is considered to be an intrinsically disordered region. Our structural and biochemical analysis revealed that this region adopts a lariat-like loop structure by interacting with the N-terminal structured region. We are investigating the structural nature of the lariat-like loop region using CD and SAXS. In addition, we are working to reveal the tertiary structure of the CagA-PAR1-SHP2 complex. These CagA researches are supported by CREST/JST. The third project is the structural analysis of the redox-dependent affinity control of the electron transfer complex, which is supported by the Photon and Quantum Basic Research Coordinated Development Program. The SBRC is studying the detailed molecular mechanisms underlying the redox-dependent affinity control between ferredoxin BphA3 and ferredoxin reductase BphA4 on the basis of their crystal structures under various redox states at high resolution.

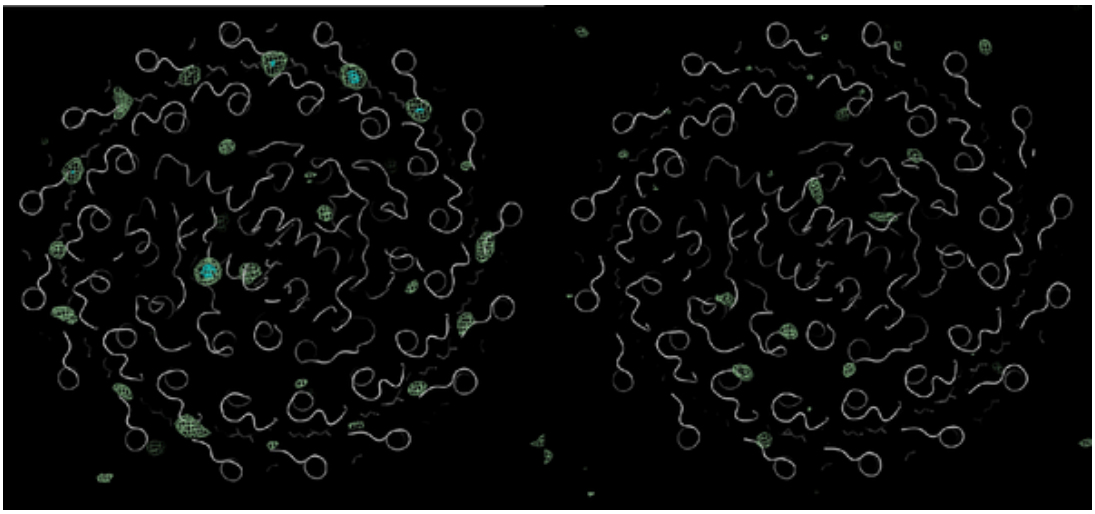

Figure 3: Determination of Ca^{2+} sites using long-wavelength X-rays in the supramolecular complex between the photosynthetic reaction center and the light-harvesting antenna core. Anomalous difference Fourier maps from datasets collected at 2.70 Å (left) and 3.15 Å (right), respectively. On the basis of these results, the positions of Ca^{2+} ions in the supramolecular complex were successfully determined. (Photo provided by Professor Kunio Miki, Graduate School of Science, Faculty of Science, Kyoto University)

In addition to these main projects, the SBRC has several other projects. In 2013, SBRC revealed the crystal structure of a reaction intermediate of an extradiol dioxygenase DesB. The crystal of the reaction intermediate was prepared under anaerobic conditions using an anaerobic chamber that was specially designed for anaerobic crystallization (Fig. 4). The crystal structure of DesB showed a large Fe ion shift in the catalytic reaction (Fig. 5). Biochemical studies revealed that this shift seemed to be related to the strict substrate specificity of this enzyme [3].

REFERENCES

[1] S. Niwa, L.-J. Yu, K Takeda, Y. Hirano, T. Kawakami, Z.-Y. Wang-Otomo and K. Miki, *Nature* **508**, 228 (2014).

[2] T. Hayashi, M. Senda, H. Morohashi, H. Higashi, M. Horio, Y. Kashiba, L. Nagase, D. Sasaya, T. Shimizu, N. Venugopalan, H. Kumeta, N. N. Noda, F. Inagaki, T. Senda and M. Hatakeyama, *Cell Host & Microbe.* **12**, 20 (2012).

[3] K. Sugimoto, M. Senda, D. Kasai, M. Fukuda, E. Masai and T. Senda, *PLoS One* **9**, e92249 (2014).

Figure 4: Anaerobic chamber used to prepare crystals of reaction intermediates of DesB.

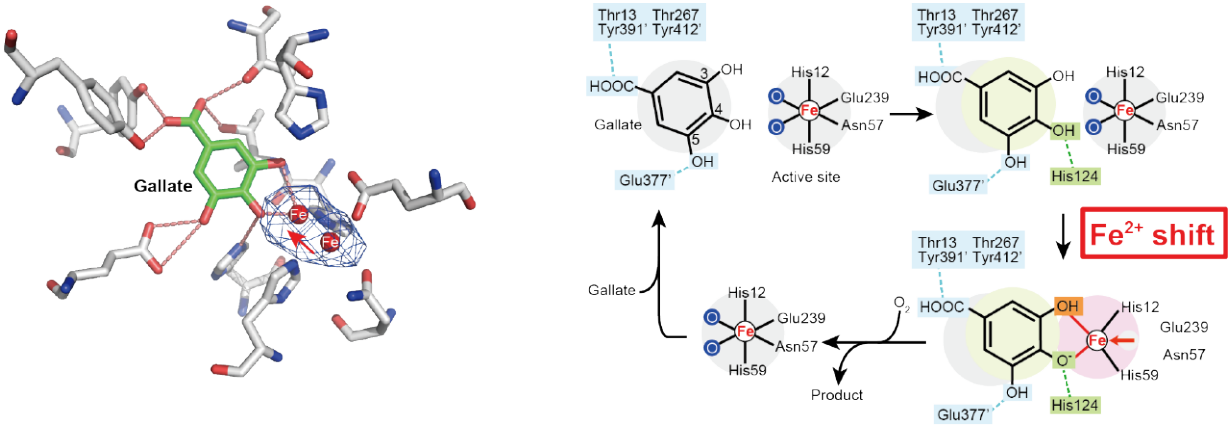

Figure 5: Active site of DesB (left) and its proposed catalytic reaction mechanism (right). In the catalytic reaction, the Fe^{2+} ion in the active site shifts by approximately 2 Å. In the left panel gallate (substrate) and Fe^{2+} ion are shown in green and red, respectively. This Fe^{2+} shift is critical to the catalytic reaction of DesB and contributes to the strict substrate specificity of this enzyme.