3 Structural Biology Research Center

3-1 Overview

The Structural Biology Research Center (SBRC) was started in May 2000, in the Photon Factory (PF), Institute of Material Structure Science (IMSS). The main tasks of the center are the user support of synchrotron radiation X-ray crystallographic studies of bio-macromolecules, highly advanced technical development, and in-house structural biology research. The center now has about 30 members, including a professor (Dr. S. Wakatsuki), two associate professors (Drs. R. Kato, N. Igarashi), a vice associate professor (Dr. M. Hiraki) and three assistant professors (Drs. N. Matsugaki, M. Kawasaki, Y. Yamada) as the core members (Fig. 1). About half of the SBRC members are engaged in beamline operation and development, and the remaining half in biological research. The branch head of beamline operation and development, Dr. Igarashi, moved to another group at the PF (the Beamline Engineering, Technical Service and Safety group) from the Life Science group with his promotion in the summer of 2008, though he still belongs to the Structural Biology Research Center. Accordingly, Dr. Matsugaki is now the branch head, and a new beamline scientist, Dr. Leonard Chavas will join us as a research associate from April 2009. The in-house biological research is carried out in the structural biology building, which was newly-built in April 2001 (430 m^2) and extended twice to 765 m^2. All the steps necessary for structural biology research can be conducted in the building, including protein expression, sample purification, crystallization, and biochemical and biophysical analyses of the 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 FY2000-FY2001. We were able to build a second up-to-date beamline, BL-5A, and also prepare various items of scientific equipment for protein expression, purification, and crystallization experiments using a

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

"Special Coordination Funds for Promoting Science and Technology" fund (FY2001-FY2003) from JST (Japan Science and Technology Agency). Next, we participated in two large-scale projects: the "Protein 3000 National Project" from MEXT (Ministry of Education, Culture, Sports, Science and Technology Japan) / JST (FY2002-FY2006) and "Development of System and Technology for Advanced Measurement and Analysis" from JST (FY2004-FY2007).

During the five-year 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. Cell signaling and intracellular trafficking are the means by which eukaryotic cells deliver cargo proteins to various organelles, cell membranes, and extracellular destinations. During the trafficking process, more than half of the eukaryotic proteins undergo post-translational processing and modifications such as glycosylation. Accurate distribution and modification of the proteins are crucial for a range of cellular functions and activities. An increased understanding of the biological and biomedical function of transport and modification proteins is indispensable for progress in the treatment of human diseases. To facilitate the research program, the SBRC took the lead by coordinating the consortium, which consisted of eleven universities and four research institutes. Our initial research plan was to accomplish structural and functional analyses of more than 70 proteins in five years, but by the end, 254 structures had been determined and 296 papers published. Since the cumulative number of structures determined by the Protein 3000 Project has exceeded 3,000, the project was considered a success, to which our consortium made a considerable contribution. To further assist the Protein 3000 Project, we set aside about 30% of the protein crystallography beam time available at the PF (a total of 327 days) for users from 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 a prototype of a next-generation X-ray area detector. BL-17A is the first beamline developed at PF with a short gap undulator as the light source. The beam size can be controlled from 100 to 10 μm square, and the beamline is useful for both modest-sized and small protein crystal experiments [1]. The next-generation X-ray area detector was developed in collaboration with NHK (Japan Broadcasting Corporation) laboratory and several additional companies. Based on HARP (High-gain Avalanche Rushing amorphous Photoconductor) and FEA (Field Emitter Array) technologies, prototype detectors were made, and they showed some advantages in spa-

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

Proposal type	G (general)	U (urgent)	S2 (special)	Total
2004	84	3	1	88
2005	81	0	1	82
2006	86	0	1	87
2007	117	3	0	120
2008	101	0	1	102

tial resolution and X-ray sensitivity when compared to CCD detectors [2].

A new five-year national project "Targeted Proteins Research Program" by MEXT/JST was started in FY2007 (Fig. 2). The SBRC contributes to the project by two independent programs. One is structural and functional research of protein transport in cells in tight collaboration with Univ. of Tokyo and Kyoto Univ. The other is beamline developments in collaboration with SPring-8, Hokkaido Univ., Kyoto Univ. and Osaka Univ. The details of the programs are described later.

In addition to scientific research activities, the SBRC accepts many researchers from outside KEK who wish to collect diffraction data of their own macromolecular crystals under the PF-PAC (Program Advisory Committee) system. Academic proposals and users are increasing in recent years, as summarized in Table 1. Additionally, together with advances in structure-based drug design, pharmaceutical companies require a large amount of beam time. As a direct consequence, ten Japanese companies and one pharmaceutical consortium have been using beam time of the bio-macromolecular beamlines at KEK-PF, and a new consortium was established under the coordination of the SBRC in FY2006. In addition, Astellas Pharma Inc. financed the construction of a new beamline, AR-NE3A, for their research. The development of the beamline has been finished and user operation will start in April 2009.

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3-2 Target Proteins Research Program

The national "Protein 3000 Project" produced a large number of protein structures, and played an important role in expanding structural biology activities in the Japanese biology society. However, the structures of many proteins and protein complexes remain to be solved in order to elucidate their functions. On this basis, the "Targeted Protein Research Program" started in FY2007. It is broadly considered that protein structural and functional analyses lead to the promotion of research in such fields as medical and pharmaceutical sciences and industrial applications. To fulfill these requests, the research project has two strategies; one is the selection of research fields, and the other is the development of key technologies (Fig. 2). The selected research fields are (1) investigation of fundamental biological phenomena, (2) contribution to medical and pharmaceutical sciences, and (3) industrial applications including food and the environment. The key technologies to be developed are (1) protein production technology, (2) protein structural analysis technology, (3) protein regulation technology, and (4) an information platform. According to this strategy, numerous universities, institutes and companies form a total of 45 consortia within Japan, and are promoting each research project.

Structure-Function Analysis of Protein Complexes that Regulate Vesicular Traffic

In the field of "Investigations of fundamental biological phenomena", we have begun a project entitled "Structure-function analysis of protein complexes that regulate vesicular traffic". This project is dedicated to the study of protein targets which are of crucial importance but are extremely difficult to isolate, such as protein complexes, including membrane proteins involved

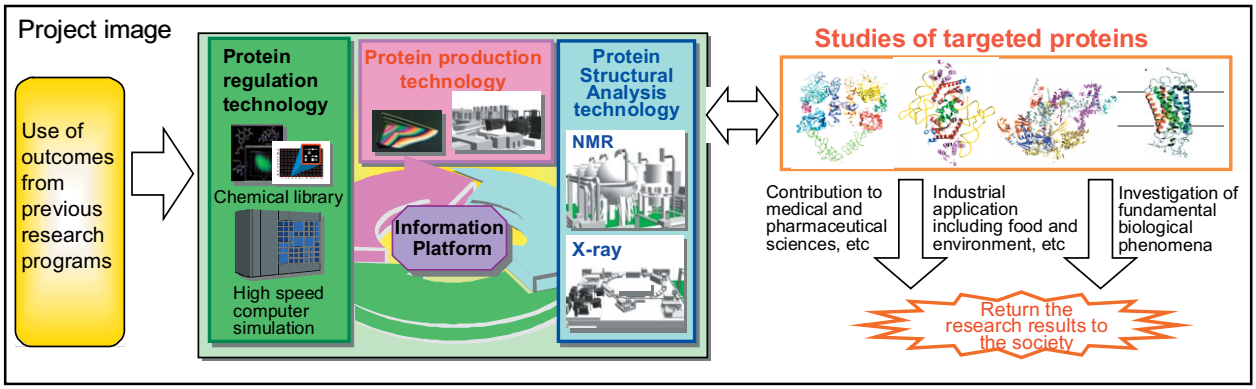

Figure 2
Scheme of the MEXT Japanese national project "Targeted Proteins Research Program" (FY2007-FY2011).

in membrane traffic in eukaryotic cells. The project is pursued in collaboration with Prof. Akihiko Nakano (Univ. of Tokyo), who is working on functional research of proteins involved in Golgi formation, post-Golgi membrane traffic and endocytosis in yeast and higher plants, Prof. Kazuhisa Nakayama (Kyoto Univ.), studying proteins involved in the regulation of cell functions such as cytokinesis, cell polarity and cell mobility, and Prof. Ken Sato (Univ. of Tokyo), who is researching transport vesicle formation from endoplasmic reticulum (ER) in yeast. With 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 form macromolecular complexes, in order to elucidate the molecular mechanisms behind their functions. The collaboration extends to Prof. Akihiko Nagano (Univ. of Tokyo) and Prof. Toshiaki Sakisaka (Kobe Univ.), who are also members of the program.

Technological Development Research of Protein Structural Analysis

One of the most important issues in the determination of three-dimensional structures of bio-macromolecules using X-ray crystallography is the phase problem. Sulfur SAD (Single wavelength Anomalous Dispersion) analysis is currently one of the most attractive methods to bypass 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 have begun to develop a new beamline dedicated to sulfur SAD experiments as a part of the “Targeted Proteins Research Program”. The new beamline, BL-1A, is designed to deliver an intense lower-energy X-ray beam at around 4 keV using the first harmonics of its short-gap undulator light source, thus enhancing the anomalous signal from light atoms such as sulfur and phosphorus. The expected beam intensity at the sample position is on the order of 10^{11} photons/sec over an area of 10 square microns, suitable for diffraction measurements from small crystals. The optics and the diffractometer will be specially designed to minimize losses for the lower energy X-ray beam. The beamline will also cover the energy range of 12-13 keV with the 3rd harmonics, allowing MAD (Multiple wavelength Anomalous Dispersion) measurements using Se, Hg, Au, or Pt atoms. The construction of the beamline is scheduled for the summer of 2009, followed by half a year of commissioning. The beamline will be opened to the members of the program in 2010.

In addition to the construction of BL-1A at the PF, the research project also includes the development of a high-energy beamline at SPring-8, with high brilliance and a small beam size, as well as some environment technology developments at other universities (Hokkaido, Kyoto and Osaka). All members of the project are involved in developing the two new beamlines and additional tools in a well-established collaboration with good communication. For example, we are developing a compatible cassette system, which will solve the inconvenience of the incompatible sample exchange systems used at the PF and SPring-8.

3-3 Highlights on In-House Structural Biology Research

Mechanism of Ca^{2+}-Dependent Recognition of ALIX by ALG-2

ALG-2 (apoptosis-linked gene 2) belongs to the penta-EF-hand (PEF) protein family, which contains five successive EF-hand motifs. PEF protein consists of eight α-helices and forms a dimer through association of the fifth EF-hand (EF5). In a Ca^{2+}-dependent manner, ALG-2 interacts with various intracellular proteins which contain proline-rich regions, such as Alix (ALG-2-interacting protein X) and TSG101 (tumor susceptibility gene 101), which are involved in endosomal sorting and HIV budding. ALG-2 binds to the C-terminal proline-rich region of Alix which contains four-tandem Pro-x-Tyr (PxY) repeats.

Using X-ray diffraction data recorded at the PF beamlines 5A, 6A and 17A, we solved the crystal structures of metal-free, Ca^{2+}-bound, and Zn^{2+}-bound forms of human ALG-2 (Fig. 3). Metal ions are coordinated in the loops of EF1, EF3 and EF5 of ALG-2. The binding of Ca^{2+} or Zn^{2+} to EF3 induces rearrangement of the loop connecting EF3 and EF4, and opens the primary binding site (pocket 1; described below) for Alix peptide. Although the complex between ALG-2 and Alix peptide (1-QGPPYPTYP-GYPGYSQ-16, corresponding to residue 799-814 of Alix), did not crystallize in the presence of Ca^{2+}, it did crystallize in the presence of Zn^{2+}, and the structure was solved at 2.2 Å resolution. An asymmetric unit of the crystal contains two ALG-2 molecules (A and B) as a dimer, and two Alix peptides (C and D). Unexpectedly, each ALG-2 protomer binds Alix peptides in a different manner. Molecule A

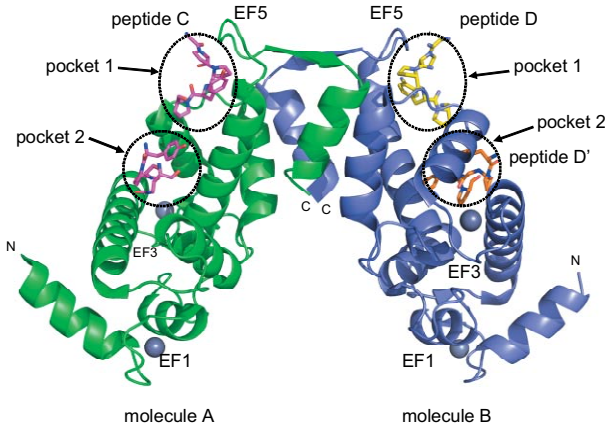

Figure 3
Crystal structure of the ALG-2/Alix peptide complex. Two ALG-2 protomers (molecules A and B) are shown in green and blue, respectively in ribbon representation. The ALG-2-interacting residues of the Alix peptides are shown in magenta (peptide C), yellow (peptide D) and orange (peptide D') in stick representation.

binds one Alix peptide (C) whereas molecule B binds two Alix peptides (D and its crystallographic symmetry mate D').

ALG-2 contains two distantly positioned peptide-binding pockets (pockets 1 and 2). The binding mode of the N-terminal part of the Alix peptide (2-GPPYP-6) by pocket 1 of ALG-2 is almost identical in both molecules of the dimer. On the other hand, the binding mode of the C-terminal part of the Alix peptide (11-YPG-13 and Q16 of peptide C; 8-YPGYP-12 of peptide D') by pocket 2 of ALG-2 is rather flexible. Comparison between the ALG-2 protomers suggests that the YP residues are the consensus bonding motif for pocket 2. The consensus binding sequence would then be PPYP(X3~5)YP. The crystal structure revealed that each ALG-2 protomer independently binds to the ligand peptide, suggesting the possibility that ALG-2 links TSG101 and Alix, and functions as a Ca^{2+}-dependent adaptor protein.

Solution of the Structure of the Cargo-Binding Domain of the Miranda Protein

The Miranda protein is involved in neuroblast asymmetric division in Drosophila Melnogaster. During the embryonic development of the Drosophila central nervous system, mitotic neuroblasts divide asymmetrically to produce different daughter cells; another neuroblast (self-renewal) and a smaller ganglion mother cell distend to differentiation. An essential cell fate determinant, the transcription factor protein Prospero binds to the Miranda central domain. As a backup mechanism, the Prospero-mRNA carrier protein Staufen also interacts with the Miranda central domain. Recently, another cell fate determinant protein, Brat, was shown to bind the Miranda central domain and colocalize with Prospero and Staufen.

Miranda protein (830 amino acids, long form) consists of three functional domains: (1) an N-terminal domain, sufficient for membrane association, (2) a central domain necessary for cargo binding of the neural transcription factor Prospero, Prospero-mRNA carrier Staufen and the tumor suppressor Brat, and (3) a C-terminal domain required for its timely degradation and cargo release.

We characterized a truncated segment of the central region of Miranda (amino acids 460-660) and obtained the first low resolution structure of it in solution using small angle X-ray scattering (SAXS). Ab initio modeling of the scattering data yielded an elongated molecule with a maximum linear dimension (Dmax) of about 22 nm (Fig. 4). The results showed that the central domain of Miranda forms an elongated, parallel coiled-coil homodimer with a "rope"-shaped structure. Further complementary biophysical and biochemical characterizations indicate that the Miranda central domain, which forms a parallel coiled-coil molecule with an extended homodimer structure, can bind multiple cargos simultaneously. Based on the these results, we modeled the full-length Miranda protein as a double-headed, double-tailed homodimer with a long central coiled-coil region, and discussed a structure-based mechanism for the cargo release and timely degradation of Miranda in developing neurobalsts (see the legend of Fig. 4).

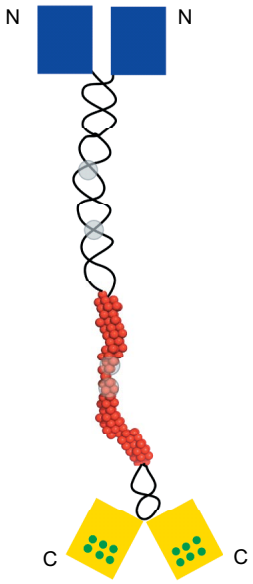

Figure 4
Model of the overall structure of Miranda protein. Cartoon representation of the full-length Miranda protein based on an ab intio SAXS model of the central domain (red balls). Miranda contains four potential destruction boxes (grey circles) - these are involved in the cell cycle dependent protein degradation. Two of these are located within the central domain whereas the other two lie in the N-terminal region. The coiled-coil structure of the central domain, along with the cargo binding, is expected to shield the destruction boxes, rendering them inaccessible to potential degradation pathways. On the other hand, the C-terminal region of Miranda was found to be essential for degradation and cargo release. In the C-terminal region, there are several protein kinase C phosphrylation sites (green circles), raising the possibility of a phoshporylation–dependent degradation. Phospohryaltion at the C termini could lead to destabilize ("unzip") the nearby coiled-coil and the central domain.

Others

The two hottest research highlights are introduced in the "Highlights" section of this volume (pp 52 and pp 56) - a structural analysis of the NF-κB signaling pathways responsible for the cell immune system [7], and a structural study of the regulation of pigment secretion in cells [8].

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3-4 Beamlines for Protein Crystallography

Current Status of Beamlines

The SBRC currently operates five beamlines for crystallography of bio-macromolecules. BL-5A, BL-17A, AR-NW12A and AR-NE3A are insertion device (ID) beamlines, while BL-6A is a conventional bending magnet beamline (Table 2). Among the ID beamlines, BL-5A, AR-NW12A and AR-NE3A are high-throughput structural biology beamlines and BL-17A is a micro-beam beamline designed for micro-crystal structure analysis and also for SAD phasing with light atoms using a lower energy X-ray beam at around 6-8 keV [1].

A further new beamline, BL-1A, is now under construction. The main purpose of this beamline is to deliver a brilliant low energy X-ray beam at around 4-5 keV (dedicated to sulphur SAD experiments), and a higher photon flux at around 12 keV when compared with BL-17A. BL-1A will start operation in April 2010. Together with the start of BL-1A operations, we are planning to close BL-6A, the oldest protein crystallography beamline at the PF. Even though the beamline is operational and extensively used by numerous users, construction of the new beamline, along with supporting its operation is leading to an increasing amount of work, and this is the main reason for the closure of BL-6A.

New and upgraded X-ray area detectors have been installed at all of the beamlines. The CCD detector at BL-5A was upgraded from an ADSC Quantum 310 to a 310r in summer 2008, leading to reduced readout noise. An ADSC Quantum 270, a brand-new, high-gain CCD detector, was installed at AR-NE3A. Another ADSC Quantum 270 was installed at BL-17A to study useful conditions for lower energy SAD phasing. When BL-1A begins operation, this detector will be moved to BL-1A, and another new ADSC Quantum 210r, the same as that already installed at AR-NW12A, will be installed at BL-17A.

For even higher throughput protein crystallography, we have improved the automation of beamline control, the operation of the sample change system, the automatic sample centering system, and unified the measurement software. In particular, a fully automated data collection and processing system has been developed and opened for user operation. We have also developed a new web-based remote monitoring system (PReMo). In this system, all experimental results (e.g. diffraction images, pictures of crystals mounted on a diffractometer, and energy scan plots) from the beamlines are stored in a database and can be viewed using a Web browser. Users who can not come to the beamline are able to join the experiment using this system. Users can also check the experimental progress of their group from a cellular phone.

AR-NE3A

AR-NE3A is a new beamline, and is dedicated to high-throughput protein crystallography for pharmaceutical applications. The construction and commissioning were completed in March 2009. As expected from ray-tracing simulations, AR-NE3A provides the most intense X-ray beam at the sample position among all of the protein crystallography beamlines in PF. The flux at 12.4 keV is about 8.0 × 10^{11} photons/sec., which is about three times higher than at one of the other high-throughput beamlines, AR-NW12A. At the end-station of AR-NE3A, a high precision diffractometer, a fast-readout

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)		

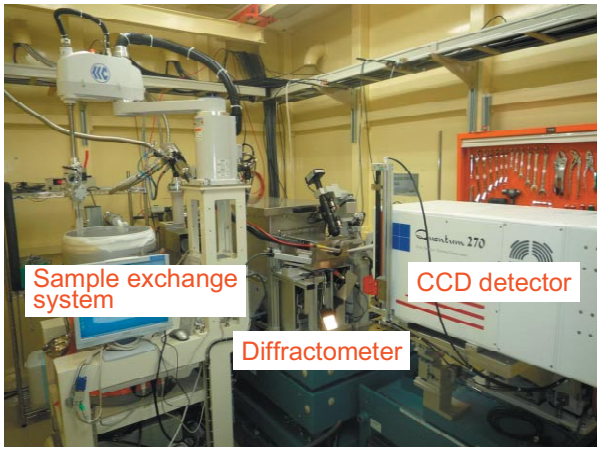

Figure 5
End-station of AR-NE3A for high-throughput pharmaceutical data collection. A high precision diffractometer, a fast-readout and high-gain CCD detector and a sample exchange system which can store 288 samples in the Dewar are installed.

and high-gain CCD detector with a large active area, and a sample exchange system (PAM) [9] are installed for high-throughput data collection (Fig. 5). In addition to these apparatus, the automated data collection system described below, enables a user to collect data sets from more than 150 samples in a day. Details of the beamline construction can be found in the "Newly Developed Experimental Facilities" section in this volume. The construction of AR-NE3A is financially supported by Astellas Pharma Inc., who have priority access to this beamline for their research. The remaining beam time will be assigned to general academic users and other industrial users. User operations begin in April 2009.

Automated Data Collection and Processing System

Fully automated data collection and data analysis have been strongly demanded by structural biology researchers. Automation of the experiments is especially essential for pharmaceutical applications, where a large number of protein/ligand complex crystals must be examined. In order to meet this demand, we have developed a fully automated data collection and processing system. In the system, a user prepares a sample description file before the experiment. This definition file contains a list of the samples, their slot number in the cassette of the 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, and spacegroup candidates) (Fig. 6).

After placing the sample cassette in the Dewar of the PAM robot, sending the sample description file to the beamline control software, and starting the automatic data collection and processing, the user does not need to do anything until all of the data sets have been collected. The samples are automatically exchanged and centred by the PAM robot, and the data set collected according to the conditions described in the description file. When a sample is centered, the loop centering procedure is carried out. This is because at the high-throughput beamlines BL-5A, AR-NW12A and AR-NE3A, the beam size is relatively large (~200 μm), and most protein crystals are in the beam after automatic

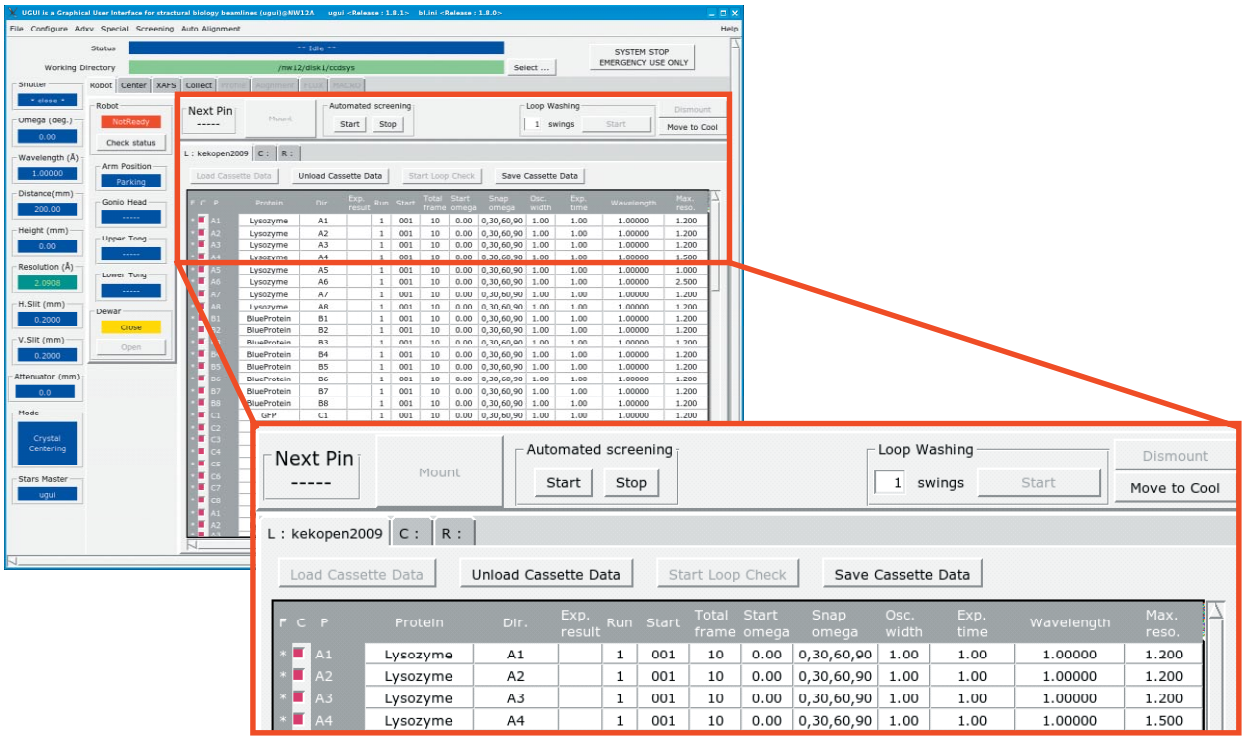

Figure 6
User interface for the automatic data collection and processing system. The user interface of the beamline control system has been extended for the automatic data collection and processing system. In the information for each sample, fields for the data collection and processing conditions have been added. Automatic data collection and processing is started with the "start" button in the "Automated screening" section.

sample centering. After data collection, the beamline control client, PROCESSOR [10], executes data processing software to process the data set, and reports the data collection statistics. Currently, PROCESSOR can execute HKL2000 and XIA2 for automatic data processing.

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3-5 Robotics for Structural Biology

Sample Exchange Robots

In order to increase the speed of protein structural analyses and drug design, sample exchange robots have been developed and installed at almost all of the protein crystallography beamlines. We have also developed sample exchange robots named PAM (PF Automated Mounting system), which have been available to users at BL-5A and AR-NW12A since October 2006, and BL-17A since May 2007. The PAMs were used by 13.2% of users in FY2008 (11.2% in FY2007), and 4,258 sample pins were mounted by the PAMs (2,282 in FY2007). During the summer shutdown of the PF/PF-AR rings, interlock systems were installed for PAM and the experimental hutch door. The PAM can be controlled only while the hutch door is closed, stopping immediately for safety if the door opens.

In parallel with the operation of three PAMs, we have been developing a new PAM for the new beamline AR-NE3A. The design of the new PAM is almost same as that of the other PAMs. The new PAM was delivered to the PF-AR NE hall in January, 2009. After setup of the software and a continuous test, we installed the PAM in the experimental hutch of AR-NE3A by the middle of February, 2009 (Fig. 5).

Development of PF/SPring-8 Reversible Cassettes

The PAM can currently only operate with the SSRL (Stanford Synchrotron Radiation Laboratory) cassette. The SSRL macromolecular crystallography group have also developed the “Uni-puck”, which is compatible with systems at both the SSRL and at the ALS (Advanced Light Source). We are modifying our control software to use this Uni-puck. In Japan, there are two types of sample exchange robots: our PAM, and SPACE (SPring-8 Precise Automatic Cryo-sample Exchanger) developed by SPring-8. The SPACE pins are designed for precise repeatability, and their shape is quite different from that of the widely-used Hampton pin. Users have to use different pins and cassettes for each robot. To solve this inconvenience, a project to develop PAM/SPACE reversible cassettes has started in collaboration with SPring-8 and Kyoto University, as a part of the Targeted

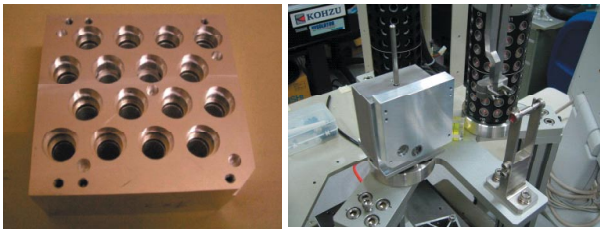

Figure 7
Reversible cassette. (A) Prototype of the reversible cassette developed by Kyoto Univ. PAM accesses the sample pins from this side, and SPACE picks up the sample pins from the opposite side. (B) One of the reversible cassettes being tested by R&D-PAM. This prototype was developed to investigate the strength of the magnet inside the cassette.

Proteins Research Program. To allow the handling of the plastic SPACE pin by the PAM, a stainless-base for the SPACE pin was developed at SPring-8. The SPACE robot picks up only the SPACE pin from the reversible cassette, but the PAM pulls the SPACE pin along with the stainless-base. Several prototypes of the reversible cassette have been tested using the PAM, and the PAM could access the stainless-based SPACE pins stably (Fig. 7).

Protein Crystallization System

To relieve one of the bottlenecks in high-throughput protein crystal structural analysis, we developed the large-scale protein crystallization and monitoring system “PXS” in 2003 [11]. PXS contributed to the Protein 3000 project, and is currently used for the Targeted Proteins Research Program and other projects at the Structural Biology Research Center. The SBRC is the main user of PXS, but 11% of the plates set to date were set by other research laboratories, including AIST (Advanced Industrial Science and Technology) in Tsukuba, RIKEN in Yokohama and Wako, several universities in Japan, Seoul University in Korea, and Manchester University in the UK. Researchers can access the image database from their laboratory using a web browser to check their crystallization drops. The PXS system has been used to set up 3,587 crystallization plates between FY2003 and FY2008.

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