2 Structural Biology Research Center

2-1 Introduction

The Structural Biology Research Group was formed in May 2000. The aims of the research group are user support of synchrotron radiation X-ray crystallography studies of macromolecules, highly advanced technical development and in-house structural biology research. The group has grown steadily during the last four years; the structural biology building was extended from 429 m^2 to 643 m^2, and the number of members increased from four (one professor and three research assistants) to about thirty (Fig. 1), including the group leader Professor Soichi Wakatsuki, an associate professor (Dr. Ryuichi Kato) and five research associates (Drs. Noriyuki Igarashi, Naohiro Matsugaki, Masato Kawasaki, Masahiko Hiraki and Yusuke Yamada) as the core staff members. While about half of the members are primarily engaged in beamline operation and development and the remaining half in biological research, the synergy between the two activities is a unique aspect of this group. In conjunction with the recent change of status of KEK from a government institute to an agency in April 2004, the Structural Biology Research Group became the Structural Biology Research Center in May 2003. Three graduate students (attached to the Graduate University for Advanced Studies, SOKENDAI) including one from France are studying and carrying out their own research projects relevant to the group's research field under the guidance of the staff members.

During FY2001-FY2003, the activities of the group were supported by a fund called "Special Coordination Funds for Promoting Science and Technology" awarded to the group together with the universities of Hokkaido, Tokyo, Kyoto and Osaka and the research institute of NHK (the Japanese national broadcasting corporation) by MEXT (Ministry of Education, Culture, Sports, Science and Technology). We built and commissioned a new high-throughput beamline BL-5A, developed an assortment of technologies for the automated handling of protein crystals, built a prototype of a next-generation two-dimensional X-ray "HARP" detector, and developed software which facilitates rapid and accurate structure determination. We also made improvements to the experimental environment of the beamlines and sample preparation laboratories using the same research fund. Subsequently, a five year national project, "Protein 3000" was begun by MEXT in FY2002. The project consists of two programs; a "Comprehensive Program" carried out by RIKEN, and "Individual Analysis Programs" carried out by eight consortia of universities and institutes including the KEK-PF Structural Biology Research Center. In addition, in FY2004 a new research and development program, "Development of Systems and Technology for Advanced Measurement and Analysis" was launched by JST (Japan Science and Technology Agency). We proposed a project to develop a next-generation detector coupled with a micro-focus beamline, which was selected and commenced in the same year for an initial 3 years. During this project we will develop a new beamline BL-17A, optimized for data collection from small crystals, and develop an advanced prototype of the next-generation two-dimensional X-ray "HARP" detector in collaboration with the research institute of NHK and associated companies.

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

Highlights of the R&D projects and biological research carried out in FY2004 are described below to illustrate the synergistic approach of the group in pursuing in-house structural biology research, developing and improving fundamental research tools for synchrotron based protein crystallography, and operating the user facilities.

2-2 Protein 3000 Project, Individual Analysis Programs

FY2004 is the third year of the five-year national project. The Structural Biology Research Center serves as the leading institute of one of the eight consortia of the Protein 3000 project pursuing structural and functional analyses of post-translational modification and transport. Our consortium consists of nine universities and four research institutes (Table 1).

Cell signaling and intracellular trafficking are the means by which eukaryotic cells deliver cargo proteins to various organelles, cell membranes, and extracellular destinations (Fig. 2). The accurate distribution of these proteins is crucial for a range of cellular functions and activities. Mutations in the genes encoding protein transport regulators underlie a number of genetic diseases. Hence, an understanding of the biological and biomedical function of transport proteins is indispensable for making progress in treating human diseases. Furthermore, more

Functional Analyses	Intracellular trafficking	Akihiko Nakano (RIKEN, Univ. of Tokyo), Kazuhisa Nakayama (Kyoto Univ. Pharmaceutical), Hiroshi Ohno (RIKEN Laboratory of Epithelial Immunobiology), Hiroaki Kato (Kyoto Univ. Pharmaceutical), Masayuki Murata (Univ. of Tokyo, Arts and Sciences), Syuya Fukai (Tokyo Inst. of Technology), Soichi Wakatsuki (KEK-PF)
	Post-translational modification	Toshisuke Kawasaki (Kyoto Univ. Pharmaceutical), Naoyuki Taniguchi (Osaka Univ. Medicine), Yoshifumi Jigami (AIST), Koichi Kato (Nagoya City Univ. Pharmaceutical), Sumihiro Hase (Osaka Univ. Science), Soichi Wakatsuki (KEK-PF)
Structural Analyses	X-ray crystallography	Takamasa Nonaka (Nagaoka Univ. of Technology), Nobutada Tanaka (Showa Univ. Pharmaceutical), Hiroaki Kato (Kyoto Univ. Pharmaceutical), Shuya Fukai (Tokyo Inst. of Technology), Soichi Wakatsuki (KEK-PF),
	NMR, Small angle X-ray scattering, Bioinformatics	Koichi Kato (Nagoya City Univ. Pharmaceutical), Mikio Kataoka (Nara Inst. of Science and Technology), Kei Yura (JAERI CCSE)

Table 1
Members of the “Posttranslational Modification and Transport” network in Protein 3000 Project.

than half of human proteins undergo post-translational processing and modification such as glycosylation, acetylation, phosphorylation, geranyl-genylation, and farnesylation. In partiulcar, glycobiology has recently gained prominence as a post-genomic science for its role in modulating protein function and transducing cellular signals. The recent interest in proteomics, the study of structure-function relationships of proteins, further increases the relevance of structural exploration in glycobiology and protein transport.

As part of the project, the SBRC has sought to maximize the efficiency of the large-scale expression, purification, and crystallization of proteins by trying to eliminate bottlenecks in each process. X-ray crystallographic studies using synchrotron X-ray radiation are conducted at KEK-PF, Kyoto University, Nagaoka University of Technology, Tokyo Institute of Technology and Showa University. NMR experiments are conducted at Nagoya City University, and small angle X-ray scattering studies at Nara Institute of Science and Technology (NAIST). From these institutes, Prof. Mikio Kataoka of NAIST and Dr. Shuya Fukai of Tokyo Institute of Technology are new members of our consortium from FY2004. Each structural analysis project maintains close contacts with the groups

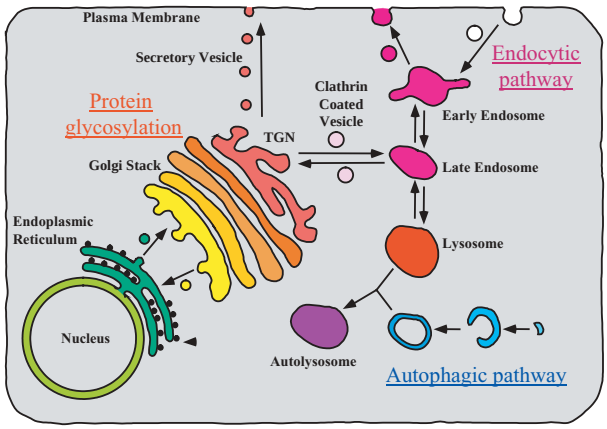

Figure 2
Network of protein glycosylation and transport in cells.

responsible for functional analyses. Our initial research plan was to accomplish structural and functional analyses of more than 70 proteins during the five years of the project. To date, we have completed structural analyses of 67 proteins and complexes and are currently working on about 100 additional targets, prompting us to increase our goal from 70 to 150 over the five-year period. Furthermore, we have submitted ten domestic and two international patent applications, based on our R&D and structural and functional studies of the target proteins. We have also established an operation scheme to reserve about 30% of the beam time available at our beamlines for users of the eight university consortia of the project and developed a web-based beam time reservation system to facilitate the Protein 3000 project.

As mentioned above, the post-translational modification of proteins is closely associated with the intracellular transport systems, and new molecular interactions are being identified and characterized, thus providing new targets for our project. A logical extension of the current proteomics studies is the incorporation of the post-translational modification and transport machineries, and in particular, membrane complexes which are key players in membrane trafficking. Our future research plan thus includes structural analyses of these complexes. With this in mind we will further develop the integrated and synergistic approaches within our consortia, and establish new research collaborations with groups in other research networks.

2-3 Development of Systems and Technology for Advanced Measurement and Analysis

Synchrotron X-ray crystallography is the most powerful technique available for determining the three-dimensional structures of biological molecules. The advancement of synchrotron facilities and experimental

techniques over the past twenty years has had major impacts in biology and other life sciences. However, as scientists try to solve significantly more difficult and complex structures, it becomes harder to obtain crystals of sufficient sizes. Even with the latest 3rd generation synchrotron facilities such as SPring-8 (Japan), APS (US) and ESRF (EU), the structural analysis of protein crystals of a few microns or smaller is currently out of reach. While every effort has been made and more advanced techniques are being developed to push the limits of ring-based synchrotron facilities, the next (4th) generation synchrotron light sources such as the X-ray free electron laser (X-FEL) and energy recovery linac (ERL), are expected to produce X-ray beams with unprecedented qualities. The higher brilliance X-rays with shorter pulse-lengths available from such light sources may be useful for the structural analysis of single- or nanometer-size crystals and various theoretical studies have been reported on the feasibility of such experiments [1]. It will be crucial to develop next-generation two-dimensional X-ray detectors with high-speed readout and high sensitivity to match the characteristics of the next-generation structural analyses. Such detectors will be also useful for collecting weak diffractions from small crystals using micro-focus beamlines.

During FY2001-FY2003, we developed a prototype high-speed X-ray area detector based on a visible light HARP (High-gain Avalanche Rushing amorphous Photoconductor) camera in collaboration with NHK-ES (NHK Engineering Service) by funded of MEXT. Following this program we are now improving the X-ray HARP detector, funded by a JST "Development of Systems and Technology for Advanced Measurement and Analysis" program (FY2004-FY2009, Fig. 3). The purpose of the new program is to develop a new measurement system for biological macromolecules based on a high-speed and high-sensitivity X-ray detector system coupled with a micro-focus beamline optimized for small crystals. The micro-focus beamline for the project, BL-17A, is being constructed at the 2.5 GeV PF-ring, and is described in detail elsewhere [2]. The detector system is also currently being developed, and is described below. When a new prototype of the X-ray HARP detector is developed, it will be immediately be installed at BL-17A for a series of test experiments. Based on the experience gained from these studies, the high-speed and high-resolution structural analysis system will be further improved. Upon completion, the system is expected to provide a solid base for developing X-ray detectors for the next generation of synchrotron light sources. The new detector will also be used for phase-contrast imaging, medical imaging diagnosis, and many other applications which require high-speed and high-sensitivity X-ray imaging systems.

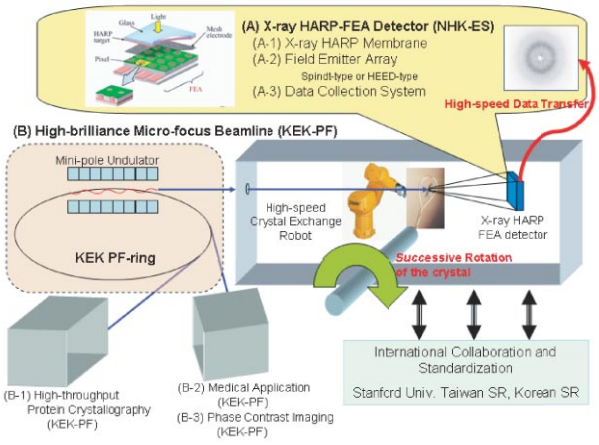

Figure 3
An outline of the "Development of Systems and Technology for Advanced Measurement and Analysis" program. In the project, we will mainly develop (A) an X-ray HARP-FEA detector in collaboration with NHK-ES and (B) a high-brilliance micro-focus beamline.

References

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2-4 Structural Biology Research Highlights

Molecular mechanism of ubiquitinated-cargo recognition by GGA

Lysosome is an acidic compartment found in all animal cells, and contains digestive enzymes for degrading unnecessary proteins and glycolipids. Mannose-6-phosphate receptors (M6PRs) transport newly synthesized lysosomal enzymes from the trans-Golgi network (TGN) and the plasma membranes to endosomes. GGA (Golgi-localizing, γ-adaptin ear domain homology, ARF-binding) proteins mediate the sorting of these lysosomal cargo receptors at the TGN. The GGA protein is composed of four functional regions; VHS, GAT, hinge and GAE. Since the discovery of GGA in 2001, we have been pursuing structure-function analyses of each domain of GGA in complex with its partners (Fig. 4). Lysosomal cargo receptors interact with the VHS domain of GGA. We have reported the structure of GGA1-VHS in complex with the cytoplasmic tail of M6PR [1]. We have also reported the interaction mode between GGA1-VHS and β-secretase which cleaves the Alzheimer's amyloid precursor protein [2].

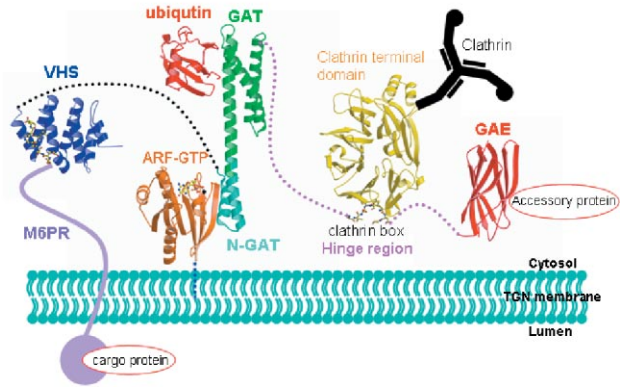

Figure 4
The interaction network between GGA and other proteins which are involved in intra-cellular protein transport.

The GAT domain of GGA consists of two subdomains; the amino (N) terminal (N-GAT) and the carboxyl terminal (C-GAT) domains. The N-GAT domain has a helix-loop-helix structure that is responsible for ADP-ribosylation factor (ARF) binding. In 2003, we reported the complex structure between N-GAT and ARF, explaining how GGA is recruited to the TGN membrane by ARF [3]. On the other hand, the binding partners of the C-GAT subdomain remained unknown until 2004 when ubiquitin was found to bind C-GAT. The ubiquitination of cell surface receptors serves as a signal for lysosome degradation after the internalization of receptors *via* endocytosis. Thus knowing that ubiquitin interacts with C-GAT uncovered a novel role for GGA in the ubiquitin-dependent sorting of cargo proteins in the endocytosis pathway. Quite recently, we solved the crystal structure of the complex between GGA3 C-GAT and ubiquitin [4].

In the GGA3 C-GAT/ubiquitin complex crystal, a hydrophobic patch of helices α1 and α2 of C-GAT interacts with the hydrophobic ubiquitin Ile44 surface, which is commonly used as a binding site by a variety of ubiquitin-binding modules. The ubiquitin Ile44 surface can be divided into three hydrophobic pockets. We compared the known structures of ubiquitin in complex with various ubiquitin-binding modules, and found that the three pockets of the Ile44 surface generally accommodate hydrophobic residues in all cases. We also found that Arg42 of ubiquitin is a key residue for determining the shape and charge distribution of the Ile44 surface, facilitating interaction with the structurally divergent ubiquitin binding modules. In addition to the ubiquitin-binding site mentioned above, biochemical and NMR data on the C-GAT/ubiquitin interaction suggested that another hydrophobic patch of helices α2 and α3 of C-GAT also interacts with the ubiquitin Ile44 surface. This double-sided ubiquitin binding allows the efficient recognition of ubiquitinated cargos by GGA.

First structure of human sialidase, Neu2, and potential drug design for influenza viruses

Sialidases are glycohydrolytic enzymes widely distributed among species ranging from viruses to mammals. Neu2 has been recently identified as a human cytosolic sialidase, among four known human sialidases. We have determined the first crystal structures of a mammalian sialidase from human, both in its free form and also in complex with an inhibitor, 2-deoxy-2,3-dehydro-N-acetyl neuraminic acid (DANA) [5]. The structure of the Neu2 and DANA complex shows the inhibitor lying in the catalytic cleft surrounded by ten conserved amino acids (Fig. 5, left panel). The interaction between Neu2 and DANA shows similarities with bacterial and viral counterparts, but also exhibits significant differences in active site arrangement and the dynamic nature of the loops which contain residues responsible for catalysis and substrate recognition (Fig. 5, right panel). Tamiflu® (oseltamvir) and RELENZA® (zanamivir) are recently-developed drugs which target influenza viruses [6]. They bind to neuraminidase, an influenza virus sialidase, and inhibit the release of virus particles from the host cell. There have been reports of some side-effects from the use of these drugs, and a detailed structural comparison between the virus and human sialidases is expected to provide clues for developing more effective drugs with fewer side-effects. More details are described in Highlights in this volume (See pp.44-45).

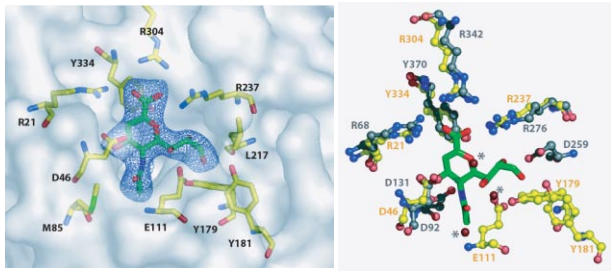

Figure 5
Left: Molecular surface representation of Neu2 active site showing residues involved in its inhibitor, DANA coordination. DANA is represented as a green stick model with its omit map contoured at 2σ (blue). Right: Ball-and stick representation of the active site residues of Neu2 (yellow) and viral sialidase (gray) around the DANA molecule (green).

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2-5 Beamlines

Overview of the current development of beamlines

We completed the construction of two insertion device (ID) high-throughput multiple-wavelength anomalous dispersion (MAD) beamlines for protein crystallography, AR-NW12A and BL-5A, in FY2003 and FY2004, respectively. In addition, we continued operating two bending magnet (BM) beamlines, BL-6A and BL-18B. Beamline operation during FY2003-FY2004 is summarized in Table 2. Owing to the high efficiency of data collection and high demand for beam time, we began to assign beam time in half-day units (9-hr for the daytime, 15-hr for the nighttime) on the ID beamlines in the 3rd period of FY2004. This allowed for more efficient beam time assignment and timely access to the synchrotron for users. At the end of February 2005, we stopped operation of BL-18B and closed it in order to construct a new ID beamline BL-17A, specifically designed for measurements of small

size crystals and low-energy experiments. The newly developed ID beamlines (AR-NW12, BL-5A and the under-construction BL-17A) have the following common features; (1) high-speed data acquisition using CCD detectors, (2) fast and reliable X-ray energy tunability using double-crystal monochromators (DCMs), (3) extremely precise sample rotation axes, and (4) motorized stages in the experimental stations. The updated specifications of the beamlines are summarized in Table 3.

To operate all the beamlines efficiently, a network-based beamline control system has been developed, providing not only a common user interface but also a function to enable experiments to be carried out remotely using secure TCP/IP communication. As part of the system, software using a relational database is being developed for the storage of all the necessary information related to the experiments. Together with the sample exchange robots installed on the ID beamlines, fully automated experiments will become possible. Detailed descriptions of the beamlines and software are provided in the following sections.

Table 2 Summary of Beamline Operation.

	FY	2003			2004		
	Period	1st	2nd	3rd	1st	2nd	3rd
PF	E+B	37	68	52	57	67	28
5A	User experiment	construction and commissioning			43	60.5	25.5
	Setup				12	6	2.5
	Not used				2	0.5	9
6A	User experiment	29	41	28	40	50	26
	Setup	7	13	12	12	4	1
	Not used	1	14	12	5	13	1
18B	User experiment	33	33	22	30	26	22
	Setup	2	12	14	18	26	1
	Not used	2	23	16	9	15	6
AR	E+B	52	69	54	64	52	44.5
NW12A	User experiment	37	52.5	43	44.5	44	42.5
	Setup	13	5	7	10	5	2
	Not used	2	11.5	4	10.5	3	0

(Days)

High-throughput ID beamlines, BL-5A and AR-NW12A

The newest beamline, BL-5A, has been available since April 2004, and FY2004 is the second year of public-use for AR-NW12A. Both beamlines have been quite stable and productive throughout the year. As for beamline development, we have installed one more set of four-blade slits immediately upstream of the sample position at AR-NW12A, forming part of a double-slit system along with the existing four-blade slits. This allows users to easily change the beam divergence, useful for measurement of crystals with large cell dimensions. Another important improvement of AR-NW12A is the use of the 1st harmonic of the undulator beam for low-energy experiments (wavelengths from 1.5 Å to 2.0 Å). This decreases the heat load on the optical elements drastically compared to the previous situation, where the 3rd harmonic was used with a short undulator gap.

The micro-channel crystal in the DCM at BL-5A caused problems several times as the cooling efficiency gradually decreased during operation. Finally, the beam intensity at the sample position decreased to about half of the normal intensity. We had to replace the crystal after each operation period, and used two precious crystals.

Table 3 Structural Biology Beamlines at Photon Factory.

	BL-6A	AR-NW12A	BL-5A	BL-17A
Starting year	1987	2003	2004	2006(planned)
Synchrotron ring	PF	PF-AR	PF	PF
Injection	once a day (9:00)	twice a day (10:00, 21:00)	once a day (9:00)	once a day (9:00)
X-ray source	Bending Magnet	Undulator	Multi Pole Wiggler	Short Gap Undulator
Wavelength range (Å)	0.91-1.33	0.7-1.9	0.7-1.9	0.95-1.1 1.4-2.0
Energy resolution (ΔE/E)	1×10^{-3}	2×10^{-4}	2×10^{-4}	3×10^{-4}
Photon flux (photons/sec @ 1.0 Å)	1.0×10^{10}	2×10^{11}	2×10^{11}	$> 10^{10}$(@1.0 Å), $> 10^{11}$(@2.0 Å)
Slit size (mm)	0.1	0.2	0.2	0.02
Detector	Quantum 4R	Quantum 210	Quantum 315	not decided
Type	CCD	CCD	CCD	-
Active area (mm^2)	188 × 188	210 × 210	315 × 315	-
Pixel size (μm^2)	81.6 × 81.6	51 × 51	51 × 51	(The specifications of BL-17A are estimated values.)
Pixel number	2304 × 2304	4096 × 4096	6144 × 6144	
Frame data size (MB)	11	34	75	
Readout time (sec)	8	1	1	
Typical exposure time (1.0° oscillation)	30 sec	5 sec	5 sec	-
Typical data collection time (180 frames)	2.0 hr	20 min	20 min	-
Buckup time for 1 data set (180 frames) (using the IEEE1394 interface)	3 min	6 min	6 min	-
Camera distance (mm)	60 ~ 400	60 ~ 1000	60 ~ 1000	-
Detector vertical offset	0 ~ 25 deg	0 ~ 100 mm	0 ~ 180 mm	-
Software for image processing	HKL2000, DPS/mosflm			

In addition, we had some trouble with the X-ray CCD detector (ADSC Quantum 210) at AR-NW12A at the beginning of June 2004. Several stripe-like noise lines were frequently observed in one quadrant of the read-out images, although the level of the noise was not serious. The trouble, however, was fixed during the summer shutdown and has not occurred since.

BM beamlines, BL-6A and BL-18B

BL-6A is a conventional beamline for protein crystallography using BM as a light source. It is the oldest protein crystallography beamline at the PF, and has been operational since 1987. In FY1999, we began a refurbishment program for BL-6A, which was completed at the beginning of FY2004. Currently, BL-6A functions as a modern beamline with a CCD detector data acquisition system and a high-precision optical bench. In 2005, the following further upgrades are scheduled. (1) Installation of the "FancyBox" interface already in use at beamlines AR-NW12A, BL-5A and BL-17A (under construction). (2) Development of a compact type high-precision sample rotation axis with spherical confusion of a few microns. (3) Development of a compact high-speed X-ray shutter. (4) Installation of a new graphical user interface already in use at the other ID SBRC beamlines (see below). With these improvements, all four beamlines will have the same architecture and look-and-feel.

BL-18B (Fig. 6) was closed on the morning of February 28, 2005. It was initially built in 1993 as a beamline for anomalous dispersion work using a rapidly tunable DCM and the millisecond time-resolved Laue method with a bent-cylindrical focusing mirror. Since then, it has contributed significantly to the progress of structural biology as a pioneer beamline for MAD experiments in Japan. After over 10 years of operation, its mission has been completed, producing many experimental results leading to numerous publications.

Figure 6
Homage to BL-18B. BL-18B was built in 1993 and was the first successful MAD beamline in Japan. The original detector was an offline imaging plate system with manual exchange of IPs which was later replaced by a CCD detector (ADSC Q4R). The beamline was closed in February, 2005. Left: Control PCs at the front of the door of the experimental hutch. Right: Inside view of the experimental hutch.

Construction of a new ID beamline, BL-17A

At the end of February 2005, we started to construct a new protein crystallography beamline, BL-17A. The existing BL-17A, B and C will be moved to a new BL-18B. The light source of BL-17A is a short gap undulator which will be installed in the 2.5 GeV PF ring after the improvement of the straight sections. This beamline is designed for measurements of very small protein crystals and for low-energy experiments. In FY2004, the construction of two optics hutches and the deck were completed (Fig. 7). The first beam will be delivered in October 2005, and operation for general users will begin in FY2006. The details of new BL-17A are described elsewhere [1].

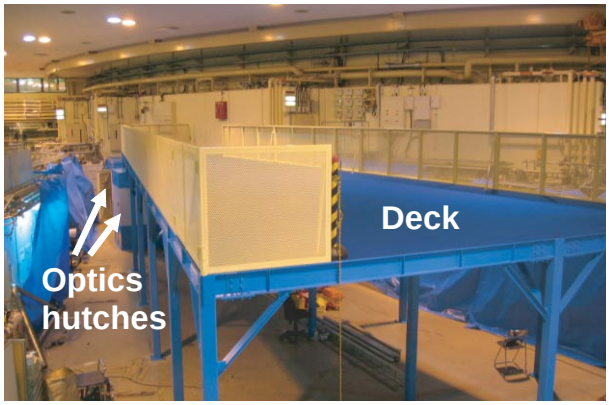

Figure 7
View of BL-17A under construction.

Development and improvement of the beamline control software, graphical user interface and experimental environment

A unified graphical user interface (GUI) for beamline control has been newly developed and made available for public use (Fig. 8). Previously, the GUI for each experimental operation (for example XAFS and sample alignment) had been developed separately. Although such concurrent development is one of the advantages of our STARS system, we have unified the three GUIs for crystal centering, XAFS measurement and diffraction image collection in order to prevent users from making mistakes during beamline operations. The new interface provides users with a smooth and intuitive operating environment. For instance, it is possible to extract the wavelength values for MAD data collection automatically from the XAFS measurement. The unified GUI will be installed at all the structural biology beamlines, so that users can carry out their experiments in the same manner at each beamline. In addition, automatic loop centering software and a control module for the crystal exchange robot are under development. In the near future, these functions will be also be installed in the system, to achieve a higher level of user-friendliness and semi-automated data collection.

We are also developing an integrated control system based on the unified database, PCCS (Protein Crystallography experiment Control System, Fig. 8). This system is designed to manage all experimental information, including structural biology research activities such as overexpression, purification and crystallization. In addition, the system allows fully automated measurements and multiple access from various places at the PF. We began to commission and debug the beamline part of the software at the end of FY2004. In the future, PCCS will be accessible from outside the PF via a web-server, allowing users to collect and process data from their laboratories. The system is developed on a central server [2] connect-

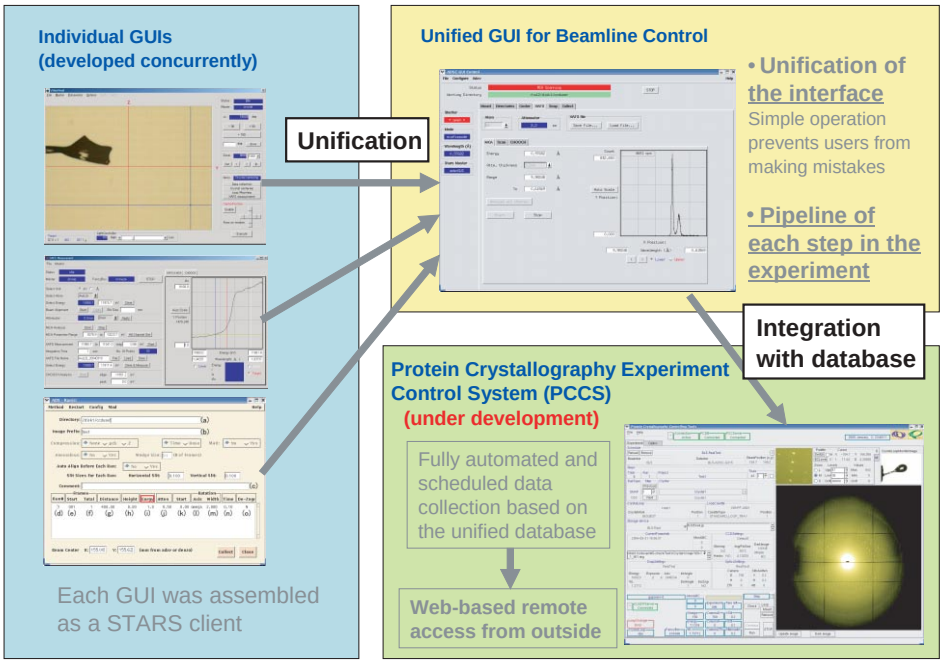

Figure 8
Schematic representation of the development of the beamline control software, graphical user interface, unified database system and overall integration.

ed with the beamlines and the experimental facilities with a high-speed optical fiber network.

An intelligent multilayer modular switch, Cisco Catalyst 6503, was installed as the gateway for our LAN, improving the network performance to a level ready for future remote access from sites outside the PF. LDAP/NFS was also installed to consolidate user accounts and the measured data. Users can access their data under the same environment from any PCs connected to our LAN. A new room for data processing and analysis was prepared in the PF-AR northwest building (Fig. 9), supported by Protein 3000 project. Users who have finished their beam time can use the room to process, analyze and backup their data efficiently, under the same environment as at the beamlines. A similar workspace will be installed on the deck of the new beamline, BL-17A.

Reference

[1] *Photon Factory Activity Report* **22A** (2005) 65.
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Figure 9
A new computer room for data processing and analysis located near AR-NW12A in the PF-AR northwest building.

2-6 Robotics for High-throughput and Automated Protein Structural Research

Large-scale protein crystallization and monitoring system

Protein crystallization remains one of the bottlenecks in the crystallographic analysis of macromolecules. We have developed a large-scale protein crystallization and monitoring system (Fig. 10) that is unrivalled in terms of speed by any other system in the world. The system has shortened the time required for crystallization setup by more than 100-fold; the time required to setup 480 crystallization conditions using the new system is only 10 minutes, whereas the same task performed manually would take 20 hours. Users can view images of the crystallization drops remotely using web-based browsing software and quickly search for the appropriate crystallization conditions. About 900 crystallization plates (each plate contains 96 crystallization drops) were setup for the initial screening of crystallization conditions at the beginning of operations in the autumn of 2003, and we have succeeded in making a large number of crystals using

Figure 10
Exterior view of the large-scale protein crystallization system in operation.

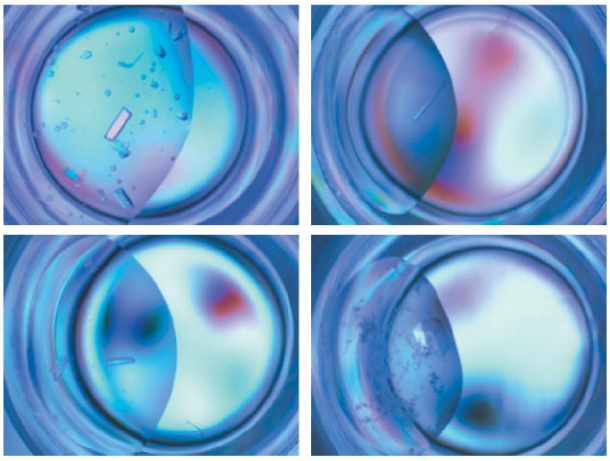

Figure 11
Some examples of protein crystals obtained using the large-scale crystallization system.

this system. In some cases, we could determine the protein structures directly from the crystals grown by the system without further optimization (Fig. 11). In other cases, crystals of size and quality sufficient for X-ray diffraction experiments were obtained by further optimization (manual screening) based on the conditions found using the crystallization system.

To improve the system, we are developing a high-speed observation system with 96 cameras operating in parallel to capture images of all the drops on the crystallization plates on a timescale of tenths of a second. Moreover, we plan to develop a nanolitre dispensing system to reduce sample volume and dispensing time, and an automated crystal scoring system based on various image processing techniques. As a longer-term development project, we also plan to develop a fully automated system which encompasses the whole range of crystal preparations: protein crystallization, crystal observation, crystal scoring, picking up the crystal, soaking the crystal to a cryoprotectant, and freezing and storing the crystal into a cassette. To this end, we have already developed a seal cutting system which can cut out the seal of the specified wells in which prospective crystals are found, a cryoprotectant exchange system and a subsidiary system for supplying consumables.

Automated sample-exchange robots

The high-flux ID beamlines BL-5A and AR-NW12A need significantly shorter exposure time for X-ray protein crystallographic experiments. Combined with fast-readout CCD detectors and shutters, a high quality dataset can be collected within 10 to 30 minutes for 180 degree oscillations. This means that each user (group) can collect 20 to 30 high quality X-ray diffraction datasets during 24 hours of beam time. To further improve the overall throughput, the time required for the offline experimental steps must be reduced as much as possible, in particular the time taken for manual crystal exchange in the experimental hutches. To this end, we have adopted an automated system developed by Stanford Synchrotron Radiation Laboratory (SSRL) [1] for exchanging samples from a liquid nitrogen Dewar, mounting it on the diffractometer

Figure 12
Overview of the sample exchange robot installed at AR-NW12A. The inset shows an inside view of the Dewar in which three SSRL cassettes are held in place.

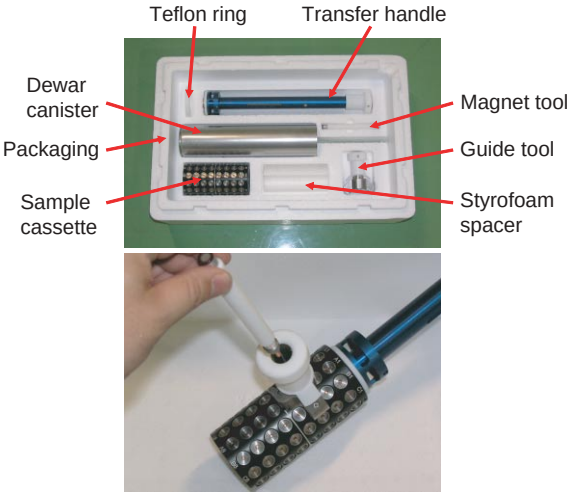

Figure 13
Upper: Sample loading tools developed by SSRL. Lower: How to use them.

and centering the crystal. We selected this system based on its reliability, its track record of about three years of user operation, its large capacity (continuous operation of 288 crystals using three cassettes with 96 crystals each), and its compatibility with commercially available cryo-loops. In addition, we modified the SSRL system from a single cryo-tong to a double cryo-tong in order to reduce the number of trips between the Dewar and the diffractometer by a factor of two. We have developed two exchange robots with double tongs, and installed them on BL-5A and AR-NW12A (Fig. 12) during the summer shutdown of 2004. In order to improve the stability of the system, we have been modifying the position calibration and the orientation of the cryo-loops in the cassettes in the Dewar. These robots will increase the throughput of the beamlines from 20 to 30 data sets per day to over 100. Easily-operated tools for inserting the protein crystals into the cassette are available for users (Fig. 13).

References

[1] A. E. Cohen, P. J. Ellis, M. D. Miller, A. M. Deacon and R. P. Phizackerley, *J. Appl. Cryst.* **35** (2002) 720.

2-7 Development of a Next-Generation X-ray Area Detector

X-ray detectors are one of the key components of synchrotron X-ray protein crystallography beamlines. In recent years, tapered fiber optics coupled CCD detectors have become widely used throughout the world. They match most of the current requirements but fall short for the expected characteristics of future light sources and ever increasing demands of structural analyses of complex structures. To prepare for such high demands in the near future, we have been working on a project to develop a next-generation X-ray area detector in collaboration with NHK-ES. The core of the detector system consists of a matrix field emitter array (FEA) and an X-ray HARP. A prototype FEA-HARP has been developed for HDTV applications by the research institute of NHK (Fig. 14) [1]. In the FEA-HARP, the FEA is faced to the photoconductor plate. After exposure to light, the photoconductor stores the image in the form of a surface charge distribution on the back of the photoconductor plate, which is scanned by electron beams generated by the FEA and readout individually. The photoconductor plate is comprised of a HARP target layer formed on a glass face plate. The HARP target layer, consisting mainly of amorphous selenium, uses an avalanche multiplication effect across the high-electric field to amplify the signals. These characteristics show the following advantages over the currently available CCD and other area detectors; (1) higher sensitivity, owing to the amplification effect by the HARP membrane, (2) higher spatial resolution, achieved by the single driven FEA, (3) higher framing rate of 10-120 images/second, and (4) other characteristics such as a higher dynamic range, lower noise and radiation hardness.

The maximum framing rate of 120 images/second matches the proposed frequency of LCLS, an X-FEL being developed at SLAC (Stanford, California, USA). The X-ray FEA-HARP detector system will be used for experiments at next generation light sources such as LCLS. Fig. 15 shows a view of a test experiment using the prototype X-ray HARP detector with an electron tube to collect diffraction images from a typical protein crystal

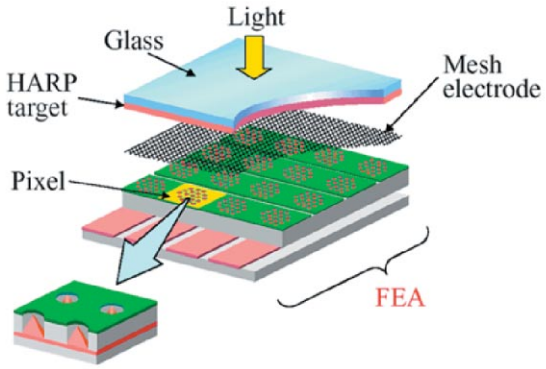

Figure 14
Schematic representation of the HARP area detector with FEA. The glass plate is replaced by beryllium, silicon or other material for an X-ray detector.

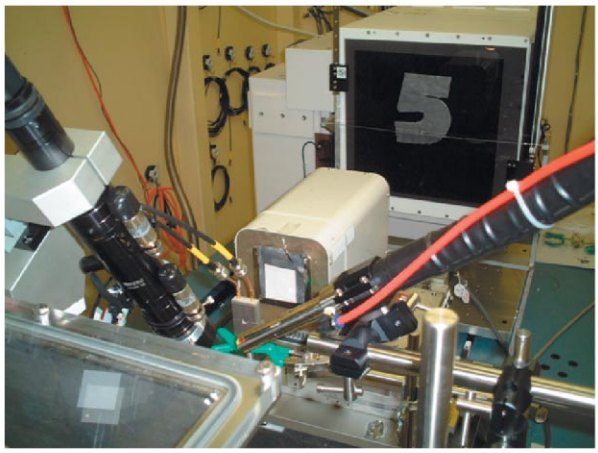

Figure 15
View of the test experiment of the prototype X-ray HARP tube camera at BL-5A. A protein crystal diffraction experiment with 180 degree rotation was completed within 12 seconds.

Table 4 Milestones of X-ray HARP-FEA Area Detector Project.

	First term (1-3 year)	Second term (4-6 year)
Pixel number	1000 × 1000	6000 × 6000
Active area (mm)^2	22.6 × 22.6	300.0 × 300.0
Framing rate (images/sec)	30 – 90	10 – 120
Dynamic range (bits)	14	16

at BL-5A. Diffraction images covering a 180 degree range could be recorded in only 12 seconds. The new detector system has shown tremendous possibilities which could bring a breakthrough for protein crystallographic analyses of macromolecules.

The development of this detector system is a six-year project starting from FY2004 and is divided into two terms with specific milestones (Table 4). The detector is also planned to be used for other applications such as low-dose medical imaging and real-time phase-contrast imaging methods, which will benefit from the high sensitivity and high framing rate of the detector.

References

[1] T. Yamagishi, M. Nanba, S. Okazaki, K. Tanioka, F. Sato and N. Egami, *Proc. of SPIE* **3019** (1997) 137.