3 Structural Biology Research Center

3-1 Overview

The Structural Biology Research Center (SBRC) was started in May 2000 in the Photon Factory (PF) at the Institute of Material Structure Science (IMSS). The main tasks of the center are providing user support for synchrotron radiation X-ray crystallographic studies of bio-macromolecules, highly advanced technical development, and in-house structural biological research. The center now has about 30 members, including a professor (Dr. S. Wakatsuki), two associate professors (Drs. R. Kato and N. Igarashi), a vice associate professor (Dr. M. Hiraki), and four assistant professors (Drs. N. Matsugaki, M. Kawasaki, Y. Yamada, and L.M.G. Chavas) as the core members. About half of the SBRC members are engaged in beamline operation and development, and the remaining members in biological research (Fig. 1). Dr. L.M.G. Chavas joined us in April 2009. The in-house biological research is conducted in the structural biology building, which was built in April 2001 (430 m^2) and has been extended twice to 765 m^2. All the processes necessary for structural biological research can be conducted in the building, including protein expression, sample purification, crystallization, and biochemical and biophysical analyses of targeted proteins.

Our research activities are mainly supported by scientific funds. The first high-throughput beamline, AR-NW12A, was built using a PF supplemental budget during FY 2000–2001. We built a second state-of-the-art beamline, BL-5A, and also prepared various scientific equipment for protein expression, purification, and crystallization experiments using a grant from "Special Coordination Funds for Promoting Science and Technology" (FY2001–2003) of JST (Japan Science and Technology Agency). We subsequently participated in two large-scale projects: "Protein 3000 National Project" of MEXT (Ministry of Education, Culture, Sports, Science and Technology Japan)/JST (FY2002–2006) and "Development of System and Technology for Advanced Measurement and Analysis" of JST (FY2004–2007).

Figure 1
Members of the Structural Biology Research Center (SBRC) photographed at the entrance to the Structural Biology Building.

During the five years of the Protein 3000 Project, the SBRC operated in one of the eight consortia aimed at pursuing structural and functional analyses in the field of post-translational modification and transport. In order to conduct the research program, the SBRC of KEK-PF took the lead by coordinating the consortium, which consisted of eleven universities and four research institutes. Our consortium determined 254 protein structures and published 296 papers in five years. Since the cumulative number of structures determined by the Protein 3000 Project exceeded 3,000, the project was considered a success. To further assist the Protein 3000 Project, we set aside about 30% of the bio-macromolecular crystallography beam time in PF (327 days in all) for the users belonging to the eight consortia.

During the "Development of System and Technology for Advanced Measurement and Analysis" project, we developed a micro-beam beamline, BL-17A, and the prototype of a next-generation X-ray area detector. BL-17A was the first beamline developed at PF to have a short gap undulator as a light source. The beam size can be controlled from 100 to 10 μm on the horizontal axis and is useful for both modest and small protein crystal experiments [1]. The new generation X-ray area detector was developed in collaboration with NHK (Japan Broadcasting Corporation) Laboratory and several other companies. Based on HARP (High-gain Avalanche Rushing amorphous Photoconductor) and FEA (Field Emitter Array) technologies, prototype detectors were developed, which showed the advantages of special resolution and X-ray sensitivity as compared to CCD detectors [2].

The current five-year national project "Targeted Proteins Research Program" by MEXT/JST started in FY2007 and will end in FY2011 (Fig. 2). The SBRC is contributing to the project via two independent programs. One is the structural and functional research of protein transport in cells in close collaboration with the University of Tokyo and Kyoto University. The other is beamline developments in collaboration with SPring-8

Table 1 Numbers pf proposals for protein crytallography beamlines in recent five years.

Type	G	P	U	S2	Total
2005	81	0	0	1	82
2006	86	0	0	1	87
2007	117	2	3	0	123
2008	101	0	0	1	102
2009	115	1	0	0	116

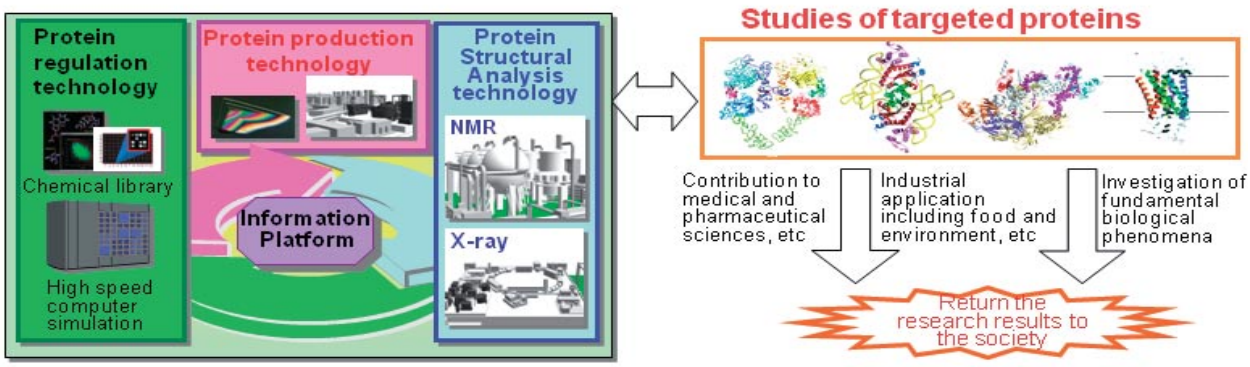

Figure 2
Scheme of the Japanese national project "Targeted Proteins Research Program" by MEXT (FY2007–2011).

and Hokkaido, Kyoto, and Osaka Universities. Details of the programs are described later.

In addition to the local scientific research activities, the SBRC assists a large number of researchers from outside KEK, who wish to collect diffraction data of their own macromolecular crystals under the PF PAC (Program Advisory Committee) system. The numbers of academic proposals and users have been increasing in recent years as summarized in Table 1. Additionally, along with advances in structure-based drug design, pharmaceutical companies are requiring a larger amount of beam time. As a direct consequence, twelve Japanese companies have been using beam time of bio-macromolecular crystallography beamlines at KEK-PF. In addition, Astellas Pharma Inc. financed the construction of a new beamline, AR-NE3A, for their research. The operation of the new beamline began in April 2009, and many diffraction data have been collected already.

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3-2 Targeted Proteins Research Program

Structure-Function Analysis of Protein Complexes that Regulate Vesicular Traffic

In the field of "Investigations of fundamental biological phenomena," we have begun the "Structure-function analysis of protein complexes that regulate vesicular traffic". This project is dedicated to protein targets of crucial importance but presenting extreme difficulty to deal with, such as protein complexes, including membrane proteins involved in membrane traffic in eukaryotic cells. Cell signaling and intracellular trafficking are the means by which eukaryotic cells deliver cargo proteins to various organelles, cell membranes, and extracellular destinations. Accurate distribution of proteins is crucial for a range of cellular functions and activities. An increased understanding of the biological and biomedical functions of transport and modification proteins is indispensable for further progress in the treatment of human diseases.

In order to develop the project, SBRC has collaborated with Prof. Akihiko Nakano (University of Tokyo), who is working on the functional research of proteins involved in Golgi formation, post-Golgi membrane traffic, and endocytosis in yeast and higher plants; Prof. Kazuhisa Nakayama (Kyoto University), who is studying proteins involved in the regulation of cell functions, such as cytokinesis, cell polarity, and cell mobility; and Prof. Ken Sato (University of Tokyo), who is researching transport vesicle formation from endoplasmic reticulum (ER) in yeast. Through close cooperation among these researchers, SBRC is currently working on the expression, purification, crystallization, and structure determination of target proteins that are either membrane proteins or form macromolecular complexes. The collaboration also includes Prof. Nagano (University of Tokyo) and Prof. Sakisaka (Kobe University), who are members of the program. Recent outcomes of the research are described later.

Technological Development Research of Protein Structural Analysis

In determining the three-dimensional structures of bio-macromolecules by X-ray crystallography, the phase problem is one of the most important issues. Sulfur SAD (Single wavelength Anomalous Dispersion) analysis is currently one of the most attractive methods to bypass this problem, especially when heavy atom (or selenomethionine) derivative crystals of the targeted macromolecules are difficult to prepare, e.g., integral membrane proteins and macromolecular complexes. We have developed a new beamline dedicated to sulfur SAD experiments as part of the national project "Targeted Proteins Research Program". The beamline, BL-1A (Fig. 3), has been designed to deliver an intense lower energy beam at about 4 keV using the first harmonics

of the short gap undulator as the light source in order to enhance the anomalous signal from light atoms, such as, sulfur and phosphor. The beamline also covers the energy range of 12–13 keV with the 3rd harmonics, allowing MAD (Multiple wavelengths Anomalous Dispersion) measurements using Se, Hg, Au, or Pt atoms. The beamline will be open to the members of the program in FY 2010.

In addition to the construction of BL-1A at PF, this research project contains another high-energy, brilliant, and small-size beam beamline development at SPring-8 as well as some environmental technology developments by other universities (Hokkaido, Kyoto, and Osaka Universities). Compatibility of the sample cassette has been one of the most important problems, e.g., the SPACE pin developed at SPring-8 cannot be used at any other facilities; our PAM (PF Automated Mounting) system can handle Hampton-type general sample pins with an SSRL cassette but not a Uni-puck cassette, which is now widely used globally. In the project, SBRC is developing a compatible cassette, which can deal with both SPACE and Hampton pins, in collaboration with the universities. We have modified the PAM system to be able to handle Uni-puck cassettes and the system was opened for users at the end of FY 2009 (Fig. 4).

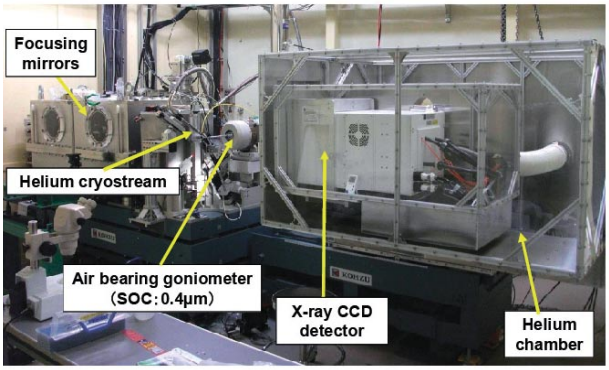

Figure 3
Internal view of the end-station of BL-1A.
The vacuum section of the beamline has only one beryllium window, followed by a diffractometer, equipped with a helium cryostream, and a specially designed helium chamber to minimize the loss of the lower energy beam. Simple optics (a cryo-cooled channel-cut monochromator, which is placed upstream of this photo, and bimorph KB focusing mirrors) are adapted to deliver a well-focused small-sized beam with good stability.

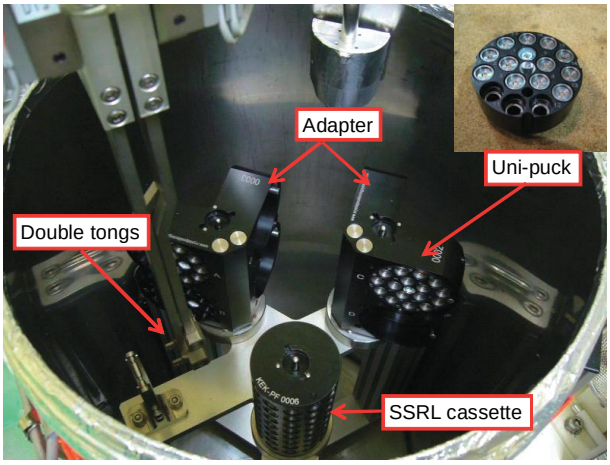

Figure 4
Uni-puck cassette in the PAM system.
View of the liquid nitrogen Dewar in which sample pins are stored. Three cassettes can be set in the Dewar; one SSRL cassette and two Uni-puck adaptors are set in the figure. One SSRL cassette can store 96 sample pins and one Uni-puck adaptor can contain four Uni-pucks, which can store sixteen sample pins each.

3-3 Highlights on In-House Structural Biology Research

Many GTPases regulate the intracellular transport and signaling in eukaryotes. Guanine nucleotide exchange factors (GEF) activate GTPases by catalyzing the exchange of their GDP for GTP. ARA7, a plant homolog of the Rab5 GTPase, controls endosomal fusion and is specially activated by VPS9a, a GEF for ARA7. Crystal structures of ARA7/VPS9a complex in three different nucleotide states: no nucleotide-free, GDP-bound, and GDPNH2, which is a GDP analog, were determined (Fig. 5). Moreover, the structure of the ARA7-GDP/VPS9a (D185N) mutant was also determined. The four complex structures provide a mechanistic description of the intermediates of guanine nucleotide exchange in Rab5/Vps9, in which GEF directly interacts with the nucleotide. Upon complex formation with ARA7, VPS9 wedges into the interswitch region of ARA7, inhibiting the coordination of Mg^{2+} and decreasing the stability of GDP binding. The aspartate finger of VPS9a directly recognizes GDP β-phosphate and pulls the P-loop lysine of ARA7 away from GDP β-phosphate towards switch II to further destabilize GDP in order to release it during the transition from the GDP-bound to nucleotide-free intermediates in the nucleotide exchange reaction. The aspartate finger of VPS9a and the P-loop lysine of ARA7 are supposed to elaborate the nucleotide exchange mechanism.

Another research highlight is introduced in the “High-

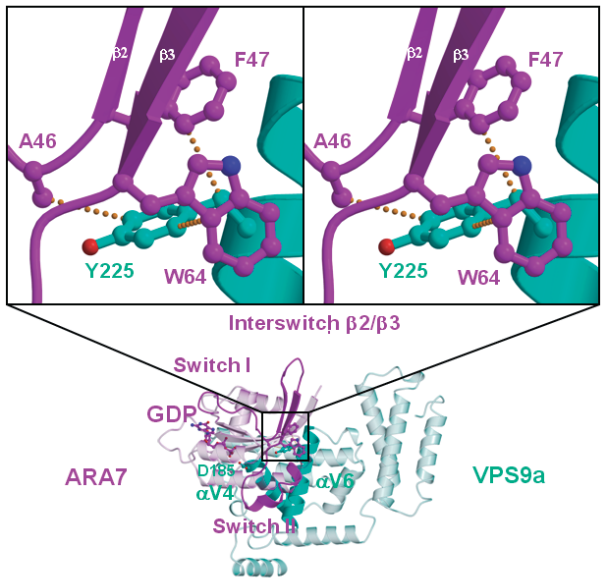

Figure 5
Structure of ARA7-GDP/VPS9a.

lights" section of this volume (pp 44). CERT is responsible for the ceramide transfer. We solved the structures of CERT protein with some inhibitors. The crystal structures reveal how CERT recognizes ceramide specifically, and how CERT interacts with membrane. [3, 4].

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3-4 Beamlines for Bio-Macromolecular Crystallography

Dr. N. Igarashi had been the branch head of beamline operation and development until 2008. Since he moved to a group for beamline engineering, technical service, and safety, Dr. N. Matsugaki has been appointed as the branch head and a new member, Dr. L.M.G. Chavas, has also joined us. Although Dr. N. Igarashi moved to another group, he still belongs to the SBRC and plays some important roles.

The SBRC currently operates four insertion device (ID) crystallographic beamlines for bio-macromolecular crystallography (Table 2). Among these ID beamlines, BL-5A, AR-NW12A, and AR-NE3A are high-throughput structural biology beamlines. BL-17A is a micro-beam beamline designed for micro-crystal structure analysis and SAD phasing with light atoms using a low energy beam of about 6–8 keV [1]. BL-6A, which was a bending magnet beamline, was closed at the end of FY 2009. A new beamline, BL-1A, has been constructed and will open in FY 2010. Rearrangement of the X-ray area detectors installed at each beamline is being conducted. The ADSC Quantum 270 moved to BL-1A from BL-17A at the end of FY 2009. Exchange of the ADSC Quantum 315r at BL-5A and ADSC Quantum 210r at BL-17A has been scheduled for the summer shutdown of FY 2010.

AR-NE3A, which began operating from FY 2009, is a new beamline and is dedicated to the high-throughput bio-macromolecular crystallography required for pharmaceutical applications. AR-NE3A can generate the most intense X-ray beam at the sample position among all the bio-macromolecular crystallography beamlines at PF [5]. Its flux is about three times higher than the high-throughput beamline, AR-NW12A. In addition, a high precision diffractometer, a fast-readout and a high-gain CCD detector with a large active area, a sample exchange system (PAM) [6], and an automated data collection system realize high-throughput and precise data collection. Users can collect more than 150 data sets in a day. The construction of AR-NE3A was financially supported by Astellas Pharma Inc. and they have priority access to this beamline for their research. The remaining beam time is assigned to general academic users and other industrial users.

For further high-throughput and more convenient bio-macromolecular crystallography, we have been improving the automation of beamline control, operation of the sample change system, automatic sample centering system, unified measurement software, and automated data processing. In a fully automated data collection system, a user prepares a sample description file before the experiment. This description file contains a list of the samples with the slot number on the cassette of the

Table 2 Summary of protein crystallography beamlines at KEK-PF.

	BL-5A	BL-6A	BL-17A	AR-NE3A	AR-NW12A
Starting year	2004	1987	2006	2009	2003
Synchrotron ring	PF (2.5 GeV, 300 - 450 mA)			PF-AR (6.5 GeV, 40-60 mA)	
Injection	once a day (9:00) or continuous (top up)			twice a day (10:00, 22:00)	
X-ray source	Multi Pole Wiggler	Bending Magnet	Short Gap Undulator	Undulator	Undulator
Wavelength range (Å)	0.7-1.9	0.91 - 1.33	0.95-1.3, 1.6-2.2	0.7-1.9	0.7-1.9
Energy resolution (ΔE/E)	2.5×10^{-4}	1×10^{-3}	2.5×10^{-4}	2.5×10^{-4}	2.5×10^{-4}
Photon flux (photons/sec @ 1.0 Å)	3.0×10^{11}	1.0×10^{10}	6.6×10^{9}(@1.0 Å) 1.3×10^{10}(@2.0 Å)	8.0×10^{11}	2.9×10^{11}
Slit size (mm)	0.2	0.1	0.02	0.2	0.2
Detector	Quantum 315r	Quantum 4R	Quantum 270	Quantum 270	Quantum 210r
Type	CCD	CCD	CCD	CCD	CCD
Active area (mm^2)	315 × 315	188 × 188	270 × 270	270 × 270	210 × 210
Pixel size (μm^2)	51 × 51	81.6 × 81.6	64.8 × 64.8	64.8 × 64.8	51 × 51
Pixel number	6144 × 6144	2304 × 2304	4168 × 4168	4168 × 4168	4096 × 4096
Frame data size (MB)	75	11	34	34	34
Readout time (sec)	0.9	8	1.1	1.1	0.9
Typical exposure time (1.0° oscillation)	3 sec	30 sec	5 sec	1 sec	3 sec
Typical data collection time (180 frames)	14 min	120 min	24 min	11 min	14 min
Camera distance (mm)	60 ~ 100	50 ~ 400	40 ~ 700	60 ~ 500	60 ~ 1000
Spindle axis precision	1.0 μm	10 μm	0.37 μm	1.0 μm	2.2 μm
Sample exchanger	PAM	-	PAM (288 samples / Dewar)		

sample exchanger and the data collection and processing conditions (e.g., exposure time, number of images, wavelength of the X-ray beam, camera distance, slit size, spacegroup candidates, and so on). Once the user sets the sample cassette in the Dewar of PAM robot, the sample description file is sent to the beamline controlling software, which begins automatic data collection and processing. The user does not need to do anything until all data sets have been collected. During the procedure, the samples are exchanged by PAM, centered, and the data set is collected according to the conditions described in the description file. When a sample is centered, the loop centering procedure is performed. After data collection, a beamline control client, PROCESSOR [7], executes the data processing software to process the data sets and reports the data collection statistics.

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3-5 Homage for BL-6A

BL-6A ended its long history as a structural biology beamline in March 2010. BL-6A has been dedicated to structural biology for many years. However, the beam intensity from the bending magnet of the second-generation synchrotron is no longer sufficient for current structural biology targets such as membrane proteins and protein complexes, so the KEK decided to close BL-6A and use it for other science activities.

BL-6A was opened to users in 1987 [8]. The beamline was equipped with a quartz plane mirror more than 1 meter long for vertical focusing. An optical bench was installed in the experimental hutch, equipped with an asymmetric Johann-type monochromator for horizontal focusing, a slit system and a horizontal 2θ table used for mounting and aligning a diffractometer. Depending on the diffractometer, BL-6A was divided into two periods (Fig. 6). The diffractometer was a Sakabe Weissenberg camera (until 1999) [9] or an X-ray CCD detector on a goniostat (since 2000).

BL-6A was one of the oldest structural biology beamlines in the world and has seen a huge number of users from not only Japan but worldwide. Prof. Ada Yonath, who won the Nobel Prize in Chemistry in 2009, was one of the first three user groups of BL-6A. The highlight of the first season of BL-6A was perhaps the publication of the structures of cytochrome c oxidases from two different species in *Science* and *Nature* in the same week in 1995 [10,11]. We should also not forget that BL-6A was used for material science, and an important paper in the field was published in *Nature* the next week [12].

At the almost same timing in the second season, the SBRC started at KEK. The first paper from the SBRC using the new BL-6A diffractometer was published in *Nature* [13]. The diffractometer was improved year by

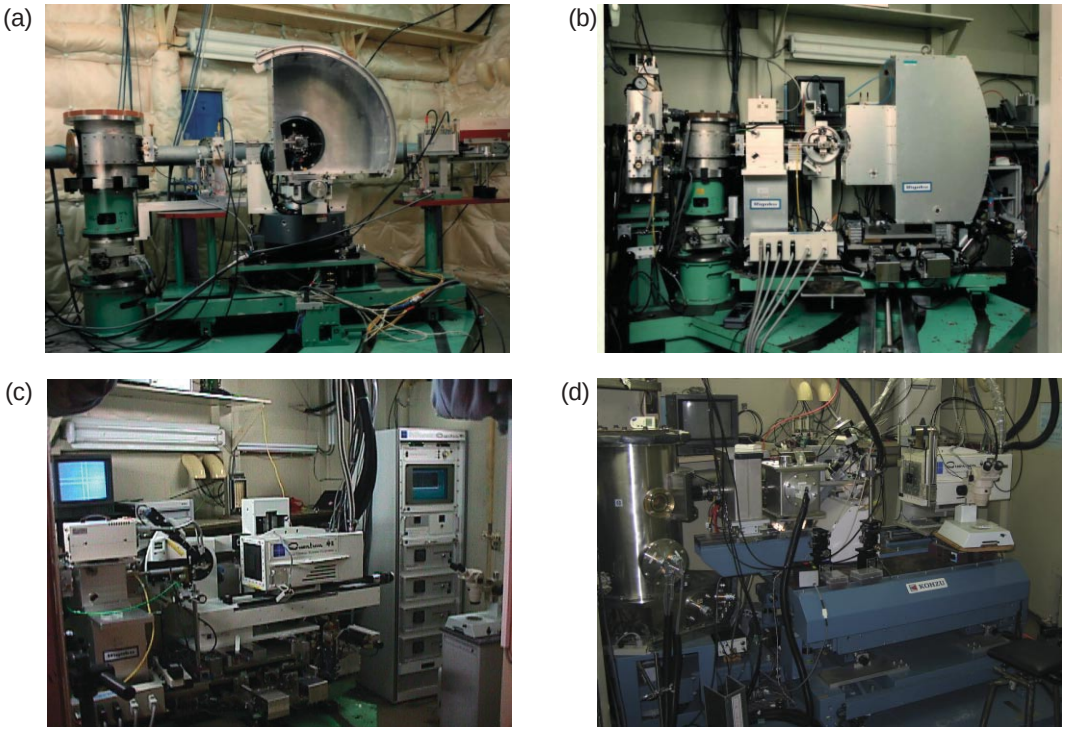

Figure 6
BL-6A diffractometers. (a) Sakabe camera No. 2 until 1990, (b) Sakabe camera No. 3 until 1999, (c) X-ray CCD goniostat on 2θ table until 2001 and (d) new diffractometer, monochromator and 2θ table until 2010.

year and the devices, control software and many techniques were widely applied to other PF structural biology beamlines. Currently we operate five insertion device beamlines dedicated to structural biology and almost all apparatuses have been developed based on the BL-6A diffractometer.

Many beamline staff supported the BL-6A activities (Fig. 7). First, Prof. Sakabe's group developed the Sakabe Weissenberg camera with imaging plates. Drs. A. Nakagawa and N. Watanabe were managers of the BL-6A beamline. Then, Drs. M. Suzuki and N. Igarashi managed BL-6A and developed the measurement system based on the CCD detector. The beamline staff of the SBRC group, Drs. N. Igarashi, N. Matsugaki, Y. Yamada, M. Hiraki and S. Wakatsuki, completed the integrated data collection system. Also, many other PF staff and beamline assistants were involved in BL-6A operations, and a number of users contributed significantly to the progress of structural biology using BL-6A for many years. To mark the closing of BL-6A, a PF study meeting was held with many participants on July 12-13, 2010. We talked about the many contributions and good memories of BL-6A and discussed the next steps for structural biology research based on the BL-6A achievements. Although BL-6A is now closed, its links to the future of structural biology will remain.

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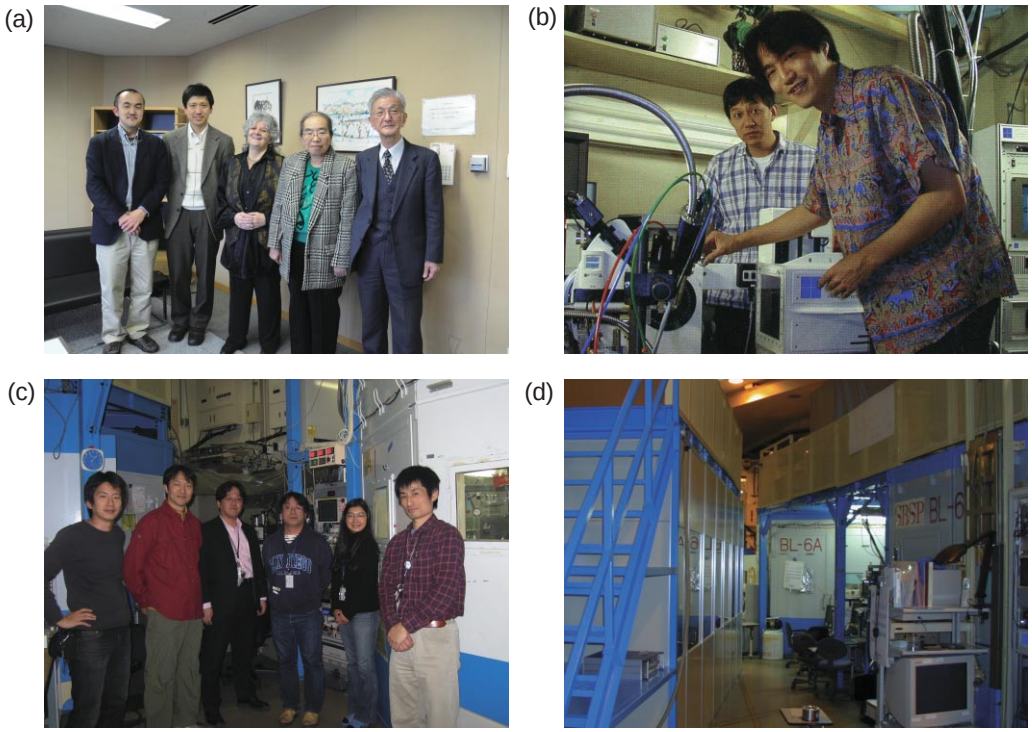

Figure 7
Beamline staff dedicated to the BL-6A. (a) Prof. Sakabe's group with Prof. Yonath, (b) Drs. Suzuki and Igarashi, (c) SBRC beamline staff with the last users (Drs. Senda) on March 9, 2010 and (d) BL-6A experimental hutch viewed from the PF corridor.