

Progress Report

February 15, 2007



prepared by
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for the X6A Team

MISSION

The East Coast National Institute of General Medical Sciences (NIGMS) Structural Biology Research Facility mission is to provide first class resources to the biological-, biochemical-, and biophysics- communities to explore all aspects of structural biology. It is the goal of this facility to provide assistance to expert and non-expert crystallographers. These goals are:

- Beam line access to a structural biology community at large.
- Fast access to beam time for the user community.
- Crystal screening and high-throughput data collection.
- Assistance and training for academic and professional users.

INTRODUCTION

The NIGMS East Coast Structural Biology Research Facility at the National Synchrotron Light Source (NSLS) was created in response to the National Institute of General Medical Sciences Initiative for Synchrotron Facilities. The main motivation of this program is to increase beam time access to the general structural biologist at synchrotron facilities. At the NSLS this program provided funding for the reconstruction of a bending magnet beam line. As the sole source of support the NIGMS effort assures the full operation and staffing of the Facility.

The NIGMS investment at the NSLS has secured fast beam time access to numerous young investigators. The support and training provided by the staff of the Facility has allowed these scientists to excel in a number of research programs aligned with the NIH Roadmap for Medical Research and the Health and Human Services (HHS) Department strategic plan. The science developed at the Facility reflects the significance of the NIGMS support. Four science projects are highlighted:

1. Hydrophobic transport across the outer membrane

Little is known about the transport of hydrophobic molecules across biological membranes. Van den Berg's group, University of Massachusetts Medical School at Worcester, has been characterizing outer membrane (OM) proteins belonging to the long chain fatty acid transporter family, FadL. Widespread in gram-negative bacteria, members of this family are the only known to transport hydrophobic compounds across the OM. The goal of van den Berg's group is to understand the role of members of this family in the uptake of long-chain fatty acids (LCFA) and xenobiotics in structural and mechanistic terms. Studies being carried out at X6A are assisting in the construction of a model for LCFA transport across bacterial OM.

2. Visualizing the Immune System

The ability of the immune system to discriminate between that which should be destroyed (microbial pathogens and cancer) from that which should not be destroyed (normal cells and tissues) is a fundamental property of the vertebrate immune system. Ostrov's group at the University of Florida, College of Medicine, has been focusing on questions regarding immune recognition. Understanding the relationship between the variable region-containing chitin-binding proteins (VCBPs) found in amphioxus and vertebrate adaptive immune receptors could lead to immune boosting drugs in the fight against cancer and disorders such as rheumatoid arthritis. Studies at X6A lead to the first high resolution (1.15 Å) V-type immunoglobulin structure.

3. Structural Biology of Nucleo -Cytoplasmic Transport

Any protein with a Nuclear Localization Signal will bind strongly to importin and together will move into the nucleus through the nuclear pore. This import pathway also requires the small protein GTPase Ran that allows the movement of the import complex through the nuclear pore. Similarly some proteins need to be exported from the nucleus to the cytoplasm following an analogous mechanism. The Group of Dr. Cingolani, State University of New York, Upstate Medical University, proposes to determine the structure of HIV-Rev Nuclear Localization Signal in complex with importin b. HIV Rev is a short phosphoprotein that regulates the expression of HIV proteins by controlling the export rate of viral mRNAs from the nucleus. In order to activate HIV expression, Rev protein is imported into the nucleus by the receptor importin b. At X6A the group proposes to study the interaction between HIV-rev and importin b using X-ray crystallography.

4. Intermembrane proteases

Intermembrane proteases include a diverse family of proteins, which present multiple transmembrane domains with the active site embedded within the hydrophobic region of cell membranes. The main characteristic of this family of proteins is their property to cut their targets in the transmembrane region. Recently Dr. Ha's group, Yale University School of Medicine, determined the crystal structure of a rhomboid intermembrane protease, the first of its kind, with data collected at X6A. Part of the family that includes human gamma-secretase, an intermembrane protease thought to contribute to the release of amyloid fragments in the brain the rhomboid protease under study may provide new clues on Alzheimer's disease.

In addition to the exciting science the Facility has provided beam time access to teams of researchers requiring interdisciplinary approaches to solve their problem. As an example the team lead by Nadrian Seeman, New York University Chemistry has been awarded beam time at the Facility for extended periods of time to develop the formation of specific 3D structural designs based on branched DNA. In the last calendar year collaboration with members of the pharmaceutical sector contributed to the increased sample throughput at the Facility leading up to an improved user environment.

PROGRESS SUMMARY

The X6A beam line was initially designed and built without automation. After an initial operation period it became clear that automation is a necessary step for the facility to stay competitive.

The first step to create a fully automated screening and data collection environment at the beam line was the acquisition of the *ALS* (Advanced Light Source) *automated sample changer*. Fully operational throughout Calendar year 2006 the *automated sample changer* has been available to all users. Integrated into the data collection environment through a BluIce-like graphical user interface and a Device Control System (DCS) software package (Fig. 5) the *automated sample changer* has been requested by approximately 12 groups, academic and pharmaceutical, of the Facility. These users were responsible for screening 1500 samples with less than 3% failure. Academic users in general prefer to borrow the special sample holders that are available through the Facility. The second step was the inclusion and commissioning of an automated crystal centering software. Integrated into the data collection environment this function will require hardware upgrade for its continuous and reliable operation by the inexperienced experimenter.

The automation of the sample environment was key to the first true remote data collection during the 36th Mid-Atlantic Macromolecular Crystallography Meeting. General users can now choose between three different data collection modes: **local**, with members of the group being present at the beam line; **virtual**, with sample ship-in ship-out and staff assisted; and **remote**, accessing the beam line hardware with limited staff assistance.

To improve user access and streamline the Facility administration and management the user database and webpages were re-designed (Fig. 7). As a consequence the first steps towards the integration of an user-experiment database allows users to access the history of their beam time through an online experimental logbook. WebPages powered by MediaWiki allow for the continuous update of online manuals and maintenance files. On the administration front the new user database allows staff track user access and beamline productivity improving significantly the preparation of the annual survey.

The current Facility staff (Table 4) consists of two full time scientists, a physicist and a biochemist, a post-doctoral fellow with a chemistry background and a computer science associate. As an NSLS operated beamline, substantial technical support to enhance and test novel experimental procedures is provided by the NSLS Control and Detectors group and the Beam Line Support group.

In Calendar year 2006, the Facility has reached its saturation with 102% of available beam time for operations scheduled by users (Fig. 8). The beamline productivity has grown dramatically with 43 structures deposited in the Protein Data Bank, doubling the total number of structures and 14 publications in high impact journals direct or indirect

result of beam time access (Fig. 13; Fig. 14). The user community has matured with a number of groups visiting at least twice through out the year (Fig. 10). The beamline continues to attract new users with 20 new groups joining the X6A user base this year. One of the key operational features of the Facility that has contributed to its growth is the rapid access program that is provided through the X6A web based functions (<http://protein.nsls.bnl.gov>) and allows user access to beam time within a week of proposal submission. Following an external review process, users can schedule their own beam time on the online beam line calendar. This has been the sole mechanism chosen by users to access beam time at the Facility in 2006 in detriment of the NSLS on-line proposal submission system. Another factor contributing to successful operations is the increased support to users by providing assistance with data collection and analysis. Finally, in close coordination with other structural biology beam lines the X6A Facility was host to the Case Center for Proteomics macromolecular crystallography users while their beam line was moved from the X9 port to the X3 port on the NSLS X-ray ring.

In the following sections the technical developments and the facility management, are addressed in more detail. User access to beam time and the impact factors are presented and upgrade plans for the beam line discussed. Overall the Facility has reached saturation over the last year as reflected by the growth of its user community and increased PDB deposits and publications.

FACILITY DESCRIPTION

Instrumentation

The NIGMS Facility at the NSLS includes a bending magnet beam line, X6A, specifically designed to pursue the structure determination of macromolecules using the Multiwavelength Anomalous Diffraction technique (MAD) in a user-friendly environment and an associate Laboratory. Major Optics and End-Station components are summarized in Table 1 and Fig. 1. A detailed description can be found in the report for calendar year 2005.

Table 1. Characteristics of the X6A bending magnet *optics*.

Optical Element					
	<i>crystal channel cut</i>	<i>energy range</i>	<i>band pass</i>	<i>Intensity*</i>	<i>spot size</i>
monochromator	Si(111)	6 - 23 keV	1.9×10^{-4}	1.2×10^{10} ph/s	$0.1 \times 0.5 \text{ mm}^2$
	<i>coating</i>	<i>figure</i>	<i>magnification</i>	<i>acceptance</i>	
mirror	Rh	thoroidal	1:1	3 mrad	

* I=230 mA, 200 μ m slits at 1.2 Å



beam line control	EPICS/VME
diffractometer control	Compumotors
goniometer control	Galil
automated sample changer	Wago
in line sample viewer	Wago

Fig. 1. The X6A beam line *end-station* at the National Synchrotron Light Source is equipped with an ADSC Q210 CCD detector, an Oxford Cryostream 700R, a Crystal Logic single axis diffractometer, an ALS Automated Sample Changer and In-line viewer, and an NSLS Fluorescence detector. The control is integrated into a BluIce like graphical user interface and a Device Control System (DCS) software package.

Support Facilities

Computing and Network

The users are provided with extensive computing facilities and a complete set of up-to-date crystallographic structure determination software. Users can solve, refine, analyze and display macromolecular structures at the beam line using their preferred software. The latest versions of HKL2000, Mosflm, Scala, Solve/Resolve, Shelx, CCP4, CNS, Amore, and others are available for data analysis. Model building software includes Coot and O, Pymol and Molscrip for presentation graphics. Software is maintained and updated by the beam line personnel.

Four independent workstation computers at the beam line and a Silicon Octane 2000 workstation located on the 2nd floor of the NSLS allow for simultaneous crystal screening, determination of data collection strategies, data collection, electron density determination and model building. The Facilities hardware is summarized in Table 2 and 3. User data is kept for a period of one month. Users are encouraged to provide their own data backup during their beam time. Data can be saved onto DVD disc, firewire or USB2 enabled portable disks, laptops or transferred directly to home institutions via FTP. The local network is a Gigabit link and the external network is only 100 Mbit and soon will be upgraded.

Table 2. Computer Hardware available to the Facility user for beamline and experimental control, data analysis, and storage.

Experimental Control	1TB Raid 10 system dual AMD Opteron 242 dual Gb network
Data Analysis	1TB Raid 10 system dual AMD Opteron 242 dual Gb network
Firewall	130 Gb SCSI
Long term storage; additional data analysis	1TB RAID 5 system, Dual AMD Opteron 248 Dual Gigabit network

Table 3. Analysis software available to the X6A beam line user.

Data Collection	BluIce/DCS
Data Processing	HKL2000, MOSFLM
Data Analysis and Refinement	SHELX, CCP4, SOLVE, RESOLVE, CNS, etc...
Model Building	Coot, O

Sample preparation

A crystallization laboratory located close to the beam line is available to the users for manipulation of their samples. The Lab is equipped with an AKTA explorer system and a PHAST system (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA), a UV-VIS spectrometer, a refrigerated centrifuge (< 12000 rpm), three Pine Torres incubators, two stereo microscopes and two LN2 storage dewars. The standard equipment includes a chemical hood, a water purifier (Millipore, MiliQ), a fridge/ (-20°C) freezer, an analytical and semi-analytical balance, pH-meters, glassware, labware, stirrers, heaters, common buffers, chemicals and solvents, etc. are also provided. Users are also guaranteed access to an ice machine and a 4°C walk-in cold room maintained by the X4 PRT, New York Structural Biology Center.

Other support facilities

Special assistance is provided to users with specific experimental needs. The facility scientific staff works closely with the users to develop and install any devices needed in the performance of their experiments. Specific needs such as sample holders, flow cells, installation of light devices, small lasers for dynamical studies have been provided with the assistance of the technical and scientific staff of the Control and Detector, and Beam Line Operation Groups.

Staffing

The NIGMS provides support for four FTEs and for the operation of beam line. The NSLS provides technical and administrative assistance to the facility as needed. The staff and assignment breakdown described below is given in Table 4. Beam line activities and implementations are discussed with the Scientific Advisory Committee (SAC).

Facility scientific staff: include the PhD level scientific staff responsible for methodology and instrumentation development and direct user support. NSLS scientific staff is expected to pursue their own research 25% of the time.

Beam line operational staff: include science and engineering associates. This staff carries significant responsibility for direct support of the Facility, including experimental control data base development. This staff member Also provides maintenance of the Facility internet online services.

Research Associate: focuses on the development of new methodologies while pursuing research to enhance the beam line program. Carrying significant responsibility for direct user support, this staff maintains all scientific software use for data analysis.

NSLS Scientific Staff: include Ph.D. level scientific staff that pursues synchrotron radiation science as well as contribute and participate in the planning and development of the program. Carries out part of the development of new instrumentation and projects that enhance the beam line performance.

NSLS Technical Staff: in close collaboration with the beam line staff maintains and supports the installation instrumentation as well as provides assistance to users with special requirements.

NSLS ES&S staff: are responsible for the NSLS ES&H program. This group carries out the user safety program, and monitors beam time proposals, sample shipments, sample handling and any equipment issues.

Administrative staff: includes the NSLS user administration (user registration, foreign national user access administration, etc.), business services (financial planning and accounting staff, general administrative staff), NSLS computing managers and networking staff, NSLS Operators (perform check in - check out of beam line between experiments), facility staff (plans and organizes plant improvements as well as provides general building maintenance)

Graduate and Undergraduate Students: carry out part of the research and participate in developing new methodologies and approaches in the utilization of the beam line. While not directly responsible for user support, students may assist users and often contribute to facility outreach activities.

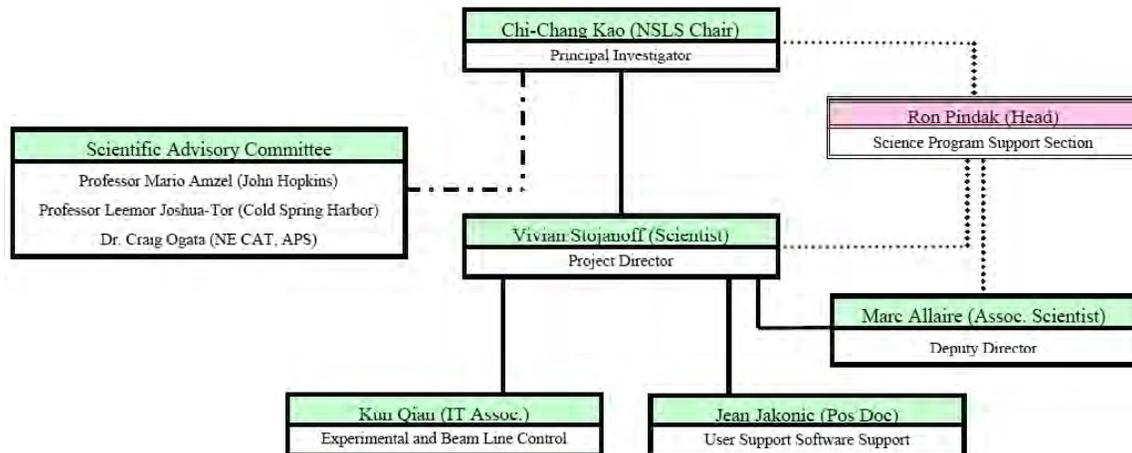


Table 4. NIGMS East Coast Structural Biology Facility staff assignment breakdown

<i>Staff category</i>	<i>FTE</i>	
Facility scientific staff	2	Marc Allaire, Vivian Stojanoff
Research Associate	1	Jean Jakonic
IT Associate	1	Kun Qian
NSLS Scientific Staff*		D. P. Siddons; S. Hulbert; C. C. Kao
NSLS Technical Staff*	0.5	B. Clay, I. So, Z. Yin, T. Kuczewski, S. Cheung, R. Greene, T. Lenhard
NSLS ES&H Staff *	0.1	A. Ackerman***
Administrative Staff *	0.1	F. Terrano***, K. Nasters***
Graduate and Undergraduate students**	0.4	A. J. Bernston, A. Jain C. Zayas, L. Piscitelli

*DOE-BES funded staff **Funded by DOE Outreach programs and SUNY at Stony Brook Outreach Programs ***team members provide assistance as required

Technical Developments

Technical development activities are intended to bring added functionality to the user program as well as continually renew the facility as it ages. In this calendar year major upgrades were introduced in the control software.. Changes in hardware supported the automation steps in the experimental control software.

End Station

Hardware

A compact 32-element solid-state detector developed by the NSLS Control and Detector group, Fig. 2, replaced the NaI fluorescence detector. The device presents improved energy resolution (~ 220 eV) and average noise level of the order of 25 electrons that will allow for higher accuracy in the measurement of small anomalous signals. The full commissioning of the device was delayed by problems with the cooling system. Energy scans of diluted (1 mg/ml) samples at the Br edge are shown in Fig. 2.

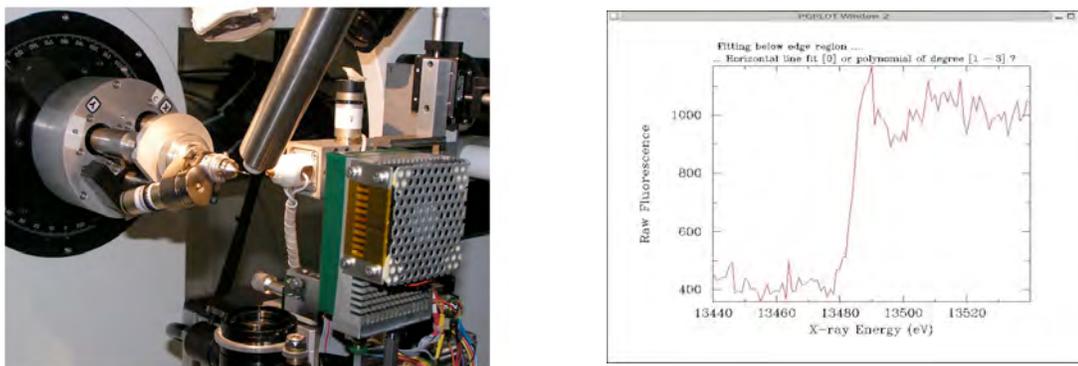


Fig. 2. The 32-element solid state detector; energy resolution 220 eV. On the left, the detector pictured in position to measure the fluorescence signal from a sample. When not in use the detector is retrieved to allow for access of the automated sample changer. On the right a fluorescence scan of a dilute (1mg/1ml) Br solution captured in a cryo-loop.

Also developed by the Detector and Control group a Beam Position Monitor (BPM) (Fig. 3) was inserted in the beam line just upstream of the Crystal Logic Diffractometer. The average signal from the BPM replaces the signal of the ion chamber used to monitor the beam intensity.

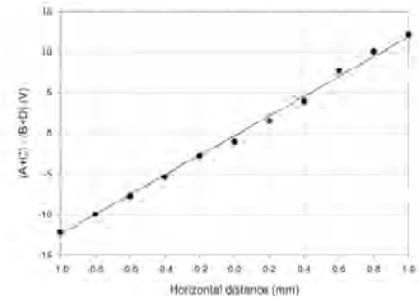
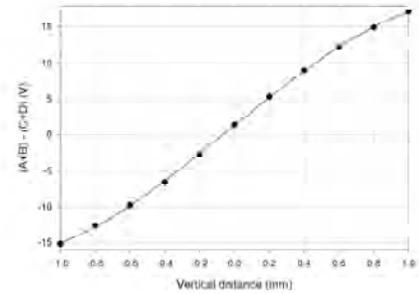
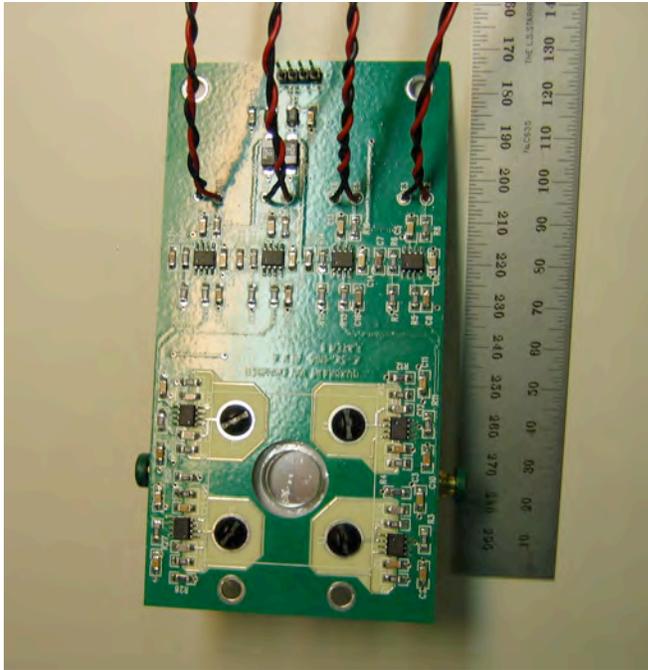


Fig. 3. The X6A beam position monitor (BPM) developed by the Detector and Control group. Left a view of the internal circuit; right calibration curves, applied voltage 500V. The resolution is of the order of 2 microns. (A. Kuczewski)

One of the main problems users encounter is centering the crystal in the beam, not only because the samples are usually small and transparent but also due to improper illumination. Also developed by the Detector and Control Group with the assistance from the Operation Group Technical staff a compact light source to be used with the in-line sample viewer has been installed (Fig. 4).

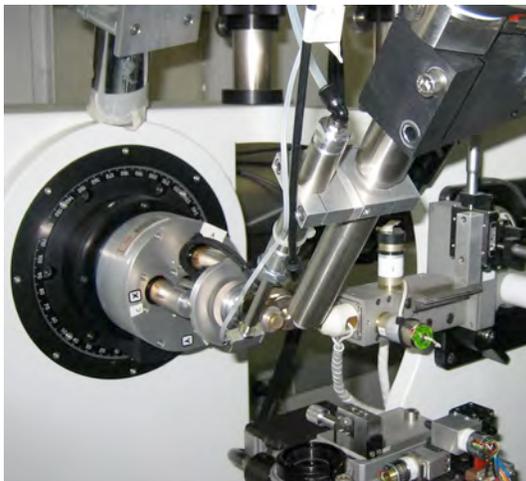
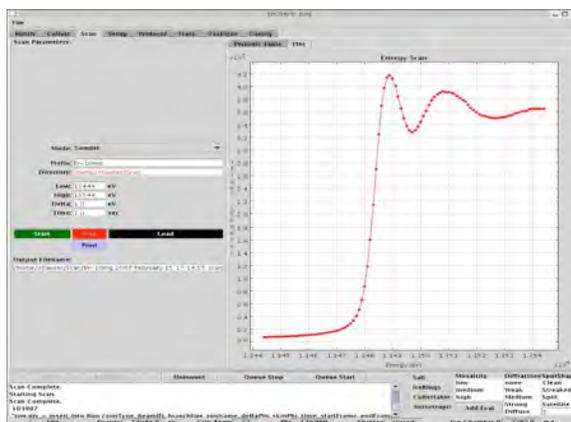
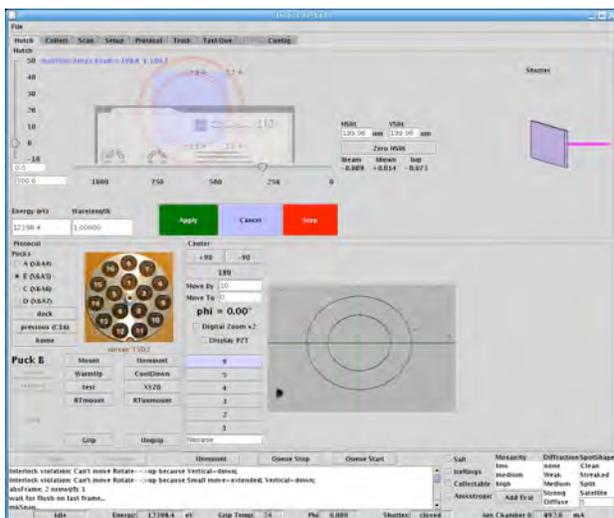


Fig. 4. The compact light source developed for the In-Line Sample Viewer. (A. Kuczewski, A. Lenhard).

Control Software

The *Device Control System (DCS)* software in use at several ALS and SSRL (Stanford Synchrotron Radiation Laboratory) beam lines *and its Blulce like graphical user interface* continues to be adapted to the X6A environment. In its current status it allows for the control of the diffractometer, the detector unit and the automated sample changer, with limited access to the beam line optics (Fig. 5). In its first year of user operation the automated sample changer served 10 different groups with 1500 crystals mounted and less than a 2% failure rate. All together 200 datasets were collected and over 30 structures are under refinement.

Significant effort went into the integration of the 32 element solid state detector with the DCS functions reserved for energy scans. This function was successfully commissioned and implemented and was recently made available to the user community. Also part of these implementations was the addition of functions that allow users to change the size of the beam and monitor the beam intensity. Users can also leave the experiment unattended, as DCS will automatically restart after any beam interruption. A whole experiment can be planned and left unattended, as users are able to establish a protocol to be followed for each of their experiments. Sample characteristics can be uploaded either using a template file in excel format or individually.



Task	Sub Project	Structure	Start	End	Status
WP1	PP1	1	0750	0755	Completed
WP2	PP1	2	0755	0800	Completed
WP3	PP1	3	0800	0805	Completed
WP4	PP1	4	0805	0810	Completed
WP5	PP1	5	0810	0815	Completed
WP6	PP1	6	0815	0820	Completed
WP7	PP1	7	0820	0825	Completed
WP8	PP1	8	0825	0830	Completed
WP9	PP1	9	0830	0835	Completed
WP10	PP1	10	0835	0840	Completed
WP11	PP1	11	0840	0845	Completed
WP12	PP1	12	0845	0850	Completed
WP13	PP1	13	0850	0855	Completed
WP14	PP1	14	0855	0900	Completed
WP15	PP1	15	0900	0905	Completed
WP16	PP1	16	0905	0910	Completed
WP17	PP1	17	0910	0915	Completed
WP18	PP1	18	0915	0920	Completed
WP19	PP1	19	0920	0925	Completed
WP20	PP1	20	0925	0930	Completed
WP21	PP1	21	0930	0935	Completed
WP22	PP1	22	0935	0940	Completed
WP23	PP1	23	0940	0945	Completed
WP24	PP1	24	0945	0950	Completed
WP25	PP1	25	0950	0955	Completed
WP26	PP1	26	0955	1000	Completed
WP27	PP1	27	1000	1005	Completed
WP28	PP1	28	1005	1010	Completed
WP29	PP1	29	1010	1015	Completed
WP30	PP1	30	1015	1020	Completed
WP31	PP1	31	1020	1025	Completed
WP32	PP1	32	1025	1030	Completed

Fig. 5. The Blulce like user interface. The *hutch* tab screen, showing the automated sample changer control and the sample auto centering centering screen. The *energy scan* screen and the *TaskQue* screen which allows to pre select the samples to be mounted.

Crystal centering is a key step in macromolecular X-ray crystallography experiments. A method using machine vision techniques that allows centering of small crystals in the X-ray beam without the need of user intervention was developed and integrated into DCS by A. Jain, a DOE summer undergraduate student. This method positions crystals even when the loop is initially out of the camera's field of view and adapts to the difficulty of the experiment. No initial information on the sample or experimental setting are necessary or are any constraints applied. The process has been tested on many diverse crystals with a 93% success rate when compared to manual centering. When used in conjunction with the automated sample changer, this method allows the performance of a fully automated experiment from crystal alignment in the X-ray beam to data collection (Fig. 6).

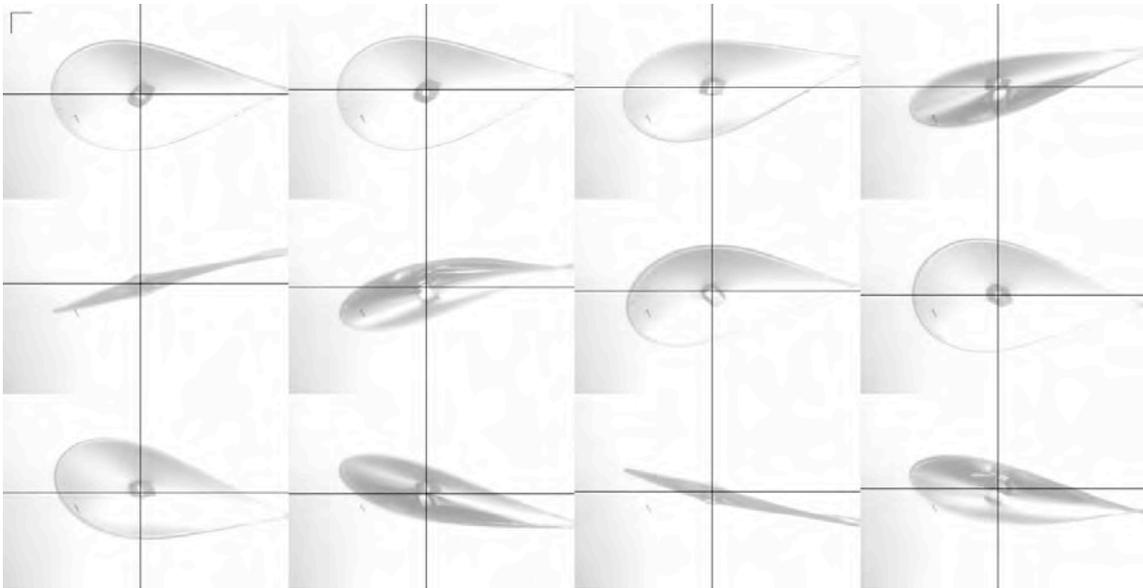


Fig. 6. The composite image created at the final stage of crystal centering shows the centered sample in twelve positions 30 degrees apart. The performance of the crystal centering process can be quickly, easily, and accurately be verified through these images. Scale bars on the top-left corner correspond to 100 μm . (submitted J. Syn.Rad.)

The X6A Web Access and Data Bases

The X6A Website underwent major upgrades. The system was designed to exclusively use Open Source software. The Facility website (<http://protein.nsls.bnl.gov>) is now powered by MediaWiki. The new website was made available to users beginning December 2006 in time for the X6A Survey. These changes are the first in a series of modules intended to build up to a fully automated data collection environment by

providing users with an improved access to the beam line as well as provide a data collection history. Users now have their own data collection space define upon each visit. For the staff the main changes were at the administration level. The new functions allow for example a real time evaluation of the beam time available and scheduled by users, the distribution of user support time amongst the staff, and the analysis of the annual survey and end of run forms. Beam line staff have also access to a visit form that contains information on the experiment to be performed, training status of visiting members, samples status, etc. The use of MediaWiki tools for the web services allows staff to easily maintain user manuals and update descriptions of the experimental control and set-up (Fig. 7). An online log file on maintenance and problem solving has increased the communication and efficiency to solve these problems. Integration with the NLSL PASS system continues as mid term goal.

The screenshot displays the X6A Beamline homepage. On the left, there are several navigation menus: 'X6A Home' (News, PDB deposits, Publications, Policy, People), 'User Corner' (Login, Schedule, Safety, Shipments, Arrival, Departure), 'Beam Line Facilities' (Optics, End Station, Automounter, Lab), 'User Guide' (Lab Facilities, Preparation, Run Experiment Manual, Data Analysis, FAQ), 'X6A Team' (SAC Login, Members Only), and a 'Search' box. The main content area features a 'Welcome To X6A Beamline' banner, a 'Main Page' with a 3D molecular structure image, and a 'MISSION' section. Below the mission is an 'ACKNOWLEDGEMENTS' section. To the right of the mission are three buttons: 'New User Register', 'User & Reviewer Login', and 'View X6A Schedule'. The bottom half of the page is dominated by a calendar for February 2007, showing a grid of days with various activities like 'NSLS Studies', 'Maintenance', and 'Conditioning' scheduled for specific times. A legend on the far right explains the symbols used in the calendar, such as 'Available', 'Maintenance', 'InHouse', 'Projects', 'Studies', 'Conditioning', 'Training', 'Commissioning', 'Template', 'Shutdown', 'Holiday', and 'UnAvailable'.

Fig. 7. The X6A Homepage Media Wiki powered and an example of a schedule page.

My Account

Edit User Profile

Change Password

Build Collaborator List

LOG OUT

Account Status

User -> Account Status

Welcome, AMOR

Schedule Status

Current or Future Schedules

You have 0 schedule(s)

Projects Status

Incomplete Projects

You have 0 Incomplete Project(s)

Choose Projects for Schedule

Currently You have 1 projects ready for scheduling
Select **AS MANY PROJECTS AS YOU WANT** to schedule your beamtime for these projects.

Project No.	Proposal Title	Submitted Date	select
x6a1	High resolution studies of a thermostable xylanase	2002-10-07	<input checked="" type="checkbox"/>

X6A Schedule Form

*Fields marked with * are required.*

Schedule available Beam Time

Projects : x6a1

Begin Date:* 1 January 2007

Shift Begin Time :* 0:00:00

End Date:* 1 January 2007

Shift END Time :* 0:00:00

Visit X6A Beamline *: YES NO

Availabe Beamtime Slots:

From	To	Duration
2007-02-19 12:00:00 (Monday)	2007-02-19 20:00:00 (Monday)	0days 8hours
2007-02-27 08:00:00 (Tuesday)	2007-02-27 12:00:00 (Tuesday)	0days 4hours
2007-03-04 00:00:00 (Sunday)	2007-03-05 06:00:00 (Monday)	1days 6hours
2007-03-06 12:00:00 (Tuesday)	2007-03-12 12:00:00 (Monday)	6days 0hours

Visit (Experiment) Information

*Fields marked with * are required.*

Visitors :*

Experimenters who will actually visit the beamLine (Press Ctrl Key to achieve multiple choices)

Stojanoff Vivian
Oliver Nelson
No visitors

(The name not shown in the List? [Build Collaborator List](#) now.)

Sample Safety

Do your samples have hazards *

Biological hazards Any Risks No Hazard Uncertain

If any hazards or risks, give details of possible hazards

Sample Shipment (ship your sample to x6a)

Date user shipped Format: YYYY-MM-DD Shipment Type Tracking Number Date X6A staff received Format: YYYY-MM-DD

Sample Back Shipment (ship your sample back)

Date X6A staff shipped Format: YYYY-MM-DD Shipment Type Tracking Number Date you received Format: YYYY-MM-DD

Fig. 7cont. Example of the X6A Web accessible User account pages. Users create, submit, and follow the status of their proposals. Approved proposals can be scheduled any time on a first come first serve basis. Proposals are valid for the duration of the research project. For each visit the Project Champion or PI is asked to fill out a visit form with general information on the experiment.

Select Fields for display duties

Select: Schedule Status Project Status

Past Schedules

Past Visits

x6a172	From: 2007-02-14 14:00:00 To: 2007-02-16 00:00:00	No need for backshipment
x6a176	From: 2007-02-13 20:00:00 To: 2007-02-14 14:00:00	Needs sample shipped back <input]<="" td="" type="button" value="[Fill BackShipment]"/>
x6a178	From: 2007-02-10 12:00:00 To: 2007-02-11 12:00:00	No need for backshipment

Current Schedules

Current Visit

Project: [x6a173] Structural studies of N-linked protein glycosylation oligosaccharyl transferases and associated sugar modifying enzymes.

Schedule: From: 2007-02-16 12:00:00 To: 2007-02-19 12:00:00

Visitors: Nelson, Olivier

Sample Shipment: No Shipment

Local Contacts: jakoncic, jean have been assigned as current Local Contact(s)

User Folder: /data_cys/User/X6969/2007-02-16

Crystals: **Crystal Form has not completed. Local Contact should ask the user to fill in the crystal in**

Fig. 7cont.. Example of the X6A Web based Administration account tools available to the staff. The *user folder* provides a direct link to the experiment data collection environment creating an individual folder for each user. A real time tracking tool allows users and staff to follow the experiment. At the end of each visit users are provided this data as a record of their visit.

Assignment -> Local Contact

Following are all the scheduled user beamtime.
Choose contact name to assign or edit local contact.

BeamTime Description	From	To	Current Contact	Duration	Assign
x6a85	2007-01-31 19:00:00	2007-02-02 12:00:00	Marc Allaire	1days 17hours	<input type="checkbox"/> Qian, Kun <input type="checkbox"/> jakoncic, jean <input type="checkbox"/> Allaire, Marc <input type="checkbox"/> Stojanoff, Vivian
x6a177 x6a179	2007-02-02 12:00:00	2007-02-03 07:00:00	Marc Allaire	0days 19hours	<input type="checkbox"/> Qian, Kun <input type="checkbox"/> jakoncic, jean <input type="checkbox"/> Allaire, Marc <input type="checkbox"/> Stojanoff, Vivian

X6A STAFF -> User Experiment Follow Up

Schedule

Visit Status: Pending

Mark Start Time 2007-02-16 12:00:00 Mark End Time 2007-02-19 12:00:00

Experiment Overview [+ More Details]

crystal	filePath	createDate	images
4d3-1	/data_cys/User/X6969/2007-02-16/Collect/4d3-1	2007-02-17 23:09:55	120
4d3-1	/data_cys/User/X6969/2007-02-16/Screen	2007-02-17 21:56:40	4
4c4-3	/data_cys/User/X6969/2007-02-16/Collect/4c4-3	2007-02-17 20:42:07	120
4c4-3	/data_cys/User/X6969/2007-02-16/Screen	2007-02-17 18:28:04	3
4c4-2	/data_cys/User/X6969/2007-02-16/Screen	2007-02-17 18:15:20	2
4c4-1	/data_cys/User/X6969/2007-02-16/Collect/4c4-1	2007-02-17 16:53:17	180
4c4-1	/data_cys/User/X6969/2007-02-16/Screen	2007-02-17 14:33:01	5
1b4-1	/data_cys/User/X6969/2007-02-16/Collect/1b4-1	2007-02-17 13:05:46	180

Statistic

- BeamTime Analysis
- Local Contact Analysis
- Reviewer Analysis
- User Analysis
- Survey Analysis

Assignment

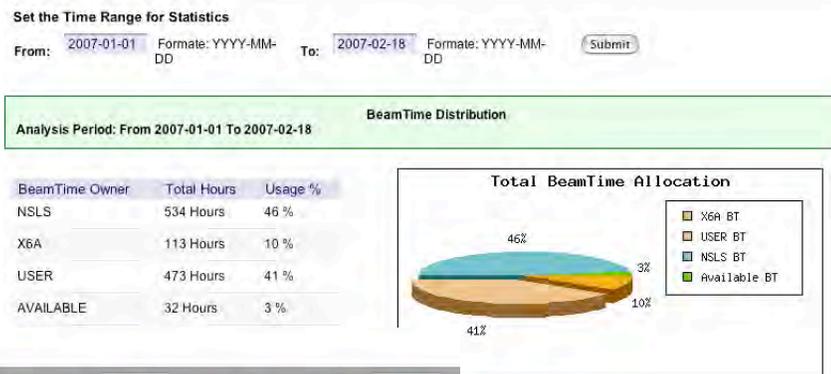
- Assign/Edit Local Contact
- Assign User Level
- Assign Reviewer Status
- Assign New Reviewers

Miscellaneous

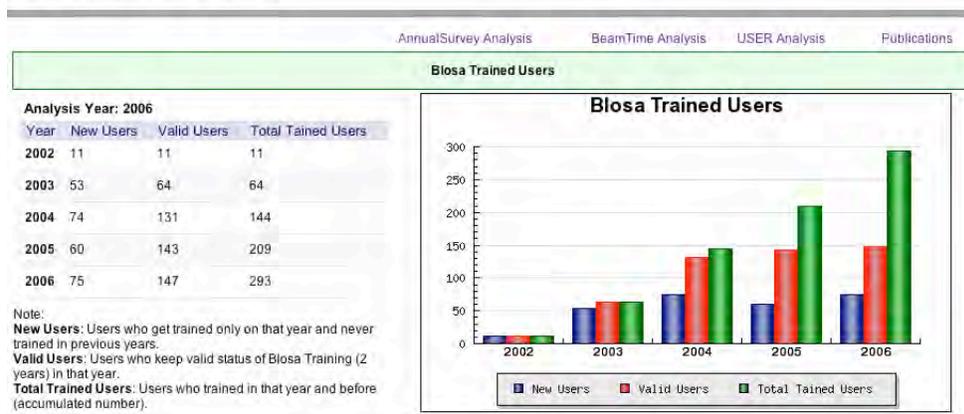
- Admin Duty
- Create X6A schedules
- Delete X6A schedules
- Add projects to Visits
- User Shipments

Analysis-> BeamTime Distribution

Fig. 7. Example of the X6A Web based Statistical tools. The beam time distribution is updated in real time. The number of Blosa trained users is update at the end of each calendar year for the annual survey.



Page-> Annual Survey Analysis



USERS

The NSLS provided approximately 205 days of user beam in calendar year 2006 from which a maximum of 25% are set aside for maintenance, beam line development and improvement of experimental methods as recommended by the US Department of Energy. At X6A 157 days, 77% of NSLS user beam, were scheduled by users. Two different modes are available to users for beam time application and scheduling:

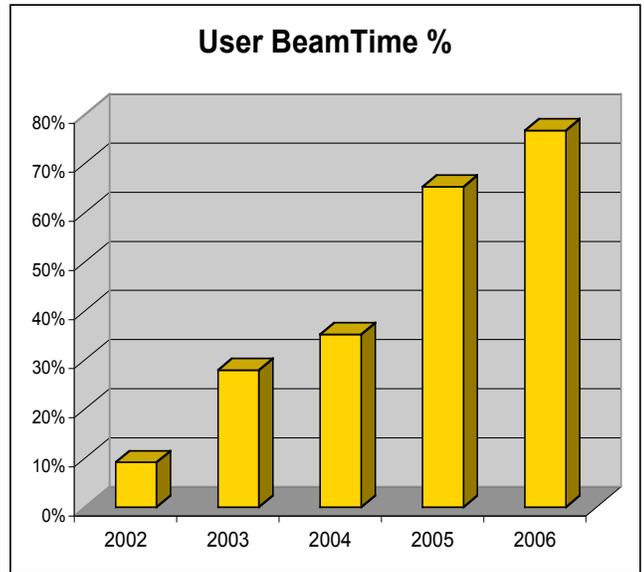
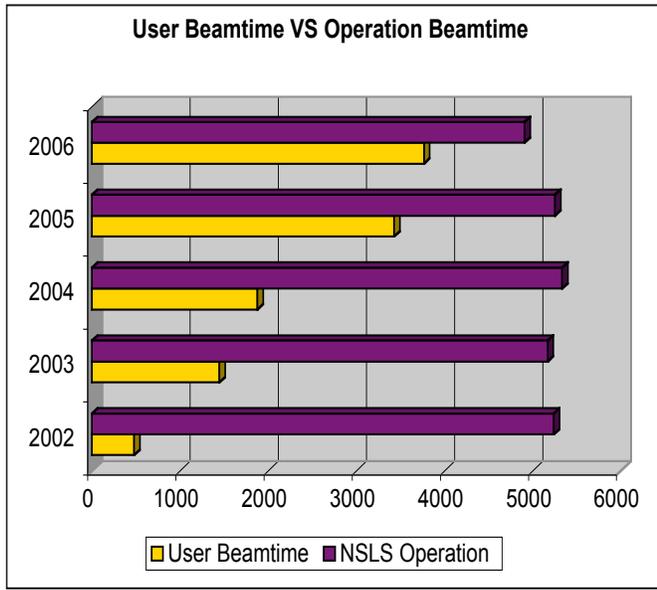
- program proposals (active for two years)
- fast access (active for the duration of a project)

Program proposals, generally referred as General User (GU) Proposals, are submitted to the NSLS User Administration three times a year. Proposals are assigned to the Macromolecular Crystallography sub-panel and rated following a peer review process. This rating (1.0 high to 5.0 low) is used for scheduling purposes. Beam time is scheduled following referral of the Allocation Committee. Users are contacted several times by the beam line staff in order to finalize their schedule. GU Proposals usually comprise a large family of proteins that are under study in the PI's laboratory.

Fast access projects are required to be protein specific and can be scheduled by the users themselves once the project has been approved for its scientific merit. A *PROJECT* as defined in the automated proposal submission form developed for the X6A beam line (<http://protein.nsls.bnl.gov>) consists of a set of native, derivatives and/or mutants of a protein from a specific source organism and expression system. Each *PROJECT* is expected to originate one or more structures and finally contribute to the understanding of the function and mechanism of the molecule. Three members randomly chosen from the 30 members that form the X6A external review panel review the projects. Beam time is awarded on a pass-fail basis; in order to pass a project has to be approved by two reviewers. The review process usually takes three days to a week. Once approved a user can schedule beam time as crystals become available.

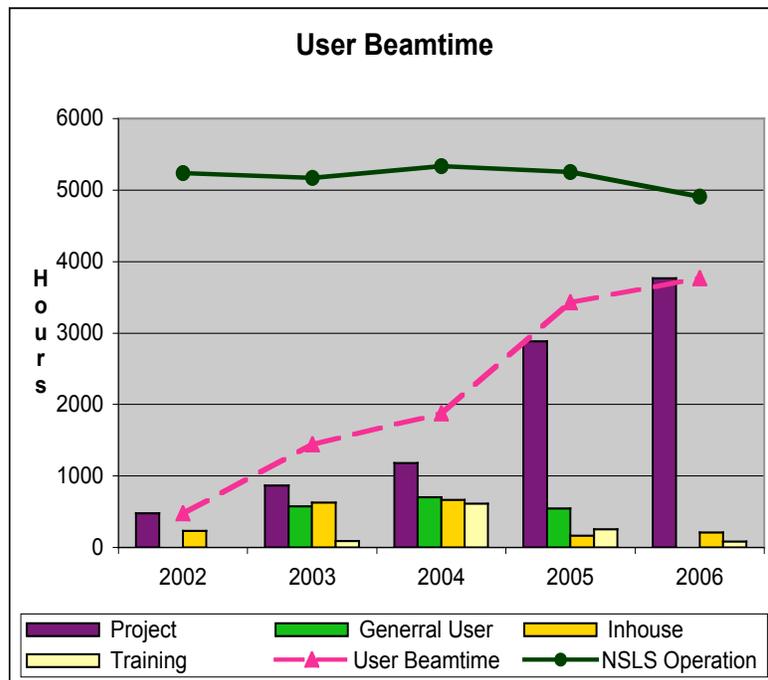
Currently all projects and proposals approved on the NIGMS Facility have been awarded beam time. The steady increase in user scheduled beam time observed over the last three years lead to the saturation of the Facility in 2006 (Fig. 8). Scheduled beam time to General User proposals has been decreasing steadily. No beam time was awarded against general user proposals in 2006 as the user community clearly prefers Fast Access to beam time rather than the more lengthy GU proposal process.

As an NSLS Facility beam line, the X6A beam line was requested to host the Case Center for Proteomics macromolecular crystallography user program while their beam line moved from the X9 port to the X3 port. 18% of the NSLS available beam time was reserved for this program, of which 38% was scheduled while the remaining 62% reverted to the X6A program.



(a)

(b)



(c)

Fig. 8. A steady increase in user scheduled beam time has been observed as the Facility reached saturation with 102% of the beam time supplied by the NSLS scheduled by users. (a) NSLS beam time availability (NSLS Operations) and scheduled beam time at the X6A beam line; (b) 75% of the beam time provided by the NSLS is dedicated to the Facility User program; as recommended by the DOE and the NSLS management 25% of the NSLS delivered beam should be set aside for maintenance, beam line development and improvement of experimental methods; (c) as User projects request more beam time, the beam time dedicated to in-house projects and training programs decreased.

A common measure of beam line usage or beam time employment has been to report the number of images collected (Table 5). This data is presently available with the new LOG capability of the experimental control software. The values reported are well above the average for similar beam lines at the NSLS.

total # images	160,360
total # images data set	145,533
total # images screening	14,827

Table 5. Beamline Use. Total number of images recorded in 2006.

The X6A user community increased significantly in 2006, as shown in Fig. 9; nearly 300 users are BLOSA (Beam Line Operation and Safety Awareness) trained on the beam line. 20 new user groups (Fig. 10) visited the beam line and 33 returned in 2006 indicating a consolidation of the user base. This trend is also reflected by a close analysis of the beam time usage; in 2004 1225 hours, in 2005 over 2719 hours and in 2006 3005 hours were dedicated to returning users out of the approximately 4000 hours available for operations. The considerable increase in number of new user groups in 2006 is a clear sign of the appeal that the program has to users that are not experts in synchrotron data collection.

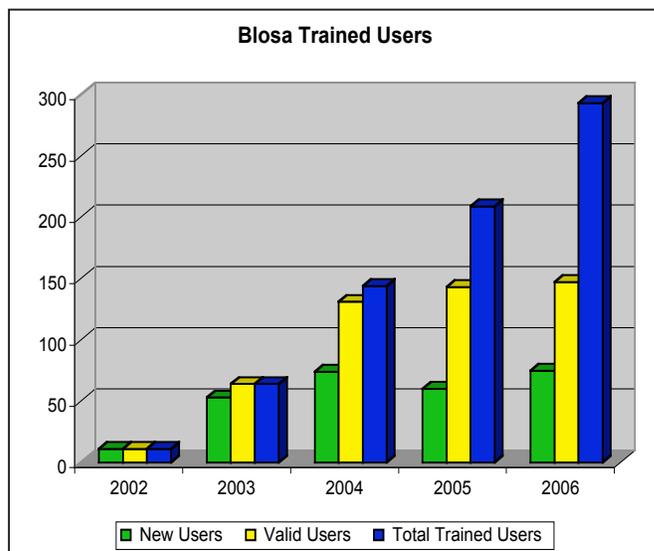


Fig. 9. Beam Line Operation and Safety Awareness training is valid for two years.

New Users: are experimenters who got trained in a specific year and was never BLOSA trained in previous years.

Valid Users: are experimenters who keep a valid BLOSA Training Status in a specific year.

Total Trained Users: are experimenters who trained in that year or before (accumulated number).

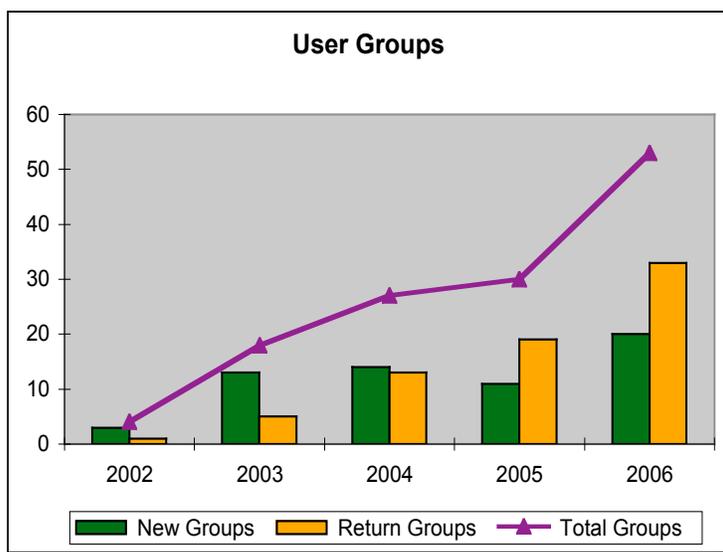


Fig. 10. The number of groups returning to the beam line has increased significantly in 2006, as has the number of new groups. As reflected in the X6A User base *New Groups* in general are young researchers starting up their Laboratories.

In 2006 two access modes, *virtual and remote*, were made available to researchers who did not wish to travel. To take advantage of this program users are requested to have visited the beam line at least once in order to become familiar with the beam line and learn about its advantages and limitations. *Virtual Users* are users who apply for fast beam time access, send their samples, are not present during the measurement, receive image files and scaled data and usually also receive an electron density map if they provided the sequence for their project. *Remote Users* also apply for fast beam time access, send their samples, and are not physically present during the measurement. The difference is that this user can control the experiment. In spite that remote access has been available users have preferred the virtual access sending their samples to the Facility and scheduling with the scientific staff the beam time as available. In calendar year 2006 7 groups have taken advantage of this capability for data collection with approximately 6 more groups leaving the samples behind that they could not measure in the time they had reserved for their visit. Some of these requests take advantage of the automated sample changer and the loan program for the sample holders. Up to now approximately 10 groups have used the automated sample changer on a regular basis in their experiments, three of which chose to send their samples by courier.

In a recent survey it was possible to track the progress of 91.4% of the 221 projects being developed at the facility. Funding (Fig. 11) for nearly 45% of these projects is provided by different NIH Institutes with most being supported by the NIGMS. Of these 221 projects 124 were solely developed at the X6A beam line, 90 were taken to other beam lines and 6 did not report the use of any other facility. 70% of the projects were reported as part of a PhD thesis or are part of a Post-Doctoral research project while approximately 20% are related to basic and applied research and may contribute to grant applications, optimization of crystallization conditions, and other applications.

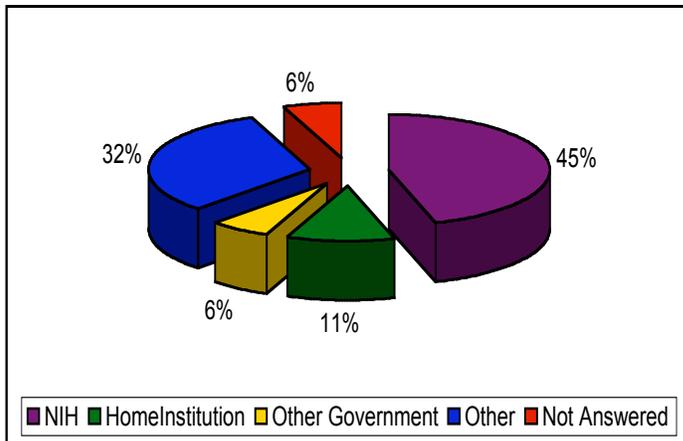


Fig. 11. Users are mostly funded by the NIH and other government agencies, such as, the NSF. A significant number of users are also funded by their home institutions; such is the case of new investigators as they establish new facilities at academic institutions.

An analysis of the user demographics reveals that although for nearly 70% of the projects the principal investigators are expert crystallographers a significant number of project champion's are new to the crystallography field. Nearly all users come from academic institutions; most are located in the northeast region of the US, but a significant number comes from other US states as presented in Fig. 12.

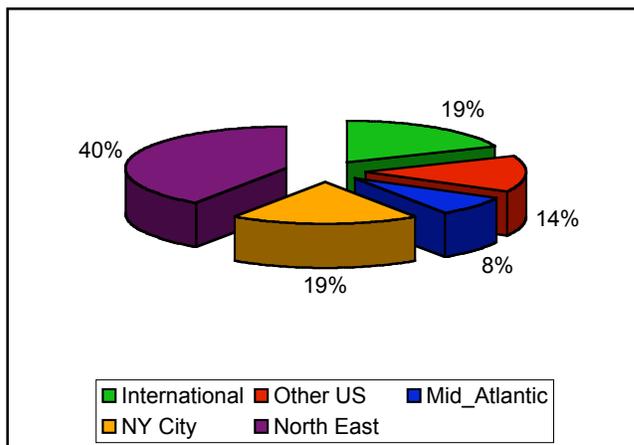


Fig. 12. Most Users come from academic institutions located in the northeast United States. Actual users usually come from Laboratories that are lead by experts in the structural biology field but come from a very diverse background, including molecular biology and biochemistry and had little exposure to structural crystallography methods. Facility users reflect the general NSLS user community, as 55% are located in the northeast.

To attract new users the effort to offer an increased support through assistance with data manipulation and data collection has continued. Dissemination at conferences and workshops as well as through bulletin boards such as CCP4 seemed to have called the attention of potential users, especially those with no ready access to beam time. Also as part of this increased user support several projects with specific needs, special experimental set-ups, and need for longer periods of beam time have been supported at the beam line. Typical examples of such projects are the projects developed by Genick's group on Photoactive Yellow Protein, Moreno's group on crystallization and Seeman's group who seeks to exploit the architectural properties of DNA.

IMPACT / COST INDICATOR

A common measure of the productivity of a beam line is the total number of structures deposited in the protein data bank. As reported in a recent survey 43 structures were deposited in 2006 in the protein data bank (Fig. 13) duplicating the number of structures related to work performed at the Facility and 56 further structures are currently reported under refinement. A list of publications based fully or in part on work performed at the X6A beam line as reported in the end of calendar year survey, or found through electronic searches is appended at the end of this report. The list for 2006 is not complete as not all the information was available at the time of the survey as it takes an average of 16 month for users to obtain a final model.

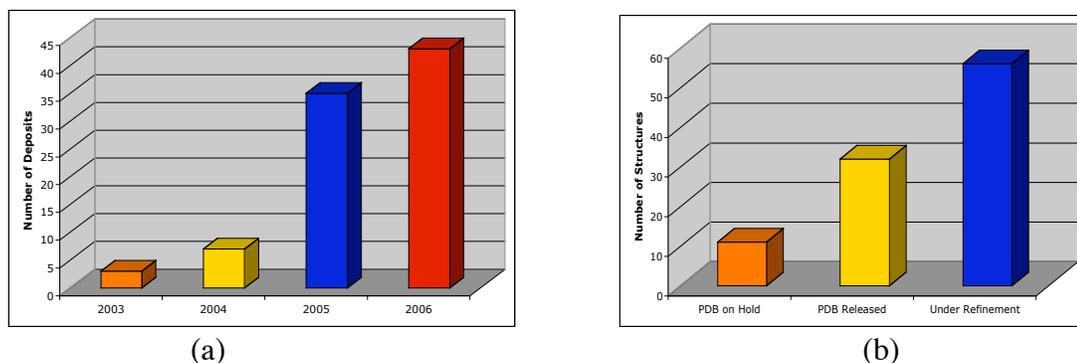
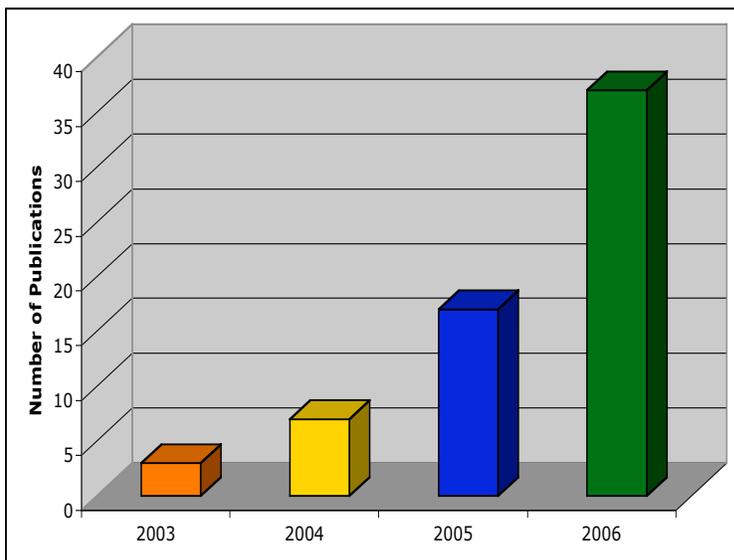


Fig. 13. 3D Structures* related to work performed at the X6A beam line. The number of deposits in the Protein Data Bank increased significantly in 2006 nearly doubling the total number of structures related to research performed at the Facility. (a) Structures deposited in the Protein Data Bank (b) 2006 Structures on hold, released or under refinement. Source X6A Survey, December 2006. (*numbers do not include structures solved by proprietary users)

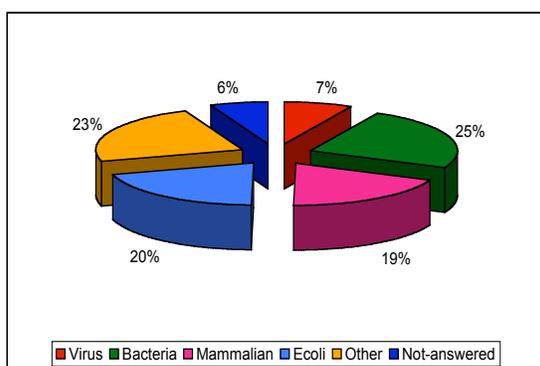
High profile publications are generally considered a measure of impact (Fig. 14). Approximately 40% of the 37 publications reported have appeared in high impact journals. The citation index included in the file was generated from the Web of Science at the Brookhaven National Laboratory Library service. It should be pointed out that for articles published late in the year 2004 and 2005, citations will not yet have appeared and the list is therefore somewhat biased.

It can be argued that another measure of the quality and impact of the research being developed at the facility is the number of projects that advance the NIGMS mission or that are significantly aligned with the NIH Roadmap activities (Fig. 15). 18% of the projects being developed are focusing on particularly challenging proteins: membrane proteins and proteins from humans. Further 41% of the proteins being researched are related to studies of the immune system and cancer or to the development of new drugs and vaccines. Still other projects are related to the study of anesthetics, RNA and DNA polymerase, infectious diseases, neurodegenerative and asthma. Other measures are shown in Fig. 15 and show the support in training a scientific workforce as more than half of the projects are solely developed at the facility.

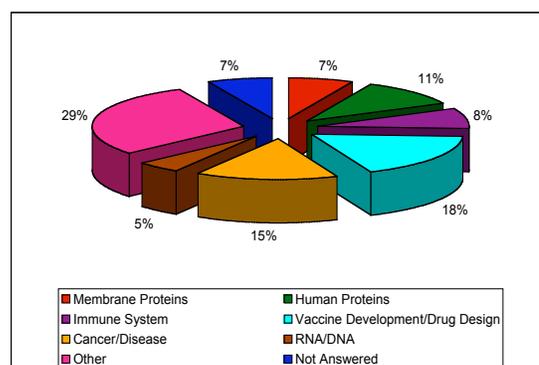


Publications	
Total	High Impact
66	29

Fig. 14. Publications related to work performed at the X6A Facility beam line. Numbers have increased as the beam line advances towards its maturity. High Impact Journals considered, indices 6 or higher according to the Web of Science at the Brookhaven National Laboratory Library service. (Source X6A Survey, December 2006).

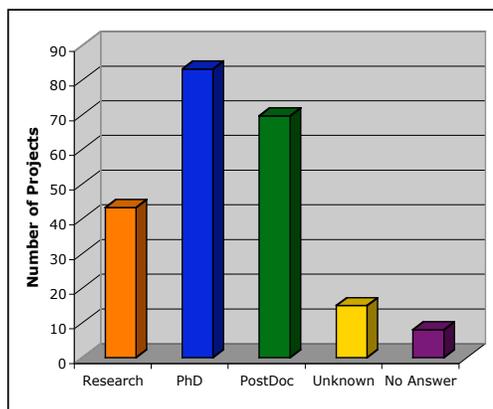


(a)

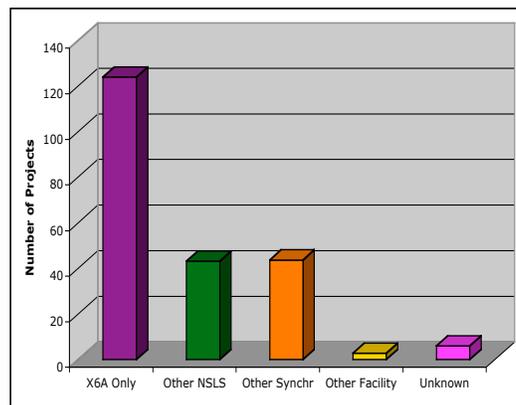


(b)

Fig. 15. Projects developed at the Facility advance the NIGMS mission and are significantly aligned with the NIH Roadmap. 75% of the projects focus on topics considered particularly challenging. (a) projects according to source organisms (b) projects by subject area. (Source X6A Database, December 2006)



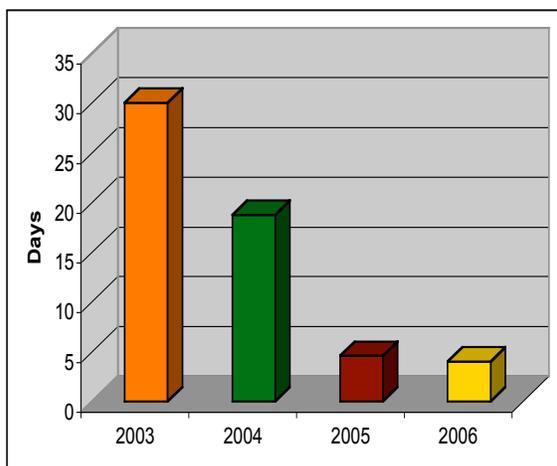
(a)



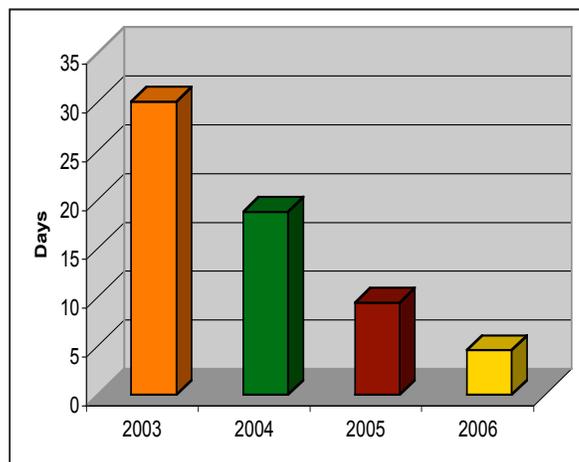
(b)

Fig. 16. As one of the NIGMS mission the Facility is also engaged in the development of a scientific workforce. (a) number of projects as a function of project champion background, 70% are PhD or PostDoctoral projects; (b) 57% of the projects, more then half of the projects, are carried out exclusively at the Facility according to the Survey. (Source X6A Survey, December 2006)

The definition on what should count in the total cost of a paper or structure is subject of different interpretations. One of the most difficult aspects to judge is what assumptions and considerations to include. Fig. 17 represents the cost per paper, per structure and per project as a function of scheduled user hours. As shown in the figure this cost decreased significantly in the last calendar year.



(a)



(b)

Fig. 17. Cost per structure or publication is subject to different interpretations. As the Facility matures the cost has been decreasing significantly. (a) The cost per structure deposited in the Protein Data Bank and (b) cost per publication, is estimated as a function of scheduled operation days.

SYNERGY

In order to provide better technical support to users and coordinate beamline activities, the NSLS recently established 18 Facility Beamlines in 2005. The NIGMS East Coast Structural Biology Research Facility at X6A was included in this grouping. The establishment of X6A as a Facility Beamline has facilitated:

- Technical and scientific exchanges with the New York Structural Biology Center,
- Collaboration with the PXRR (Protein Crystallography Research Resource) to develop high pressure freezing facility for users and a Rapid Access procedure to beam time. As consequence of this collaboration the safety approval form system for the NSLS structural biology community got streamlined and X6A users are granted beam time on an insertion device beam line, X29 (PXRR), if higher intensities and flux are required by the project being developed.
- The temporary relocation of the Case Center for Proteomics macromolecular crystallography users was hosted by X6A while the PRT beamline was relocated from the X9 port to the X3 port on the NSLS ring.

Another synergistic activities involve sharing of technologies and software resources with other synchrotron facilities. In specific the GM/CA CAT showed interest in the crystal autocentering software developed for X6A. Also underway with the ALS team and the SSRL structural biology group are several minor improvements for the automated sample changer, such as, testing a universal sample holder, improvement of the liquid nitrogen autofill system, sample tracking, etc.

FUTURE

The implementations planned for the facility over the next 12 months aim to make the facility more accessible and user-friendly. Such is the case of the diffractometer rotation axis, which we plan to change for servo motor in order to better exploit the crystal auto centering capability.

Technical Developments

Diffractometer Single Axis Replacement

The current rotation stage on the Crystal Logic Diffractometer is powered by a step motor. Under the current setting the crystal autocentering routine does not improve significantly the time spend in centering a crystal. As shown in Table 6, 67% of the time is spent in moving the rotation stage. Discussions are underway with the company to replace the current motor by a servo motor. Two options are being considered not to disrupt the user program. Most probably the change will be performed during the winter shutdown of the NSLS.

Task	Percentage of time (%)
Moving Omega Axis	67
Moving XYZ Heads	16
Computing	11
Communicating with Camera server	4
Moving Camera Zoom	2

Table 6 Crystal Autocentering. Percentage of total centering time spent performing various tasks for a 'difficult' centering case in which the loop was originally out of the camera's field of view, even at low magnification. Total centering time was 5' 36". The actual time spent processing the images is less than a second per image.

Development of user-friendly data collection environment

To provide a user friendly environment to the X6A user community the long-term goal is to pursue an automated, crystal in – structure out, approach. This goal will be achieved in several independent steps. In each step straight implementation of developments pursued and proven at other facilities or available as open source will be preferred.

The first step towards this goal, the installation of an automated sample changer has been completed. To increase the sample changer functionality a reliable liquid nitrogen fill system for the automounter needs to be implemented. Several designs in use at other facilities are being considered; although the most reliable unit seems to be the gravity feed system developed by the SER CAT group at the APS this solution may not be viable at the NSLS.

Further development of the BluIce/DCS graphical user interface and distributed control system software is required to allow users to take full advantage of the automounter capabilities. Several beam line hardware functionalities and the DCS database have to be modified and additional functionalities need be added to the data collection software.

The second step in this sequence is the integration of the automated sample changer, the diffractometer-detector data collection unit, and the beam line control unit into a single data collection platform. This step will require the integration of both the DCS database, the beam line control database and the user database. This step, a mid term goal, will be accomplished with the assistance of the NSLS Control and Detector group and collaborations with other facilities.

A third step, a long term goal, will allow for the automated usage of packages such as those being developed by the HKL2000, PHENIX or DNA projects, for the automatic determination of the three-dimensional structure of macromolecules. As software becomes available it will be integrated with the data collection environment. Data will be

automatically scaled and continuously transferred to appropriate software packages until a model is build.

CONCLUSION

Overall the Facility has matured over the last year as reflected by the technical enhancements for automated operation, the growth of its user community and increased PDB deposits and publications.