National Research Program for Genomic Medicine

Core Facility Project：**D4**

Operation and Upgrade of the Synchrotron Radiation Protein Crystallography Facility (SPXF)

Progress Report (2007.09.01~2008.08.31)

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Institution: National Synchrotron Radiation Research Center

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- **Service Statistics:**

Over the reporting period (2007/09/01~2008/08/31) the number of users accommodated at the SPXF has grown significantly up to 26 groups for BL13B1, and 20 groups for BL13C1. 173 PX experiments have been conducted and 1076 users have been trained. More than 85% BL13B1 beamtime and 80% BL13C1 beamtime are for user service which already exceeds the originally planed 65% service time and this number is expected to grow up in future. A table listed below documents this data by beamline, with the number of user groups, experiments, trained users, beamtime delivered hours, and percentage of beamtime used in user service.

Table1. Service Statistics

	# of Visit User Groups	# of Experiments	# of Trained Users	# of Beamtime Hours Delivered	% of Beamtime Used for Service
BL13B1 MAD-PX	26	110	625	4973	86.9% (31.4%)
BL13C1 Mono-PX	20	63	451	4762	83.1% (33.7%)
Sum		173	1076	9732	

( ) means the beamtime percentage used by NRPGM users.

- **Geographical Distribution of User Groups:**

Because the beamline performance of BL13B1 is comparable to many famous PX beamlines in the world, many international users in Asia are interesting in using this facility. A table listed below shows the regional distribution of the user groups.

Table2. Regional Distribution of User Groups

	Domestic Users	International Users

	Taiwan	Japan	Singapore	Thailand
# of User Groups	31	7	4	4

Nation	Affiliation
Taiwan, ROC	Academia Sinica National Cheng-Kung University National Chung-Hsing University National Defense Medical Center National Health Research Institutes National Ping Tung University of Education National Synchrotron Radiation Research Center National Taiwan University National Tsing-Hua University National Yang-Ming University Yuanpei University
Japan	Hokkaido University Kyoto University Nagoya University Osaka University Photon Factory Yokohama City University
Singapore	Institute of Molecular and Cell Biology, A*STAR's Biomedical Sciences Institutes Nan-Yang Technological University
Thailand	Mahidol University National Center for Genetic Engin. and Biotech. Suranaree University of Technology

- **Statistics of User Publications:**

The publication summary for reporting period (2007/01/01~2008/08/31) shows 37 SCI papers published (including Science, Proc. Natl. Acad. Sci. USA, Nucleic Acids Res, J Biol Chem, J Mol Biol, J. Virol., Biochem J., Proteins, PLoS ONE,…etc), 16 these completed and 85 conference abstract published for 138 total publications. All 138 publications represent service research where the SPXF provided facility and support only. Since SPXF is a 100% service-orientated core facility, there isn't any activity in R&D and Collaboration Research. Total 56 protein structures have been deposit to the Protein Data Bank (PDB) from SPXF beamlines. A table listed below provides a breakdown of the SCI papers in terms of total number and different Impact Factor (I.F.), conference abstracts, and PDB depositions.

Table3. Publications Statistics

	SCI Papers			Conference Abstracts	Theses	PDB Depositions
	Total	I.F. > 2	I.F. > 5.8			
2007	19	15	6	50	8	54
2008	18	16	7	35	8	2
Sum	37	31	13	85	16	56

- **Science Highlights**

#1 **Dual Binding Sites for Translocation Catalysis by *E.Coli* Glutathionylspermidine**

Synthetase: Trypanothione biosynthesis requires ATP-dependent conjugation of glutathione (GSH) to the two terminal amino groups of spermidine by glutathionylspermidine synthetase (GspS) and trypanothione synthetase (TryS), which are considered as drug targets. GspS catalyzes the penultimate step of the biosynthesis-amide bond formation between spermidine and the glycine carboxylate of glutathione. Prof. Andrew H.-J. Wang has solved five crystal structures of Escherichia coli GspS in complex with substrate, product or inhibitor. Based on these structures, they propose that GSH is phosphorylated at one of two GSH binding sites to form an acylphosphate intermediate that then translocates to the other site for subsequent nucleophilic addition of spermidine. They also identify essential amino acids involved in the catalysis. Their results constitute the first structural information on the biochemical features of parasite homologs (including TryS) that underlie their broad specificity for polyamines.

#2 **The Crystal Structure of XC1258 from *Xanthomonas Campestris*:** The CN-hydrolase superfamily proteins are involved in a wide variety of non-peptide carbon-nitrogen hydrolysis reactions, characterized by a thiol acylenzyme intermediate formed through the attack of a cyano or carbonyl carbon by a novel conserved catalytic triad of Glu-Lys-Cys, to produce important natural products such as auxin, biotin, precursors of antibiotics etc. Prof. S.-H. Chou determined the crystal structure of XC1258, a putative CN-hydrolase protein from the plant pathogen Xanthomonas campestris pv. campestris t and found a cacodylate or dimethylarsinic acid compound situate perfectly in the active region, forming a strong arsenic adduct with the active cysteine residue. Their observation entails a common CN-hydrolase reaction mechanism and suggests that its activity could be inhibited by the dimethylarsinic compound through a sulfur-arsinic covalent bond.

#3 **Complex Structures of *Bacillus subtilis* RIBG Mechanisms of Deamination and Reduction in Riboflavin Biosynthesis:** Bacterial RibG is a potent target for antimicrobial agents because it catalyzes the two consecutive deamination and reduction steps in the riboflavin biosynthesis, and because it is the only protein to date to share a similar active-site architecture to the pharmaceutically important dihydrofolate reductase. The complex structures of Bacillus subtilis RibG determined by Prof. S.-H. Liaw revealed four distinct interaction networks of the product/substrate with the deminase and reductase domains, which may be useful in guiding drug design.

- **Performance Upgrade and Technological Development:**

Over the pass one year, many technical problems have been pined down and improved, these include: (1) Development and application of X-ray sensitive beam stopper. (2) Install and commission the Sample Automatic Mounting (SAM) system that is available to users at the end of 2007. (3) Implement the automatic XANES spectrum scanning and MAD energy determination function to the beamline control and data collection system. (4) Development an automatic sample lighting system. (5) Development an automatic sample annealing system.

- **User Training and Support:**

The best hardware and software are inefficient if well-trained support staffs are not available to assist the user, therefore, the training of the user support staff will be one of the most important issues to keep this facility running in the most efficient way. An 8 months training program provide by NSRRC seniors scientists is applied to every beginning support staff to ensure he/she has enough knowledge and skills to supervise different PX experiments. Currently, 7 well-trained support staffs are helping users conducting theirs experiments on the beamline daily. The support staffs provide 12 hours a day (8 hours for weekend), 6 days a week on-site user supports to help user collecting high-throughput data. All users have to take the beamline training before they can start their experiments at SPXF. A 2~4 hours on-site training tutorials is provided for each visiting research group to help how to condition the X-ray beam, get in/out the experimental hutch, mount and center the crystal, collect diffraction data, process diffraction data, and backup data. Over the reporting period (2007/09/01~2008/08/31) 1076 users have been trained and 173 experiments have been conducted. A facility web site (http://bionsrrc.nsrrc.org.tw) has been established. This web site is modified frequently and includes expanded user information. The user training and dissemination are also included on this web site. This web site is currently experiencing about 1000 hits per month and the user guide page is especially popular.

- **Facility Dissemination:**

The goal of facility dissemination is to disseminate experimental techniques of macromolecular crystallography to researchers or graduate student who has no previous experience but with an interest in using this specific method to further the scope of their research, and to introduce the latest developments in experimental methods to the user community to promote the usage of the facility. Dissemination activities include workshop for experienced users, training course for potential and novice users, and posters describing facility result and available services at conferences and workshops. In addition to posters, facility staff also manned a booth providing DM, and illustrating video film. Two workshops for experienced users and one training course for potential users were held over the reporting period.

Activity Title	2007 Mini-Lectures on Protein Crystallography
Associated Organization	High Throughput Protein X-ray Crystallography Core Facility (D3)
Sponsor	National Synchrotron Radiation Research Center
Venue	Institute of Biological Chemistry, Academia Sinica
Date	2007/08/30
# of Tutors	3
# of Participants	94 (restricted to experienced user with a maximum of 100)
Web Page of Activity	http://bionsrrc.nsrrc.org.tw/workshop1.php?b_id=1&bb_id=1

Activity Title	2008 Automation on Protein Crystallography Beamlines at NSRRC
Associated Organization	High Throughput Protein X-ray Crystallography Core Facility (D3)
Sponsor	National Synchrotron Radiation Research Center
Venue	National Synchrotron Radiation Research Center
Date	2008/04/09~10

# of Tutors	3
# of Participants	23 (restricted to one representative per group with a maximum of 24)
Web Page of Activity	http://bionsrrc.nsrrc.org.tw/workshop1.php?b_id=2&bb_id=8

Activity Title	2008 Protein Crystallography Training Course
Associated Organization	High Throughput Protein X-ray Crystallography Core Facility (D3)
Sponsor	National Synchrotron Radiation Research Center
Venue	National Synchrotron Radiation Research Center
Date	2008/06/25 ~ 2008/07/02
# of Tutors	11
# of Applicants	64 (restricted to potential user with a maximum of 14)
# of Participants	14 (4 Prof., 3 Post Doc., 5 Ph.D. students, 2 MS students)
Web Page of Activity	http://bionsrrc.nsrrc.org.tw/training_open2.php?b_id=17&bb_id=273

- **Committee Activities:**

The 2007-2nd User's Committee Meeting is held at National Synchrotron Radiation Research Center on Oct. 30th 2007, 8 of the 10-committee members attended this meeting. The committee recommended that (I) The effective period of the beamtime application proposal should extend from one year to two year. (II) It is not necessary that a user has to prove he/she has crystals in hand already before he/she submits the proposal..

The 2008-1st User's Committee Meeting is held at Institute of Molecular Biology, Academia Sinica on April 23th 2008, 9 of the 10-committee members attended this meeting. The committee recommended that since the responses of trainees of training course for potential user are very positive, the core staff should consider a training school for novice user.