2 Structural Biology Research Center

2-1 Overview

The Structural Biology Research Center (SBRC) was set up in May 2000, in the Photon Factory (PF) of the Institute of Material Structure Science (IMSS). The main tasks of the Center are to support users conducting X-ray synchrotron radiation studies of bio-macromolecules, highly advanced technical development, and in-house structural biology research. The Center now has over 30 staff, including a professor (Dr. S. Wakatsuki), four associate professors (Drs. R. Kato, N. Igarashi, M. Kawasaki and N. Shimizu), a vice associate professor (Dr. M. Hiraki) and three assistant professors (Drs. N. Matsugaki, Y. Yamada, L. Chavas) as the core members. About half of the SBRC members are engaged in beamline operation and development, and the remaining half in biological research (Fig. 1). In the beamline operation and development, Drs. Shimizu and Igarashi are responsible for small-angle X-ray scattering (SAXS), and Dr. Hiraki for robotics and automation. X-ray crystallography activities are led by Drs. Matsugaki, Yamada, Chavas, Hiraki and Igarashi, while in-house biological research is led by Drs. Kato and Kawasaki. In the structural biology building, which was built in April 2001 (430 m^2) and extended twice to 765 m^2, all the steps necessary in structural biology research can be conducted, including protein expression, sample purification, crystallization, and biochemical and biophysical analyses of targeted proteins.

Our research activities were mainly supported by scientific funds. We built an up-to-date beamline, BL-5A, and also prepared various scientific equipment for protein expression, purification, and crystallization experiments using "Special Coordination Funds for Promoting Science and Technology" (FY2001–2003) from the Japan Science and Technology Agency (JST). Next, we participated in two large-scale projects: "Protein 3000 Project" of the Ministry of Education, Culture, Sports, Science and Technology Japan (MEXT) and JST (FY2002–2006) and "Development of System and Technology for Advanced Measurement and Analysis" of JST (FY2004–2007). In the Protein 3000 Project, the SBRC operated in one of eight consortia and conducted structural and functional analyses of post-translational modification and transport. Our consortium determined 254 protein structures and published 296 papers. To assist the Protein 3000 Project, we set aside about 30% of the bio-macromolecular crystallography beam time in the PF (a total of 327 days) for users of the eight consortia. In the "Development of System and Technology for Advanced Measurement and Analysis" project, we developed a micro-beam beamline, BL-17A, and a prototype next-generation X-ray area detector. BL-17A is the first beamline developed at the PF with a short gap undulator as a light source. The beam size can be controlled from 100 to 10 μm square, and is useful for both modest and small protein crystal experiments.

Figure 1
The members of the Structural Biology Research Center (SBRC) pictured at the meeting room of the Structural Biology Building.

In the next five-year national project "Targeted Proteins Research Program" of MEXT and JST (FY2007–2011), the SBRC contributed via two independent programs: structural and functional research of protein transport in cells in close collaboration with the University of Tokyo and Kyoto University, and beamline development in collaboration with SPring-8, Hokkaido University, Kyoto University and Osaka University. The details of the programs are described later. The next project, Platform for Drug design, Informatics and Structural life sciences (PDIS), which provides user support and beamline development of two SR facilities, SPring-8 and PF, will start in April 2012. In addition to protein crystallography, the project includes solution scattering for macromolecules.

In addition to the above scientific research, the SBRC accepts many researchers from outside KEK who wish to collect diffraction data of their own macromolecular crystals under the Program Advisory Committee (PF PAC) system. The number of academic proposals and users has ranged from 100 to 120 in recent years as summarized in Table 1. In addition, together with advances in structure-based drug design, pharmaceutical companies require a large amount of

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

Type	G	P	U	S2	Total
2007	117	2	3	0	122
2008	101	0	0	1	102
2009	115	1	0	0	116
2010	103	2	1	0	106
2011	121	1	0	1	123

beam time, and thus many Japanese companies have been using beam time of the bio-macromolecular crystallography beamlines at KEK-PF. The Tsukuba Consortium (seven companies), four companies from the Pharmaceutical Consortium for Structure Analysis and three other companies are using our beamlines. Among them, Astellas Pharma Inc. financed the construction of AR-NE3A for their research.

2-2 Targeted Proteins Research Program (FY2007-2011)

Structure-Function Analysis of Protein Complexes that Regulate Vesicular Traffic

In the field of "Investigations of fundamental biological phenomena", we have started "Structure-function analysis of protein complexes that regulate vesicular traffic". This project is dedicated to protein targets of crucial importance but involving extreme difficulty, 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 the 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 progress in the treatment of human diseases.

To accelerate the project, the SBRC is collaborating with Prof. Akihiko Nakano (University of Tokyo), who is working on functional researches 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, the SBRC is currently working on the expression, purification, crystallization and structure determination of target proteins that are either membrane proteins or forming macromolecular complexes. We have determined several structures of protein complexes and elucidated the molecular mechanisms of membrane traffic and cytokinesis [1-5].

Technological Development Research for Protein Structural Analysis

To determine 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 overcome the 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 developed a new beamline dedicated to sulfur SAD experiments as a part of the national project "Targeted Proteins Research Program". The beamline, BL-1A, is designed to deliver an intense lower energy beam at around 4 keV using the first harmonics of the short gap undulator as the light source, 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 third harmonics, allowing MAD (Multiple wavelength Anomalous Dispersion) measurements using Se, Hg, Au, or Pt atoms. The beamline was opened to the members of the program in FY2010, and some useful results have been obtained [6]. In addition to the construction of BL-1A at PF, this research project includes the development of another high-energy beamline at SPring-8 with a brilliant and small size beam, as well as the development of other environment technologies by other universities (Hokkaido, Kyoto and Osaka Universities).

2-3 Highlights on In-House Structural Biology Research

Structure and Function of ARF6-MKLP1 Complex which is Required for Cytokinesis

We have solved the structure of ARF6-MKLP1 complex which is required for cytokinesis [4]. A summary is reported in the Highlight Section of this volume (pp 52).

Crystal Structure of Linear Ubiquitin Chain

Ubiquitin regulates a wide range of biological processes such as protein degradation, DNA damage repair, and immune responses. Ubiquitin molecules polymerize into polyubiquitin chains; the C-terminal glycine of one ubiquitin can link to one of the seven lysine (Lys) residues in the second ubiquitin. Linear polyubiquitin chains are characterized by yet another type of linkage; the C-terminal glycine of one ubiquitin (distal ubiquitin) is covalently attached via a normal peptide bond to the

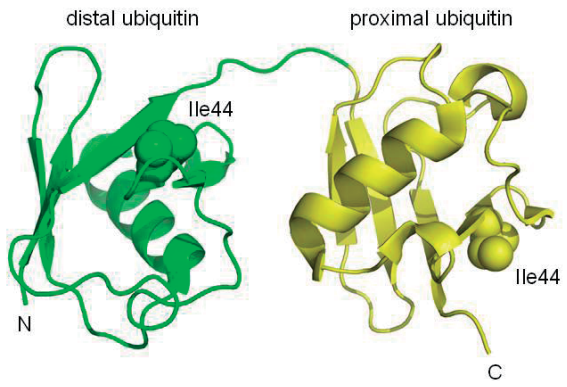

Figure 2
Crystal structure of linear diubiquitin. The C-terminus of the distal ubiquitin (green) is covalently attached to the N-terminus of the proximal ubiquitin (yellow) via a normal peptide bond. The central hydrophobic Ile44 residue of each ubiquitin is shown as a sphere model.

N-terminal methionine of the second ubiquitin (proximal ubiquitin). The specificity of polyubiquitin signaling is dictated by the linkage types of polyubiquitin chains. The linear polyubiquitin chain is involved in the activation of transcription factor NF-κB through its specific interactions with NF-κB essential modulator (NEMO) [7]. We also determined a crystal structure of linear diubiquitin at a resolution of 1.9 Å [8]. Although the two ubiquitin moieties do not interact with each other directly, the overall structure adopts a compact conformation (Fig. 2). This structure differs from the previously reported extended conformation, which resembles Lys63-linked diubiquitin suggesting that the linear polyubiquitin chain is intrinsically flexible and can adopt multiple conformations. Our result thus suggests the need for a wider search in conformational space in which polyubiquitin chains of different linkages might be exhibited when mediating distinct signals in various biological processes.

Structural Basis of Strict Phospholipid Binding by Human Evectin-2

Evectin-2 is a recycling endosomal protein involved in retrograde transport. One of the various kinds of phospholipids, phosphatidylserine (PS) is a relatively minor constituent of biological membranes. PS was found to be most concentrated in recycling endosomes (REs) among intracellular organelles. Evectin-2 consists of an N-terminal pleckstrin homology (PH) domain and a C-terminal hydrophobic region. The PH domain can specifically bind phosphatidylserine, which is enriched in REs, and plays an essential role in retrograde transport from REs to the trans-Golgi network [2]. We reported the structure of human Evectin-2 PH domain in complex with O-phospho-L-serine, and elucidated the molecular basis of how the head group of phosphatidylserine is recognized [2]. However, that structure could not elucidate why Evectin-2 cannot bind phosphatidic acid or phosphatidylethanolamine, which share a common moiety with phosphatidylserine. Then, we determined the crystal structure of an apo-form of human Evectin-2 PH domain whose ligand binding site is free from crystal packing [3]. Comparison between the structure of the apo-form and the O-phospho-L-serine complex reveals ligand-induced conformational change evoked by the interaction between the carboxyl moiety of the head group of phosphatidylserine and the main chain N atom of Thr14 (Fig. 3). This structural change effectively explains the strict ligand specificity of PH domain of human Evectin-2.

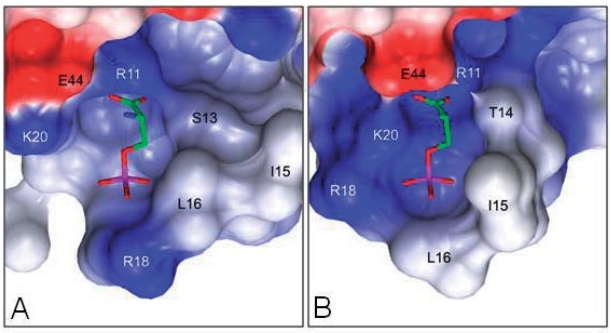

Figure 3
Ligand-binding site of Evectin-2. The apo-form (A) and the O-phospho-L-serine complex (B) structures are shown as charge distribution surface models. O-phospho-L-serine is shown as a stick model (green carbons, red oxygens and blue nitrogens). In panel A, the O-phospho-L-serine molecule from the complex structure is superimposed.

2-4 Beamlines and Technology Developments for Macromolecular Crystallography

Beamlines

The SBRC operates five insertion device beamlines for bio-macromolecular crystallography (Table 2). More than 400 days of beam time in total were provided for user experiments in FY2011 despite the disaster (Great East Japan Earthquake of March 11). Beamlines BL-5A, AR-NW12A and AR-NE3A are mainly used for high-throughput structural biology. In particular, fully automated data collection by a pharmaceutical company has been performed routinely at the AR-NE3A. On the other hand, beamlines 1A and 17A are designed for micro-

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

	BL-1A	BL-5A	BL-17A	AR-NW12A	AR-NE3A
Starting year	2010	2004	2006	2003	2009
Synchrotron ring	PF (2.5 GeV, 450 mA)			PF-AR (6.5 GeV, 40-60 mA)	
Injection	continuous (top up)			twice a day (10:00, 22:00)	
X-ray source	Short Gap Undulator	Multipole Wiggler	Short Gap Undulator	Undulator	Undulator
Photon flux (photons/sec)	4.4×10^{11} (@1.1 Å)	8.6×10^{11} (@1.0 Å)	5.5×10^{10} (@0.98 Å)	3.9×10^{11} (@1.0 Å)	1.2×10^{12} (@1.0 Å)
Wavelength (Å)	0.95 ~ 1.1, 2.7	0.75 ~ 1.9	0.9 ~ 2.1	0.75 ~ 1.9	0.75 ~ 1.9
Beam size (H × V, μm)	30 × 10	270 × 150	40 × 30	200 × 170	200 × 170
Detector	ADSC Q270 Pilatus 2M-F	ADSC Q210r	ADSC Q315r	ADSC Q210r	ADSC Q270
Purpose	low energy micro beam	high throughtput	low energy micro beam	high throughtput	high throughtput
Sample exchanger	PAM (288 samples / Dewar)				

crystal structure analysis and also for SAD phasing with light atoms using lower energy beam of around 4 keV (BL-1A), and 6–8 keV (BL-17A). As mentioned above, the lower energy SAD is currently one of the most attractive methods for solving macromolecular crystal structures. Figure 4 shows one of the successful results in which 43 kDa protein structure was solved almost completely and automatically from the data set collected at BL-1A. A pixel array X-ray detector (Pilatus 2M, Dectris) was purchased (Fig. 5), and will be installed at BL-1A in early FY2012, instead of the CCD-type detector currently in use. The replacement will improve the signal to noise ratio of a weakly diffracted beam which is critical to solve structures by the SAD method.

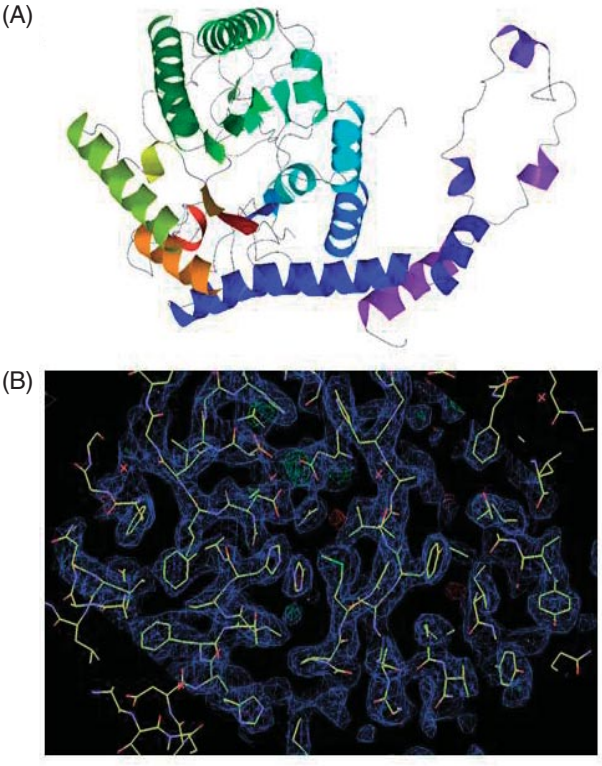

Figure 4
The protein structure solved by sulfur SAD at beamline BL-1A. The structure of glucose isomerase (MW 43 kDa) was solved by S-SAD. Ribbon diagram of the whole structure (A) and electron density map (B). Residues 4–382 out of 388 were automatically built.

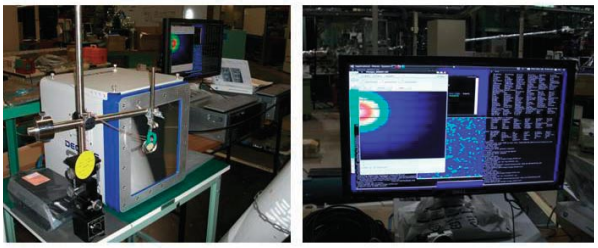

Figure 5
New pixel array X-ray detector. Commissioning of the detector (Pilatus 2M, Dectris) at the PF experimental hall using a calibration X-ray source.

Technology Developments

To assist in the implementation of fully automated crystal centering at the beam position, we investigated the potential of UV LED sources in identifying macromolecular biological crystals [9]. Automation of sample centering at protein crystallography beamlines is presently achieved by focusing on sample holder recognition, while the crystalline samples are ignored completely. The capacity of soft UV to specifically highlight biological objects makes such a light source a suitable target for future development for universal centering methods with any type of sample holder (Fig. 6).

Minimization of the absorption effects of X-rays by the air and nitrogen gas surrounding a sample during data collection is important for low-energy SAD phasing, because these effects increase at lower photon energy. The absorption effects decrease the intensity of diffracted X-rays and increase the background noise in a diffraction image. We developed an He chamber and He cryo-stream system which replace the gas surrounding a sample with He, and installed them in BL-1A (Fig. 7). The He chamber is large enough to contain an X-ray area detector and is filled with helium gas. The panel face to the sample is sealed with a thin Kapton film, and when a diffraction experiment is carried out, this chamber moves close to the sample. The He cryo-stream system consists of a gas cooler made by Cryo Industries Inc. and a gas flow controller developed by

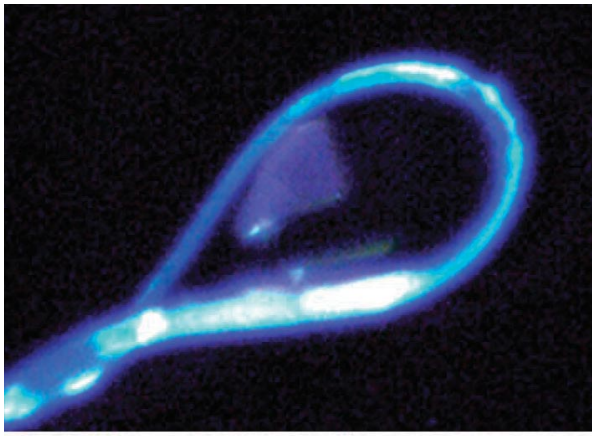

Figure 6
Protein crystals imaged by UV fluorescence. Illumination by UV LED (283 nm) of human sialidase Neu2 crystals within a nylon loop resulted in a specific fluorescence from the crystalline object.

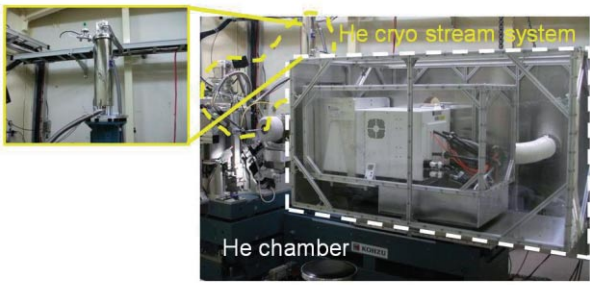

Figure 7
Helium chamber and helium cryo-stream system. He chamber and He cryo-stream system installed at BL-1A The white and yellow broken lines indicate He chamber and He cryo-stream system, respectively.

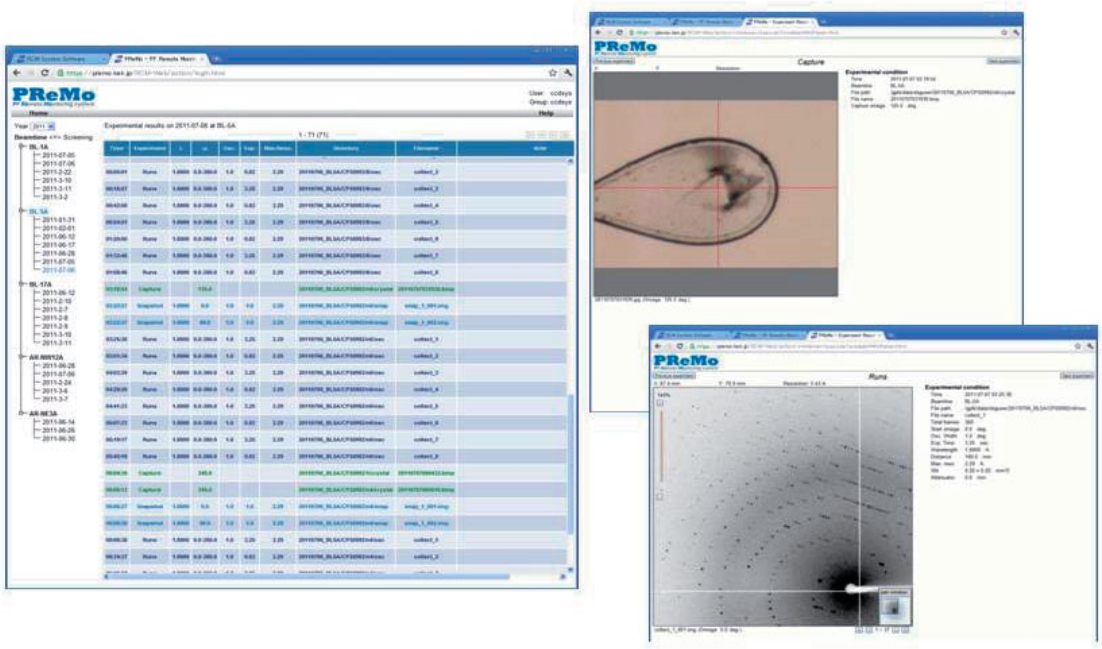

Figure 8
Upgraded web interface of PReMo. The web interface of PReMo consists of an experiment browser (left) and a results viewer (right).

us. This system can easily and quickly switch a gas flow from helium to nitrogen. This enables us to use cryo N_2 gas during sample mounting and centering, and to use cryo He gas during a diffraction experiment, thus reducing the amount of He gas consumed for the experiment.

PReMo (PF Remote Monitoring System) is a system which stores the conditions and results of diffraction experiments which are carried out at beamlines and allows users to access this information from outside of KEK via a Web browser. In FY2011, we upgraded the Web interface of PReMo to be more user-friendly (Fig. 8). A fully automated data collection system was originally developed for large-scale pharmaceutical research. In FY2011, we added a function to allow users to select a snapshot or data set collection experiment for each sample. After automated snapshot experiments of all samples stored in a sample exchange system beside the diffractometer, the user can check diffraction images via PReMo and determine data set collection conditions. Users submit information to the system through PReMo and perform automated data set collection experiments. This new automated experiment scheme was started in FY2011, and a mail-in service based on the new scheme was opened for industrial users from FY2011.

Beamlines and Technology Developments for Small-angle X-ray Scattering

There are two full-time SAXS beamlines in PF : BL-6A and BL-10C (Table 3). They have been operated by a user group because of the absence of a beamline scientist of the PF. Associate professor Dr. Shimizu arrived as a beamline scientist for SAXS in 2010, and worked with Dr. Igarashi and other support staff to refurbish BL-10C and to relocate BL-15A. Since the new BL-15A, which has a short gap undulator as the light source, is planned to be constructed for SAXS and XAFS studies, SAXS activities of BL-15A were moved to BL-6A in the summer of FY2011. Almost all the optical and experimental components were installed in the same layout as the previous BL-15A (Fig. 9A, B), but the old components and systems were replaced by new ones to improve beamline safety and user-friendliness. The PF standard control frame work (STARS) was installed to BL-6A and BL-10C and new graphical user interfaces were developed so that users can operate all the motorized stages from a remote computer.

Although there were several sets of detectors at the previous BL-15A, users had to exchange the detectors by themselves. A motorized detector stage was installed

Table 3 Summary of SAXS beamlinces at KEK-PF.

	Science Target	Light Source & Optics	Detector	Beam Size (FWHM, mm) & Flux (phs/sec)	X-ray Energy (A)
BL-6A	Hard materials Soft matters Proteins (Multi purpose, Time-resolve)	Bending magnet Flat bent mirror Asymmetric cut monochromator	2 types of XR-II CCDs PSPC (out of order) FPD for WAXS	0.6 × 0.6 ~ 1.0 × 1.0 3.6 ~ 11 × 10^{10}	1.5
BL-10C	Soft matters Proteins (Solution, Static)	Bending magnet Bent cylinder mirror Double crystal monochromator	PSPC R-AXIS 7	0.7 × 0.7 ~ 1.0 × 1.0 1.9 ~ 3.4 × 10^{10}	1.488

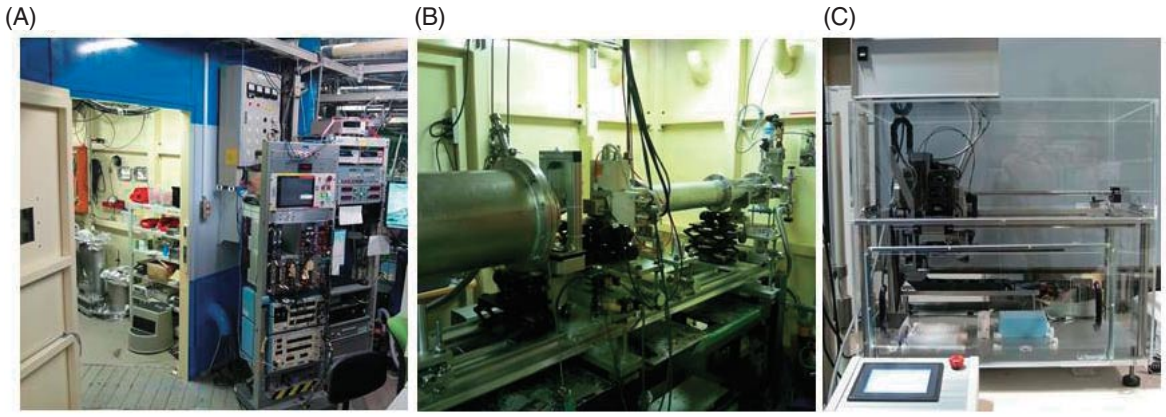

Figure 9
Refurbishment and improvement of SAXS stations. Views of BL-6A experimental hutch from outside (A) and inside (B). (C) An automated sample mixing machine was developed and installed at the side of the BL-10C experimental hutch.

at BL-6A, and users can easily set the detector position by presetting the position of each detector. Since simultaneous SAXS/WAXS measurements are generally conducted to evaluate the structure of a sample based on data observed in the broad angle region, we newly installed a flat panel sensor (C9728DK-10, Hamamatsu Photonics) for WAXS experiments and constructed a simultaneous measurement system which can measure at speeds of up to 3 Hz in combination with the preexisting SAXS detectors at BL-6A.

The data in the broad angle region can be measured with an imaging plate detector R-AXIS VII (Rigaku) at BL-10C. We replaced the size-definition slit from an old hand-operated one to a new motorized one to optimize the beam position and size by automatic scanning with an ammeter. We are upgrading BL-10C especially for high-throughput measurements of Bio-SAXS experiments. As part of this upgrade, we developed and installed an automated solution mixing machine (Fig. 9C), which will be extended to a solution-sample changer. Moreover, a micro-centrifuge, an ultra-micro spectrophotometer, an incubator, and so on were also installed on the BL-10C experimental hutch side in order to improve the sample preparation.

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