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. In the last 12 years, the SBRC has published significant research studies under the leadership of Prof. Soichi Wakatsuki. After Prof. Wakatsuki moved to Stanford University in January 2013, Prof. Toshiya Senda joined the SBRC as director. The Center has approximately 40 members (Fig. 1), including one professor (Dr. T. Senda), five associate professors (Drs. R. Kato, N. Igarashi, M. Kawasaki, N. Shimizu, and F. Yumoto), one vice-associate professor (Dr. M. Hiraki), and three assistant professors (Drs. N. Matsugaki, Y. Yamada, and L. Chavas) as the core members. About half of the SBRC members are engaged in beamline operation and development, and the others are involved in structural biology research. In beamline operation and development, Drs. Shimizu and Igarashi are responsible for small-angle X-ray scattering (SAXS), and Dr. Hiraki is responsible for robotics and automation. X-ray crystallography activities are carried out under the leadership of Drs. Matsugaki, Yamada, Chavas, Hiraki, and Igarashi.

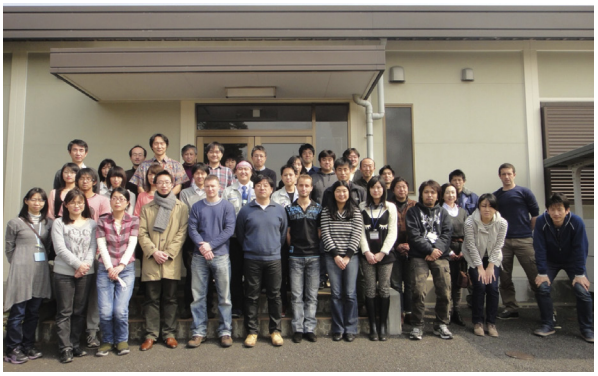

Figure 1: Members of the Structural Biology Research Center.

Figure 2: Structural Biology Research Center.

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). 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 analysis (Biacore, DLS, MALS, MS, etc.). In-house biological research is carried out under the leadership of Drs. Senda, Kato, and Kawasaki.

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 micro-beam beamline, BL-17A, which is the first beamline developed at the PF with a short-gap undulator as a light source. BL-1A was constructed in the "Targeted Proteins Research Program" by MEXT/JST (FY2007–FY2011).

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 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), is using our beamlines. Among them, Astellas Pharma, Inc. financed the construction of the beamline AR-NE3A for their research.

2-2 Leads 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), which will undergo further development in this project. In FY2012, we installed a SONICC (Second Order Nonlinear Imaging of Chiral Crystals) system as a new crystal observation system (Fig. 3). SONICC is based on the principle of second harmonic generation (SHG), where two low-energy photons combine to form a higher-energy photon under intense electric fields. The SHG signal is generated only in chiral crystals such as protein crystals. However, salt crystals, which are mostly achiral, generate no SHG signals. Therefore, protein crystals can be selectively observed with SONICC. The SONICC system will be installed into PXS in FY2013. The SBRC has also studied the sulfur-SAD method using low energy X-rays. Diffraction data for sulfur-SAD have been collected at BL-1A with a newly installed Pilatus 2M detector using the longest available wavelength of 3.3 Å (Fig. 4). We succeeded in determining the crystal structure of a test protein (glucose isomerase) by the sulfur-SAD method. Further development of the sulfur-SAD method at BL-1A will be performed in the PDIS.

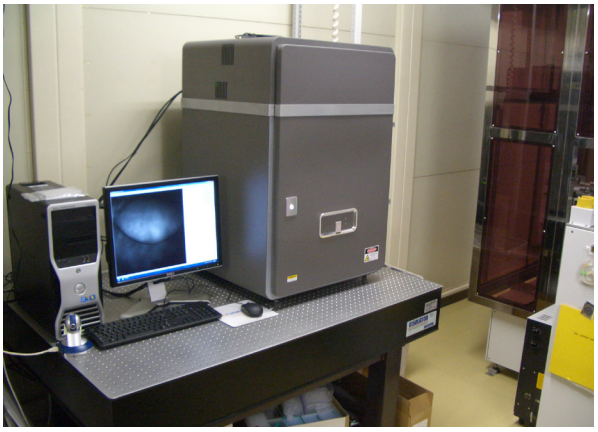

Figure 3: SONICC system installed in the Structural Biology Building.Since a second harmonic signal is generated in most protein crystals but not salt crystals, SONICC can selectively detect protein crystals.

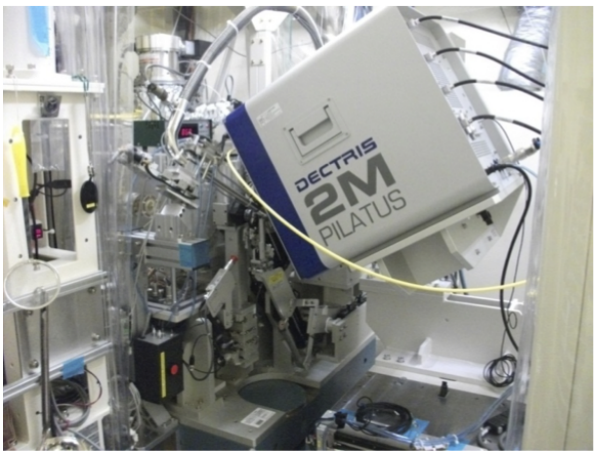

Figure 4: A newly installed Pilatus 2M detector at BL-1A.

2-3 Research Progresses under Several External Grants

In addition to the PDIS, the SBRC has obtained several external grants, such as CREST from JST, “Key strategic research for the use of X-ray free-electron lasers” from MEXT, and KAKEN-HI from MEXT/JSPS. The SBRC initiated new in-house structure biology projects in FY2012. The first one is the structural biology of epigenetic information of histone proteins to understand their mechanisms and functions. The main targets of this project are histone chaperones, which are involved in nucleosome assembly and disassembly; this project is supported by KAKEN-HI. The second project is the structural biology of the CagA oncoprotein derived from *Helicobacter pylori*, which causes some stomach diseases including stomach cancer. The crystal structure of CagA has already been determined using the beamline NE-3A in PF-AR (Highlight 5-2). [1] The SBRC is currently working to reveal the crystal structure of the CagA-PAR1-SHP2 complex with the support of CREST/JST. The CagA-PAR1-SHP2 complex perturbs normal signaling in the cell, causing the formation of stomach cancer. Tertiary structural information of the CagA-PAR1-SHP2 complex will give an insight into the molecular mechanism of stomach cancer formation by *Helicobacter pylori*. In addition to these new projects, the SBRC is continuing with existing structural biology researches [2, 3].

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