

LSBR activity report 2016-17

March 24, 2017

1 Introduction

The LSBR advisory team last met with us here at BNL in March of 2016, in the year since that meeting significant progress has been made in all aspects of the project: this work is described briefly here in this report. The diversity of the tasks accomplished, and the quality of the work performed is testament to the commitment of a large, talented group drawn principally from the LSBR program team but also from others with-in NSLS-II and BNL. We have benefited for the enthusiasm (and courage) of the scientists who have helped us through the first tentative steps taken toward routine operation of these beamlines. They, and we, recognize that the potential of these beamlines is great but that the work left is enormous! We hope that you will see from the report and the interaction you will have with the team that we are ready and able to perform this work.

The structural biology beamlines supported by the LSBR team, where built through the cooperation between NIH and DOE creating a project that was called ABBIX. This project is in the last stages of its existence, the program has delivered the base project scope and much more: this work is described in Section 2. Once construction of a beamline is over the transition to operation, through a commissioning phase, begins. In our case this also implied a transition from responsibility for these beamlines from the ABBIX construction team, to LSBR commissioning and operations team. We have worked closely to try to ensure that this transition and the changes in responsibility this entails has been as smooth as possible. For each of the three beamlines in the ABBIX program technical, scientific and administrative hurdles have been overcome. All three beamlines have achieved a degree of autonomy of operation that is allowing sophisticated experiments to take place, the status and planning is discussed for each of them below: for the LIX scattering beamline 16-ID in Section 3, the highly automated crystallography beamline AMX (17-ID-1) in Section 4, and for the frontier macromolecular crystallography beamline FMX (17-ID-2) in Section 5. In addition to these structural biology efforts the LSBR program supports the continued development of synchrotron imaging techniques for biological samples, significant progress has been made by this team in the last year and their work is described in Section 7.

In addition to the construction and commissioning of the beamlines we have been developing the infrastructure and methods to allow for the productive use of these instruments. With modern data rates the handling of data throughput is a significant challenge, subsequently making best use of these data has been the topic of a lot of work by LSBR and our collaborators. In addition to the specific examples discussed in the relevant beamline sections we highlight the work done and the challenges remaining in Sections 8, and 9.

The final part of our report covers the efforts we have put into supporting scientists who think of NSLS-II as their structural biology center. The first category is the successful program undertaken in the transition between NSLS operation and made possible by the gracious help of the SSRL management and structural biology group aided by the commitment made by our staff. The overview of this work is described in Section 10. The second element of this work has been enabling a responsive access mechanism adapted to the needs of the structural biology community. A number of initiatives have

been undertaken and are discussed briefly in Section 10, along with a summary of the growth of the program through the first cycles of operation.

2 The ABBIX project

Shortly before the previous Advisory Committee meeting held in March 2016, the LIX beamline upstream of its endstation had sustained technical commissioning, and the AMX and FMX beamlines had just attained their first light. Since then, the remaining technical commissioning of the beamlines was undertaken, and the remaining endstation apparatus on all three of AMX, FMX, and LIX were installed and commissioned. First scattering patterns from a calibration sample were measured at LIX by the end of March 2016, and from a protein in solution by the end of July 2016. First diffraction patterns from protein crystals were measured at AMX and FMX by the end of July 2016. Thereafter scientific commissioning of the three beamlines started, this was supported by external users who had submitted beam-time requests for this purpose. This mode of beamline development and operation continued until November 2016 at LIX, at which time the beamline was declared ready to support the general user science program. The counterpart milestone was achieved at AMX and FMX in February 2017.

Installation and testing of hardware at the beamlines that fell under the ABBIX Project scope were completed by the end of July 2016, except for one mirror for the FMX beamline. There followed the installation and testing of additional hardware to complete the experimental apparatus, under LSBR auspices rather than ABBIX. In regard to the one laggard component under the ABBIX Project scope, the horizontal Kirkpatrick-Baez focusing mirror for FMX, it suffered production and completion delays on the part of its manufacturer, SESO, working under a subcontract to RI, the principal contractor for the photon delivery systems of AMX and FMX. While its production continued during the fall of 2016, scientific commissioning of the FMX beamline, supported by users, was undertaken in its absence, with partial horizontal focusing achieved through suitable bending of an upstream horizontal focusing mirror (more detail below). The horizontal KB focusing mirror was delivered in December 2016 and installed in January 2017, thereby completing the formal ABBIX Project scope. Currently it is being commissioned in conjunction with the remaining FMX optical system, during planned intervals occurring around scheduled general user commitments. This commissioning is forecast to conclude by the end of March 2017.

Although the formal ABBIX Project scope has now finished, there remain leftover unassigned contingency funds that could be allocated toward appropriate beamline enhancement purposes, currently this avenue is under discussion with the NIH sponsor. Among the purposes under consideration include a possible replacement mirror for the current FMX horizontal KB focusing mirror capable of delivering a submicron horizontally focused beam at the sample position. Other options include enhanced computation equipment and detector-data buffer hardware that would allow us to take better advantage of the potential data rates at the beamlines.

3 The LiX beamline - status and perspective.

Solution scattering: LiX currently supports several flavors of solution-scattering measurements, using a flexible experimental module that can quickly switch between operation modes. Typically the module is setup for high-throughput measurements and in-line size-exclusion chromatography (SEC), sharing a 3-channel flow-through cell. This same setup can also accommodate anomalous solution measurements using a fluorescence detector, as we have explored with our DBP collaborator Karen Allen. Samples that do not flow easily can be preloaded into sample holders that accommodate multiple samples and mounted in place of the flow cells, as has been tested by Ben Hsiao's group from Stony

Brook University. We are also working with our DBP collaborator Osman Bilsel to bring in his flow mixer for time-resolved solution scattering.

Since August 2016, we have constructed two versions of the protein solution scattering experimental module. The earliest version had all the different types of sample cells in a single enclosure. The revised version sacrifices some convenience, by requiring only one cell to be installed at a time. However doing this makes it possible to establish a helium environment around the sample, resulting in lower scattering background and better data quality SAXS. To further the development of our automation we are in the process of implementing robotic sample handling. An initial design of the robot support has been completed and reviewed by NSLS-II safety staff. Our goal is eventually to have automated, unattended overnight operations, when staff and users are not at the beamline.

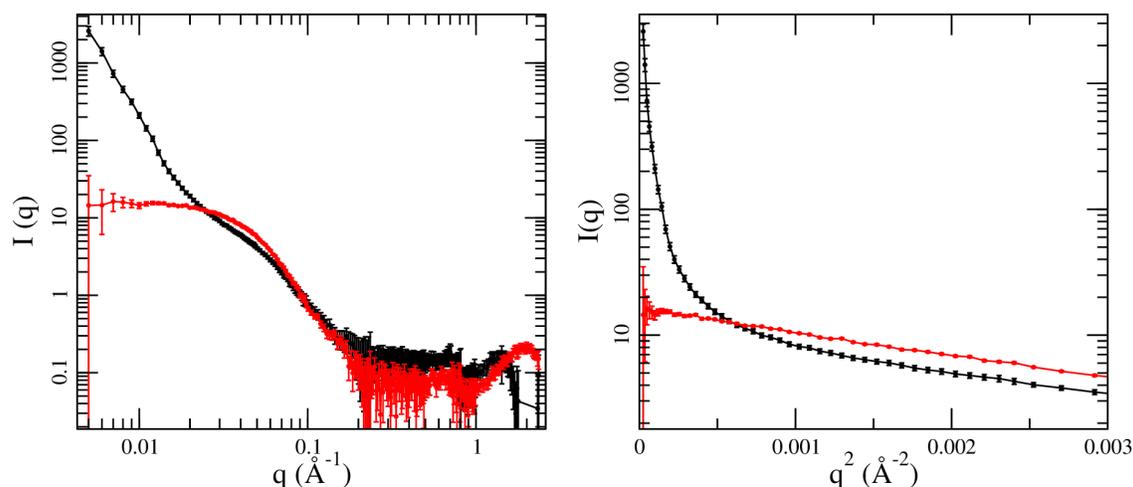


Figure 1: A comparison of solution scattering data from the same sample, collected without (black) and with (red) in-line purification. The scattering data in the full q -range are shown on the left and the corresponding Guinier plot on the right. The Hubbard group from NYU made a decision at the beamline to make use of the purification system, which removed the aggregates nicely and made the experiment successful.

In-line size-exclusion chromatography (SEC) is an important capability for our solution-scattering users, to which they can switch whenever sample purity is not up-to-standard (Figure 1). We are encouraging our users to bring in their own purification columns and are helping them plan their experiments by taking into account factors like sample dilution during the purification process.

Scanning imaging: The high brightness of the NSLS-II source makes it possible for us to perform scattering measurements on highly heterogeneous samples using small beams. We can routinely achieve a beam size of 5 micron cross-section while maintaining the same range of scattering vectors as we can achieve for solution scattering. Smaller beam sizes are possible, at the cost of lowered beam intensity.

So far our users have used microbeams to study plant tissues. The scattering intensity measured at small and wide angles provide two separate contrast mechanisms for imaging, corresponding to different length scales being probed (Figure 2). We are also exploring measuring the same tissue section at multiple angles, to reveal the orientation of periodic structures like cellulose fiber within the tissues. We have upcoming experiments to use microbeam to study other tissues as well, including the experiment by our DBP collaborator Lee Makowski on brain tissues with Alzheimer's disease.

We have developed python scripts for basic data collection and data processing. These scripts are adequate for carry out the solution scattering and microbeam imaging measurements. For solution

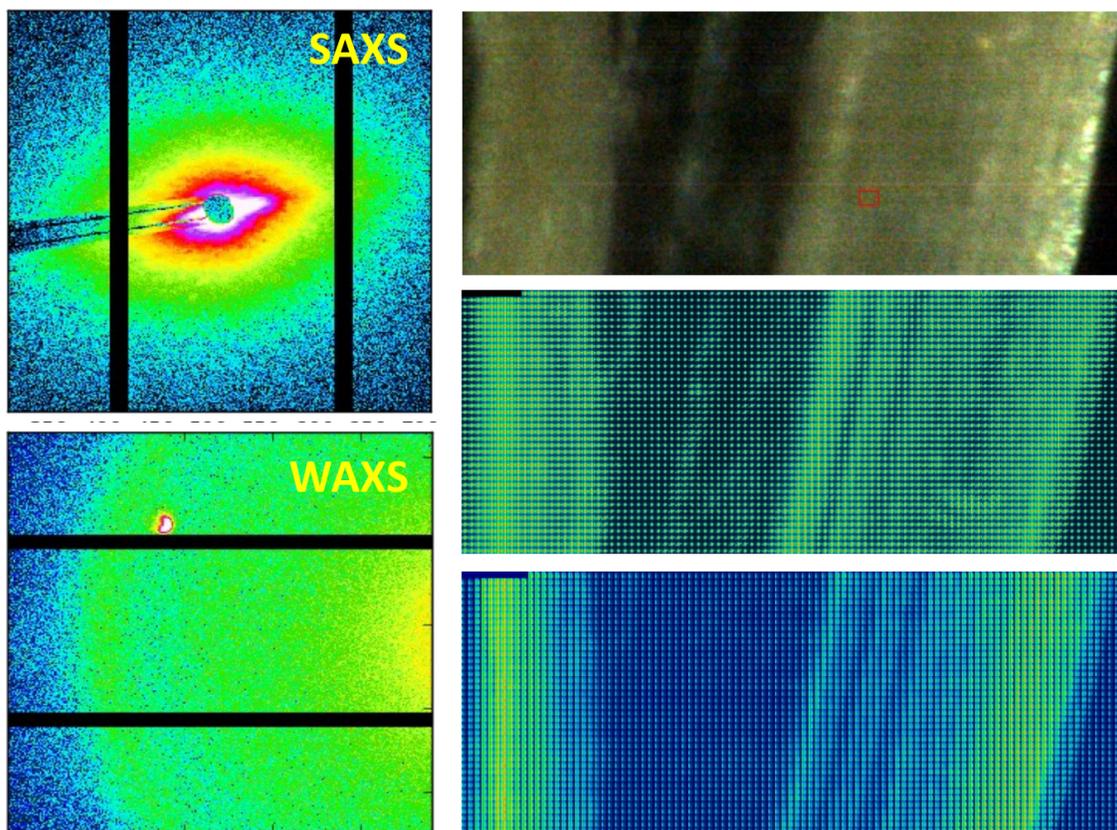


Figure 2: Representative scattering patterns and the mosaic composite from a plant section from Lee Makowski's group, together with the corresponding optical image from the on-axis microscope. The field of view is $\sim 0.5\text{mm} \times 0.2\text{mm}$.

scattering, the scripts can reduce the data into a format that users can analyze with well-known software. For scattering imaging, the scripts produce mosaic composites of scattering patterns like those in Figure 2. Extracting additional information requires programming using a python library we have developed. A major challenge we are facing now is to provide graphical user interfaces for users who are not comfortable with doing things in the “expert mode”. Unfortunately we lost the programmer who helped us develop the current scripts. We are working with other scattering beamlines at NSLS-II to continue improve software capabilities for data collection and processing.

In February 2017 we started accepting rapid-access proposals, so that users can request beam time within the same beam cycle. We are currently working with the BioSANS beamline at the HFIR neutron source at Oak Ridge to start a joint SAXS/SANS program, to give users access to both SAXS and SANS time through a single proposal. The first group of joint users are expected to run their experiments in March 2017.

4 The AMX beamline - status and perspective

Following the detection of first light at AMX and FMX in March 2016, commissioning of the beamlines progressed largely in parallel with interleaved periods of technical commissioning and an increasing number of user-assisted scientific commissioning sessions. This work, together with the gradual buildup of the experimental station apparatus, advanced the beamlines capabilities sufficiently to be-

gin general user operations in February 2017 in a third of the available beam time in the current spring operating cycle of NSLS-II. The general user available time will increase to half of the available beam time in the summer operating cycle being committed to supporting the scientific proposals received to date.

Experience gained from performing data collections with academic users remains a key driver in guiding the continuing development and testing of the AMX experimental methods. Much work on the automatic alignment of the AMX beamline optics, the experimental station instrumentation, particularly the sample-changing robotics (see following sections and Figure 9), and software for data collection and analysis, remains to be addressed in the months ahead before a full schedule of user operation can be adopted.

These are technical developments since first light: realignment of the double crystal monochromator (DCM) into the beam centerline; the calibration of beam-diagnostic screens and beam-position monitors; and the integration of the detector table into the controls system. This all accompanies the increasing completion of the experimental station: the addition of a diagnostic screen, through-beam counter, and fluorescence detector. Numerous improvements to the infrastructure were also carried out including a distribution system of liquid nitrogen at atmospheric pressure to hutches and a sample-preparation bench, and the creation of beamline-control work stations and areas.

The hot commissioning of AMX was successfully completed in November 2016 with the lead engineer from RI Research Instruments, the contractor for the photon delivery systems of AMX and FMX. At the synchrotron electron current of 250 mA pencil beams were scanned across the Kirkpatrick-Baez (KB) focusing mirrors and analyzed with software developed by the NSLS-II metrology group to optimize the voltage vectors applied to the side-attached piezo actuators of the adaptive optics mirrors. A clean focal spot of $6.6 \mu\text{m}$ (h) \times $4.8 \mu\text{m}$ (v) was readily and reproducibly obtained (Figure 3), which is close to the focal spot size of $5 \mu\text{m} \times 4 \mu\text{m}$ expected from ray-tracing models at the eventual ring emittance at 500mA. The flux too was measured at $3.5 \cdot 10^{12}$ ph/s to be close to expectations ($\sim 10^{13}$ at 500 mA). In addition, extensive beam-position stability measurements were analyzed and correlated with vibrations originating from the DCM, the tandem side-deflecting mirrors, and the KB mirrors, each presumably stimulated by ambient noise from floor and machinery.

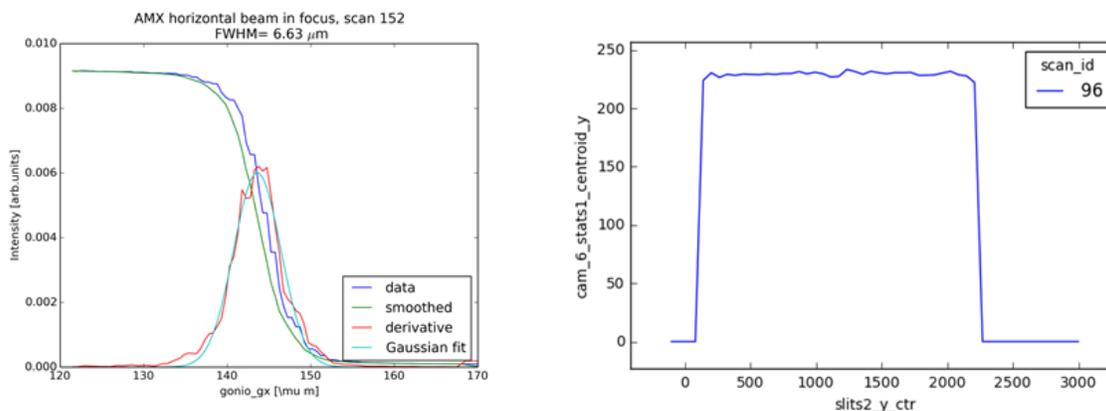


Figure 3: Left: Knife edge scan of the width of the AMX native focus. Right: Pencil beam scan across the vertically focusing KB mirror of showing that all beamlets converge to the height of the focal spot.

The Eiger X 9M pixel array detector, delivered in December, was commissioned with a Dectris engineer in January (Figure 4). Right away, its framing rate of up to 238 Hz made it possible for the first time to collect data at full flux; with the Pilatus 6M, which served during preceding commissioning runs, the beam always had to attenuated tenfold to match its 25 Hz framing rate. The Eiger 9M's maximal fram-

ing rate of 750 Hz in its 4M region-of-interest was also demonstrated to sustain data collections with 1.3 ms exposure times for more than 15k images stored locally (twice the specified sustained volume) and promptly dispatched over the recently upgraded network at 40 Gb/s to the GPFS store on the NSLS-II cluster.

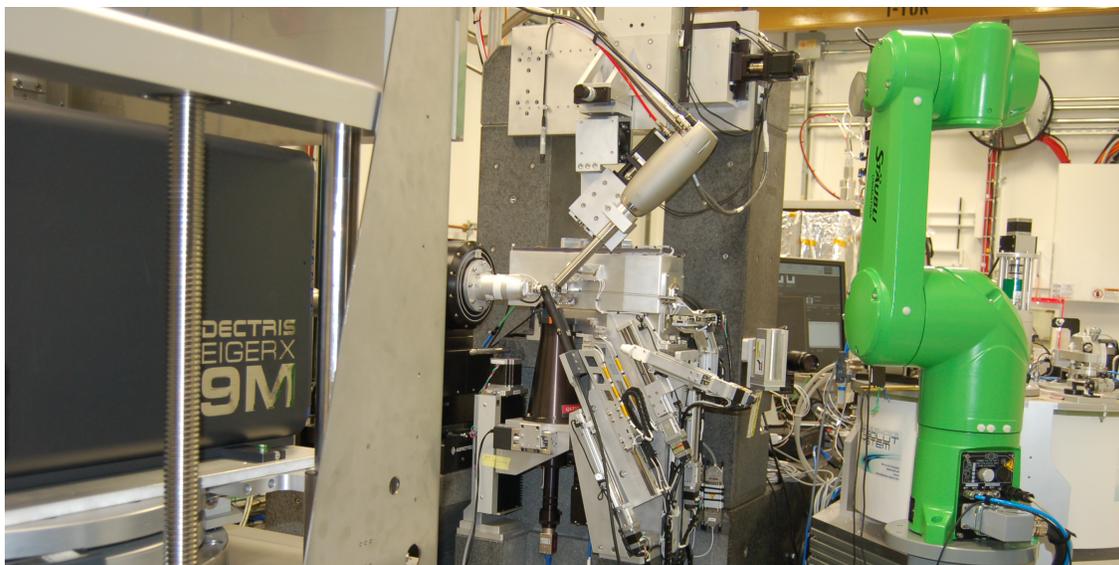


Figure 4: The AMX Eiger X 9M pixel array detector, experimental station, and sample changing robot and Dewar.

The fifteen groups who collected data at AMX in January and February all took advantage of the new detector, the small high-flux beam - and surprising to most - the very fast data collections of typically less than 5s. In many cases the small AMX beam could reveal by raster scans the domain in a larger crystal, or the one crystal, in loops containing several, that yielded the best diffraction. Followed by full flux collections this led to the determination of several new structures derived from the higher resolution of diffraction that was previously washed out on beamlines using larger beams. The on-line fastDP data reduction pipeline (and variants developed in part with Dr. Y. Yamada, a sabbatical visitor) were equally important to let experimenters promptly assess the quality of each collection.

Essential to realize fully the potential AMX affords as the premiere MX beamline for high quality data collection is a sustained development effort. Beam position stability must be improved by taking advantage of closed-loop beam steering directed by beam position monitors. Also possible might be feedback of optics-angle measurements available through existing high resolution encoders. Vibration insulation, temperature stabilization, and traffic redirection must also be considered.

Automatic beam alignment must be implemented also to make energy changes easy. Although the throughput of AMX will increase dramatically once the sample-changing robotics is unleashed (see automation Section 6), additional measures are needed including the automatic centering of loop and crystal, or for opaque specimens the automatic execution of rapid raster scans. When fully tested, and complemented by judicious streamlining of the beamline control and its tight integration with the existing data reduction pipeline, remote participation data collections can be entertained. Achieving these goals without compromising the quality of the data that can be collected at AMX will establish AMX as the best beamline to attempt structures of novel molecular systems of biomedical importance. We welcome this challenge.

5 The FMX beamline - status and perspective

After taking first light on March 8, 2016, FMX took a first room-temperature diffraction pattern on July 15, and hosted the first external users visited on August 18.

Photon delivery system:

Initial technical commissioning was conducted using only the first horizontal focusing mirror (HFM), since the horizontal Kirkpatrick Baez mirror (HKB) that would provide second-stage focusing was delivered only in December 2016. Focusing with the HFM and the vertical KB mirror (VKB) provided a $100 \text{ \AA} \times 10 \text{ \mu m}^2$ (H \times V) focal spot, which could be reduced to $20 \text{ \AA} \times 10 \text{ \mu m}^2$ using beam-conditioning slits. These specifications were the basis for all scientific commissioning experiments in the 2016-03 cycle (see below).

In-house metrology of the HKB mirror revealed that it does not meet the 0.2 \mu rad slope error specifications but was measured to $\sim 0.6 \text{ \mu rad}$. It was installed in January 2017. Commissioning of this and the other two focusing mirrors continues, including optimization of the bimorph piezo actuators via in-situ pencil-beam metrology. The initial measurement of the focal spot size before optimization was $\sim 10 \times 10 \text{ \mu m}^2$. Hot commissioning of the photon delivery system is scheduled for the week of March 20, 2017.

We have been researching alternatives for the horizontal KB mirror. The most promising replacement is a super-polished fixed ellipse mirror, which would fit into the existing mirror frame, and locate focusing control exclusively with the first horizontal focusing stage, the HFM.

Core science drivers and commissioning priorities:

When FMX reaches its target specifications, a unique combination of three properties will distinguish it - a small beam size of 1 \mu m , its high flux density, and the maximum photon energy of 30 keV.

The target beam size of 1 \mu m will make it possible to collect datasets from crystals down to $< 1 \text{ \mu m}$. High profile molecular targets like amyloid crystals are rarely larger than 1 \mu m , and FMX would be able to compete with micro electron diffraction methods to obtain structures. Membrane protein crystals from GPCRs are often in the size range of few microns and the FMX beam would be optimally matched to this. The opaque LCP buffer commonly used to grow these crystals makes rapid raster scanning and high throughput diffraction analysis a high commissioning priority. Achieving the beam size and stability for a 1 \mu m focus requires further efforts on beam diagnostics and position feedback, as well as a thorough assessment and subsequent elimination of vibration sources.

The unparalleled dose rate of up to $5 \times 10^{12} \text{ ph/s/m}^2$ at the Selenium K-edge at full target specifications will be of greatest benefit for three types of experiments. Firstly, for serial crystallographic measurements it will greatly reduce the data-collection times from hours to minutes. Secondly, it will enable time-resolved measurements with milli-second resolution. Lastly, previous experiments hint at the possibility to reduce radiation damage effects, and the achievable dose rates at FMX will allow one to verify and quantify the extent of this reduction. The expected dose rates require further efforts in sample positioning and handling (FY16 LDRD), as well as in detector readout and data processing.

The maximum photon energy of 30 keV will enable one to establish the extent of radiation damage reduction by MX measurements with high-energy photons. The initial results will give an idea of the opportunities of data collection at high energy, which will require a detector with better sensitivity at these energies to realize the full potential.

Experimental station construction:

Installation of the endstations' sub-components was staggered to optimize the support of the technical commissioning of the x-ray optics and the comprehensive radiation surveys. Following this the first diffraction experiments at room- and then at cryo temperature, and eventually the scientific commissioning experiments with external users were performed.

The current focus of endstation commissioning at FMX is on the integration of and interaction with the sample-mounting robot, the extension of raster and vector scanning capabilities (hardware triggering to synchronize the detector with crystal motion), and on completing the full beam conditioning diagnostics with active beam-position feedback. After mapping the undulator and monochromator settings of all important anomalous scattering edges, the XRF detector and edge scanning is fully functional, and is currently being integrated into the endstation. Prioritization of data quality and ease of use over secondary functionality such as the secondary goniometer and multi-axis goniometer.

With the goal to develop a high-speed and high-precision goniometer and sample supports to enable scanning serial crystallography at the full intended dose rate of FMX, we were granted an LDRD project in FY16 - "Serial microcrystallography at full flux". Yuan Gao joined this effort as a postdoc from Argonne National Lab in May 2016. He is developing a coarse/fine XYZ piezo scanner for high-speed high-precision scanning serial crystallography (PI Physik Instrumente) and designs and fabricates structured Si-wafer sample holders via nanofabrication at the BNL Center for Nanofabrication.

User Operation Summary

Scientific commissioning: In 2016, FMX has hosted a total of 13 research groups from 12 institutions. Four groups assisted the technical beamline commissioning; nine groups came to collect data with approved scientific-commissioning proposals.

Highlights included large protein complexes like 70S ribosomes with very large unit cells (PI: Jogle, Brown University) (Figure 5), membrane transporter proteins with small crystals and weak diffraction (PI: Wang, NYU; PI: Liu, BNL), and first serial microcrystallography experiments (PI: Liu, BNL) (Figure 6).

Membrane proteins tend to crystallize in micron-size crystals with weak diffraction, anisotropy, radiation sensitivity, and other problems. Dr. Wang's (also a DBP) group from NYU came to visit in Dec 2016 and screened a number of crystals of a bacterial carboxylate transporter and found one condition that produced single diffraction patterns and reasonable merged data to $\sim 4.0 \text{ \AA}$. They are planning more visits in the near future to continue on this project.

FMX received approval for General User operation in Feb 2017. As of end of Feb 2017, four user groups with general user proposals or Blocked Allocation Group (BAG) proposals have visited FMX. These included a virus crystal structure with a unit cell of $598 \times 598 \times 465 \text{ \AA}^3$, with crystals extremely sensitive to radiation damage (PI: Joshua-Tor, CSHL). Partial datasets were collected and will be used to merge with previous partial data to increase completeness.

6 Automation for Structural Biology

Over the last grant year, we have completed the commissioning and testing of one integrated system at the FMX beamline. This system is made of the Staubli TX60 robot arm, the AbsolutSystem high capacity sample changer holding up to 24 Unipucks, the new NSLS-II gripper supplemented by a commercial machine vision, a set of proximity sensors, and a force/torque sensor. This complete system, initially tested in the LOB-1 lab, was moved during the December shutdown at the beamline (Figure 7).

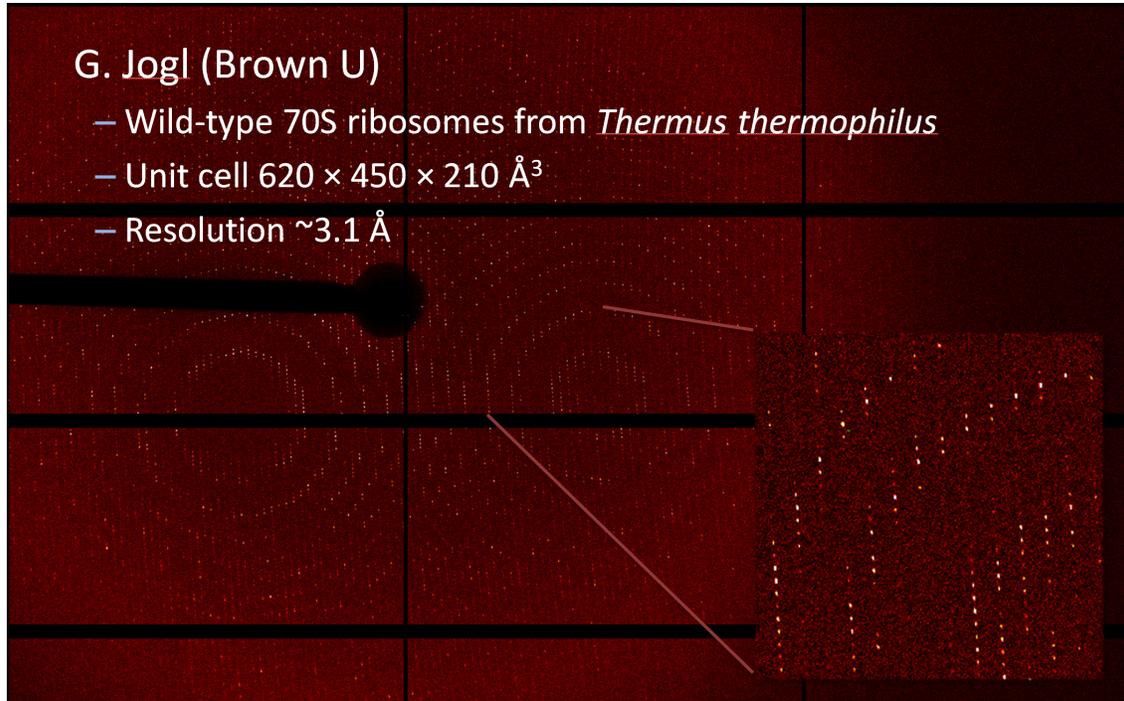
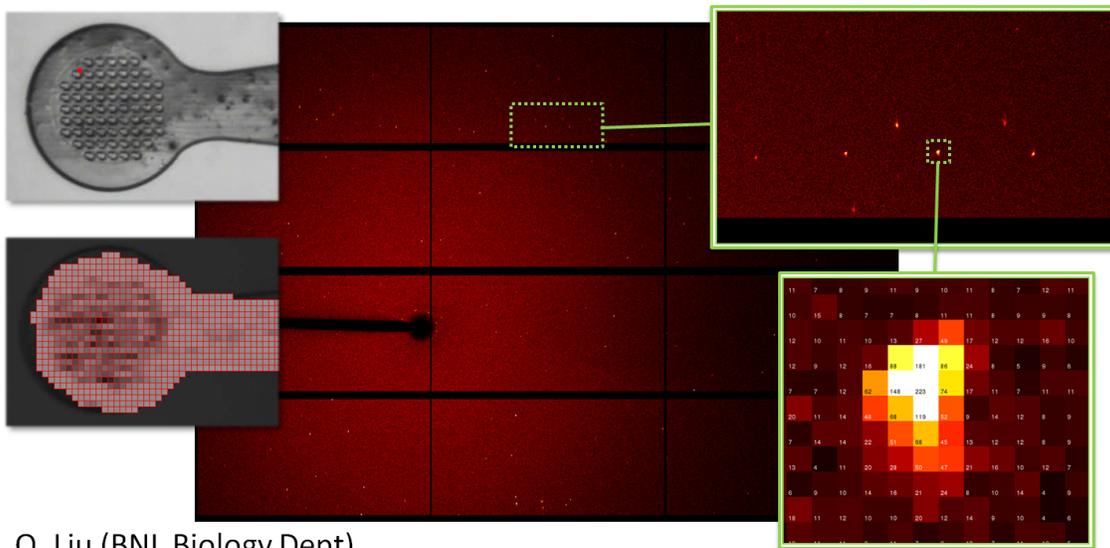


Figure 5: The 70S ribosome crystals diffracted to $\sim 3.0 \text{ \AA}$ with unit cell dimensions of $209 \times 449 \times 621 \text{ \AA}^3$. Data were collected using fine phi slicing (0.1 deg) and high redundancy (360 deg), and were successfully processed with XDS to 3.1 \AA with overall R_{pim} of 11%



Q. Liu (BNL Biology Dept)

- Developing raster data collection
- Serial microcrystallography of 1-5 μm size Thaumatococcus crystals

Figure 6: First experiments with raster based serial crystallography.

End positions and trajectories were optimized for the beamline at room temperature; motion speed was gradually increased to optimized level taking into account equipment safety and sample integrity.

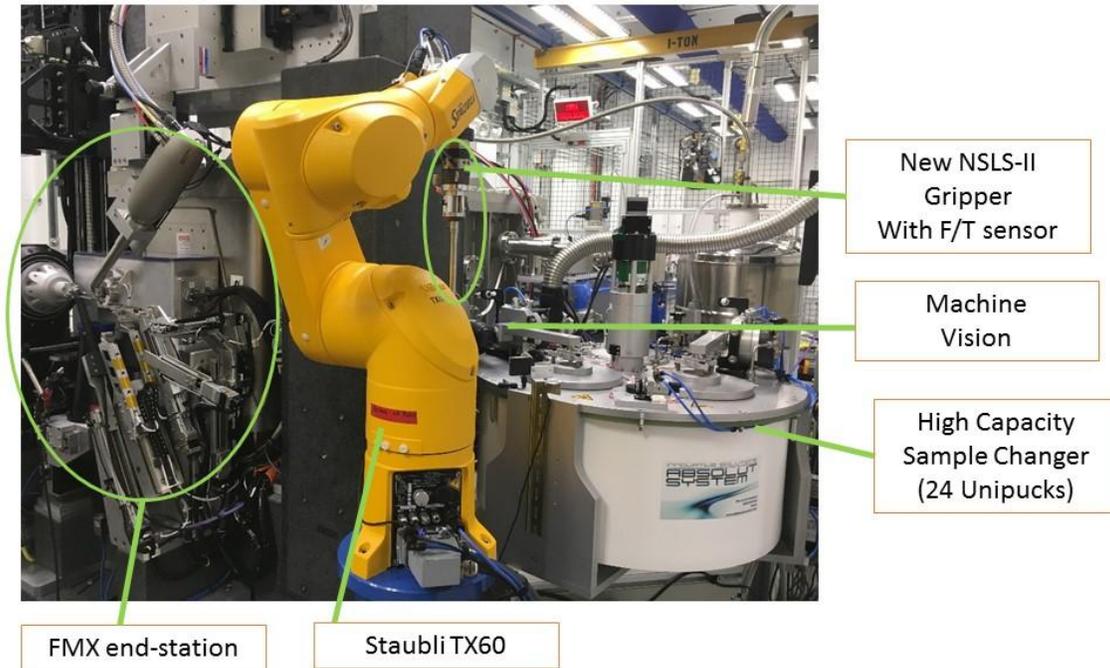
Automated Sample Changer at FMX (03/01/2017)

Figure 7: Installation of the completed automated sample changer on FMX. This system is currently in operation for users with a capacity of 3 pucks.

The transition to cold temperature was performed and extensive tests were performed, ramping up the robot speed and tweaking parameters in order to achieve a total sample exchange time lower than 1 minute (Figure 8).

Once a reasonable sample exchange time was achieved, the robot was tested for a few days in a row with test protein crystals that were examined by X-ray for quality control. We concluded that the system was ready for user operation under specific conditions. As a result, the FMX robotic sample changer was utilized by two BAG groups, and feedback from users was positive. We are currently limiting the number of puck positions available to 3 pucks, this was decided so we could perform parallel development for the second system at AMX. During last grant year, the second system, to be installed at AMX, was completed and individual components were tested in the LOB1 lab. It was moved to the beamline in January of 2017; the positions were refined and finalized in early March of 2017 (Figure 9). Teaching of the end-positions has begun and the system will be extensively tested in March of 2017. We will follow the successful protocol that was used for FMX. Since, all the individual steps are identical and the only parameters that are different are end-positions and trajectory, integration should be relatively fast. We are currently anticipating that the robot will be available to users in April of 2017.

Once the two systems are in use with users, we will work on required improvements to be deployed during the following cycle. In order to operate these two systems non-stop for several days in the row, we are currently working on three projects:

- The addition of an extra gripper (per beamline), so that they can be swapped if needed.

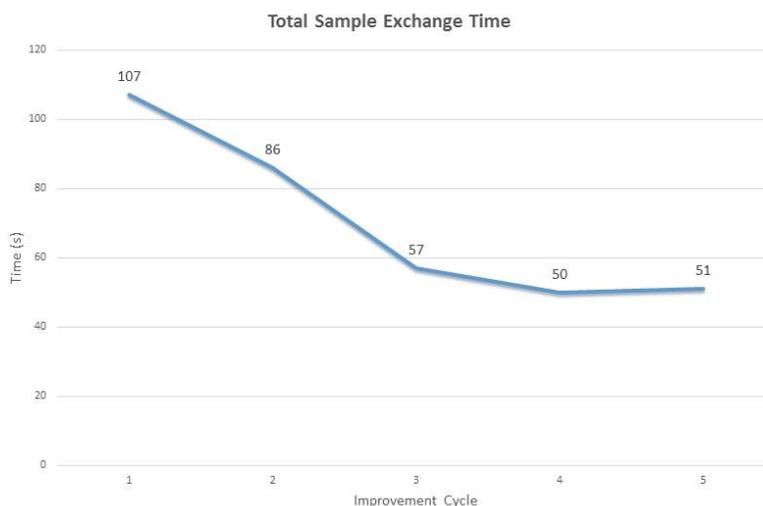


Figure 8: Progressive improvement of the total amount of time required for a sample exchange (defined as time it takes to execute the sample exchange command to moment the user can center the next sample)

- The implementation of a gripper “de-icer” that will remove ice forming on the outer shell of the gripper
- The replacement of the sample-loading port lid with a new lid that will prevent frost buildup on the lid during very long operation.

7 Imaging Opportunities within NSLS-II

A primary goal of LSBR is to enable an integrated understanding of isolated atomic-resolution macromolecular structures, multi-subunit assemblies and their dynamics, and nanoscale structural and chemical information within the context of the cell, by providing a coordinated suite of synchrotron (and other) characterization techniques to bioscience researchers. At NSLS-II, we are developing imaging beamlines that provide “tunable” spatial resolution and a range of contrast mechanisms in order to image both chemical and structural information down to the nanoscale. The primary goal of the imaging program in LSBR is to develop technology for X-ray imaging of frozen, hydrated, biological cells and tissues in their native state such that *both chemical and structural information* are obtained with nanoscale resolution and sub-micromolar elemental detection sensitivity in 3D. Specifically, we aim to (1) develop methods for imaging cryogenically-fixed cells and tissues in their natural state, (2) incorporate established light-microscopy approaches for sample visualization, and (3) develop software for image analysis, multi-technique image fusion, and statistics.

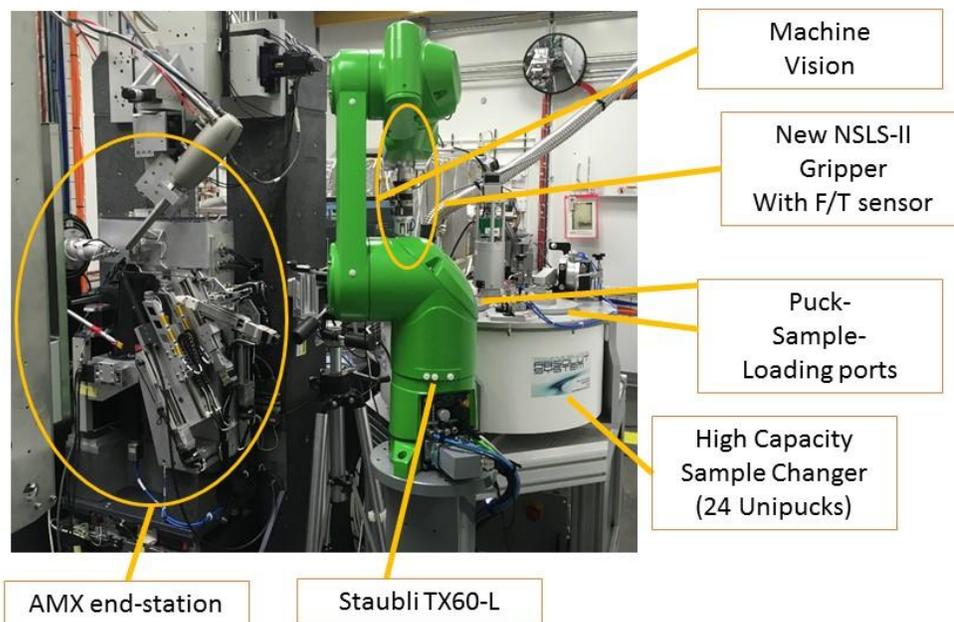
Automated Sample Changer at AMX (03/01/2017)

Figure 9: Installation of the completed automated sample changer on AMX. This system is currently being commissioned at the beamline. Robotic sample changer on AMX will begin user operation in April 2017.

Summary of progress in Year 3:

During this year, we have continued to develop Aim 1 by designing a new and improved version of our cryostage. The new cryostage has a slimmer profile and can be operated in vacuum or purged. But the significant improvement is the integration of a “door” in the top of the cryostage that enables rapid cold transfer of the sample. We also designed and built a series of sample-transfer tools that can be used with sample holders from multiple APS and NSLS-II beamlines. Lastly, we designed and built a cryostage mount for our new Nikon optical microscope so that the frozen samples can be viewed and photographed prior to mounting at the beamline. The new cryostage was installed and tested at the Sub-micron Resolution X-ray spectroscopy (SRX) beamline at NSLS-II during the summer of 2016 along with continued testing at APS beamline 13-IDE. Results showed that radiation damage is species-specific and dependent on incident photon flux density, and the new cryostage was able to limit radiation damage in even the most radiation-sensitive samples at APS but was less successful at (the more intense) SRX.

We have also made significant progress on Aim 2. Specifically, we designed a motorized visible light microscopy (VLM) accessory for the SRX beamline in order to vastly improve sample visualization at the beamline. The new VLM utilizes long working distance objectives in a normal (90 geometry) with both front and back illumination. Importantly, it incorporates standard fluorescence filter cubes for viewing natural fluorescence or fluorescent labels/tags in the biological samples. The VLM is compatible with

the cryostage and was also tested at the beamline during the summer of 2016.

Redesign of the cryostage, plunge-freezing, and rapid cold-transfer of the sample.

In Years 1 and 2 of the grant, we developed a cryostage that is compatible with X-ray Fluorescence Microscopy (XFM). We started with a commercial Linkam Scientific cryostage (Model FDCS196) and completely redesigned its housing to be compatible with XFM and XRD. This stage has an excellent cold head (Model FDCSB), controller (Model T95), and cooling pump (Model LPN95) that are needed for maintaining temperatures lower than -150 C to minimize sample radiation damage. The modified housing has an aluminum frame that was designed to hold vacuum or dry purge gas. It has large front and back kapton or ultralene windows to capture both a large fluorescence angle from the front (>45 degrees) and X-ray diffraction signal from the back (>60 degrees²). Samples are mounted on silicon nitride windows that are attached to an aluminum sample stick that is compatible with beamlines at NSLS and APS. The Al stick is cantilevered above the cold head to prevent large background fluorescence from the cooling surface itself.

In Year 3, the cryostage design was refined. It has a slimmer profile, which makes it easier to fit into the beamline space. But most importantly, the design has a slot at the top to allow transfer of a frozen sample into the cryostage at cryogenic temperatures.

The cryostage can then be placed in the VLM for viewing and photographing the frozen sample and then transferred to the beamline. Hence, with this design, the sample can stay frozen from the plunge freezer to the beamline.

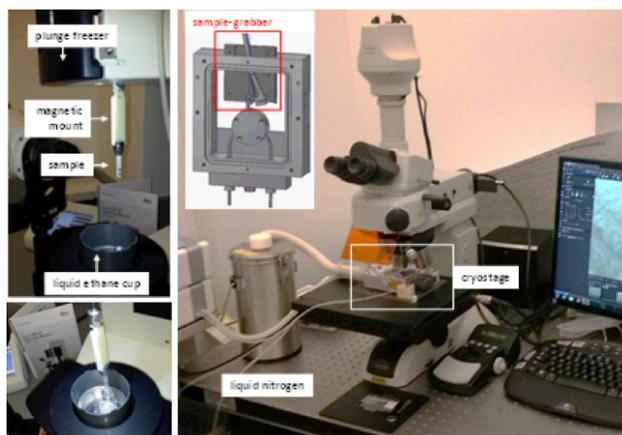


Figure 10: Cryostage and cold-transfer tools. (Top, left) Leica plunge freezer modified for freezing samples on silicon nitride windows. A magnetic mount was designed to hold and plunge the Al sample stick containing silicon nitride windows. (Bottom, left) The Al stick is plunged into liquid ethane that is kept frozen in a liquid nitrogen bath. (Top, center) Once the sample stick is plunge frozen, a sample grabber was designed to transfer the sample to the cryostage. (Right) The cryostage mounted on the Nikon microscope stage for photographing frozen samples.

In Year 3, we also developed all the tools necessary for plunge-freezing and cold-transfer of the specimen into the cryostage (Figure 10). In Year 2, we purchased a Leica Plunge Freezer (Model EM GP). We designed and built a 'gripper' for the plunge freezer that holds the Al stick in preparation for plunging. We also had to redesign the plunge cup that holds the liquid ethane to make it deeper to accommodate our silicon nitride windows. We also designed and built a modified lid to the standard cryovials for storing frozen samples until they are ready to be mounted in the cryostage. Our use of standard cryovials

allows the samples to be snapped into cryocanes and shipped/stored in dry shippers. Our goal was to minimize the number of adaptations and to make them widely compatible with commercial tools.

Design of a Visible Light Microscope (VLM) Accessory for the SRX beamline.

The current in-line VLM at SRX is inadequate for viewing biological samples for several reasons. First, the camera is monochrome and does not have epifluorescence capabilities, which prohibit viewing sample stains or labels. Second, it has a low magnification, which is insufficient to view small internal structures in complex biological materials without a digital zoom. Third, when the sample and detector are oriented at 45 and 90 to the incident beam, the optimal configuration for fluorescence data collection, the in-line camera is not normal (90) to the sample. Hence, our team designed and built a new VLM for SRX that was compatible for biological samples and the cryostage, which can be seen in Figure 11.

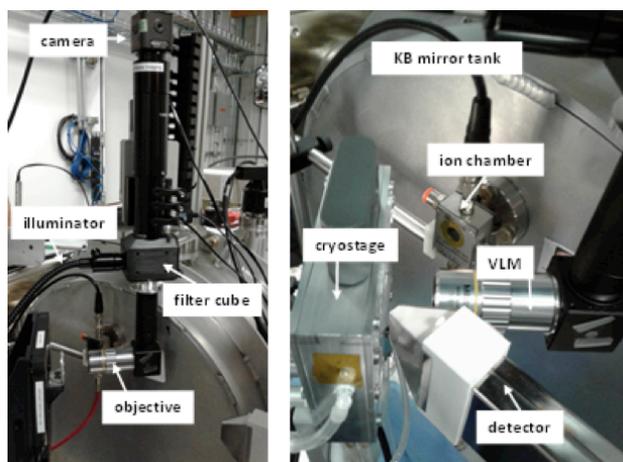


Figure 11: Left) Photo of the entire VLM assembly installed at SRX showing the camera, filter cube, long-working-distance objective, and illuminator. Both front and back illumination are possible. (Right) Zoomed-in view of the sample environment for biological samples, showing the cryostage, VLM and detector. The working distance from the KB mirror tank to the sample is 12 cm, making design of this setup challenging.

The VLM is equipped with a 10x Mitutoyo Plan Apo Infinity Corrected Long Working Distance Objective, providing a focal distance of 33.5 mm. Starting with a Navitar MTL tube lens imaging system w/ filter cube holder and video camera, we then extended the objective in a periscope configuration using Thorlabs components (lens tubes and modified right angle mirror cube) to fit into the tight space between the SRX KB mirror vacuum chamber and the x-ray focal plan (Figure 11). The VLM was then mounted to a motorized XY stage (Newport MFA-PPD) for aligning to the X-ray focus point. The VLM incorporates conventional Chroma epifluorescence filter cubes that are easily interchangeable. For the experiments described below, we used a wide-band blue filter cube. It has both a visible and UV fiber optic light source for front illumination of the sample.

The VLM was installed at the SRX beamline in the summer of 2016. Its low profile was important for the short working distance of the beamline. Results showed that it provided a dramatic improvement in the image quality of the sample and also allowed one to visualize fluorescent tags in the samples. Some examples are shown in Figure 12.

Scientific highlights and future plans

During the summer of 2016, we had beam time on the SRX beamline at NSLS-II to use the cryostage to study the speciation of Cu ions in amyloid plaques embedded within brain vessel walls from a mouse model of Cerebral Amyloid Angiopathy (CAA). This work is being done in collaboration with Professors Bill Van Nostrand and Steve Smith (Stony Brook University); it was recently funded by a new R01 grant from NIH in 2016 [co-PIs: Van Nostrand, Smith, Miller]. Similar to Alzheimer's disease, previous studies by our group have shown that amyloid plaques in CAA accumulate a high concentration of Cu (up to 100x higher than physiological). It is currently unclear how the Cu becomes bound to the amyloid, and whether it plays a role in neurodegeneration. We hypothesize that labile Cu^{2+} is present in the disease due to improper Cu transport across the vessel wall and binding to amyloid beta results in a reduction to Cu^+ and the production of free radicals and/or peroxide that is toxic to the surrounding neurons. At SRX, we examined the Cu speciation within the vessel walls in order to test this hypothesis. Without cryo protection, the Cu^{2+} would immediately be reduced to Cu^+ upon illumination with the focused X-ray beam.

For these experiments, we used the cryostage and VLM as shown in Figure 11. Initial experiments were performed on vessels isolated from the brain, deposited on 4 micron-thick ultralene film, and dried. The tissue was stained with a green fluorescent dye, Thioflavin T, which binds to amyloid. The visible and epifluorescence images of one vessel can be seen in Figure 12. Hence the bright green areas in Figure 12B represent areas of amyloid plaque in the vessel. A Cu XRF image of the same area can be seen in Figure 12C. As can be seen, the amyloid plaques are co-localized with regions of elevated Cu concentration. It is those areas in which we are interested in performing XANES spectroscopy to measure the chemical form (valence) of copper.

In a follow-up experiment, hydrated vessels were mounted on a silicon nitride window and flash frozen. The frozen, hydrated vessels were mounted in the cryostage at SRX and XRF data collection was performed. We found that, in the current step-scan mode for sample scanning at SRX, the incident flux of the SRX beam (estimated to be 10^{13} photons/sec with $\sim 0.7 \mu\text{m}$ beam) was too intense for the sample. Since the sample area was in the beam for 1-2 second / pixel for XRF mapping and >10 minutes per point for XANES, the cryostage did not have sufficient capacity to keep the sample frozen. Local melting and radiation damage occurred. While this result was disappointing, we recognize that implementation of fly-scanning at SRX will reduce the sample exposure from seconds to milliseconds without any loss in data quality. This beamline improvement is expected in the summer of 2017. In addition, we have received beam time at APS beamline 13-ID for similar experiments, which will also be run in the summer of 2017. While the APS beam size is slightly larger ($1-2 \mu\text{m}$), the flux is lower (10^{12} photons/sec), which we know has been successful at prior beam times.

8 Dealing with data.

During last grant year we have upgraded the network at AMX to a 40 Gb/s line to deal with the successful and rapid integration of the Eiger 9M detector. This detector can run at 238 Hz in the 9M mode and at 750 Hz in the 4M ROI mode. We have tested both modes during the detector commissioning and during user operation. Thus AMX and FMX are now equipped with state of the art detectors capable of data rates as high as 2 GB/s or even more. As a consequence, we are confident that the network we have in place will be sufficient to deal with data rate for the years ahead.

The NSLS-II computing facility hosts "our" medium term storage, a ~ 1 PB GPFS disk farm made of 180 spinning drives to eventually be used by all beamlines accessing this facility. Initial tests with the two beamlines operating at the same time offered sufficient insights that we have ordered a dedicated SSD buffer disk with ~ 20 TB capacity and sufficient IO performance to deal with not only writing the

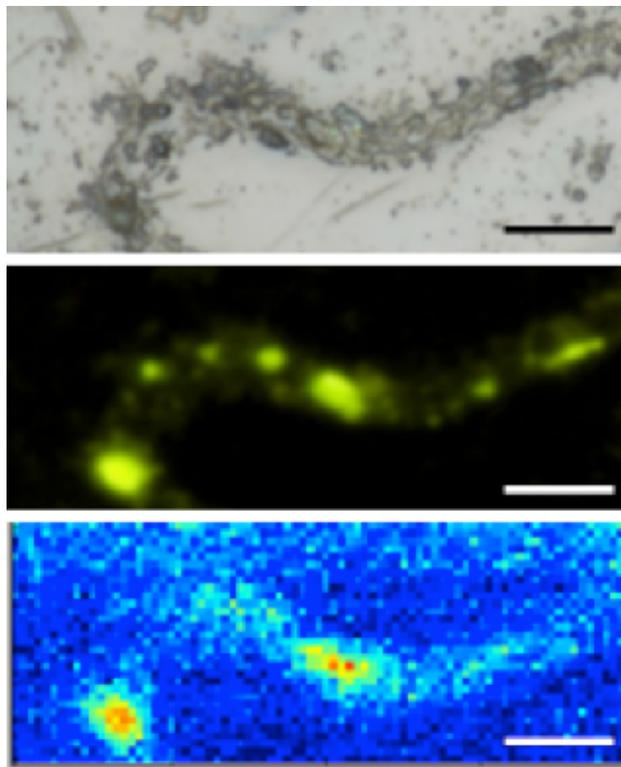


Figure 12: (Top) Visible, (Center) green epifluorescence, and (Bottom) Cu X-ray fluorescence images of a brain vessel from a mouse model of cerebral amyloid angiopathy (CAA). With the new VLM installed at SRX, the green fluorescence from the amyloid tag is visible. Results showed a strong co-localization of the amyloid and high Cu content in the vessel.

data from one beamline but reading the data multiple times. Once demonstrated (April / May), we will acquire a second system, so that each MX beamline will have its own dedicated buffer, starting during the next cycle. Nevertheless, we made significant progress using the existing disk and CPUs. We currently utilize eight dedicated nodes, four high-core nodes and four fast-core nodes; the high-core nodes are used for the initial and demanding computing steps: spot finding for efficient and fast crystal centering using X-ray diffraction, and data reduction to rapidly provide feedback such as completeness, CC, Rmerge, and resolution. In our current implementation, each beamline has two dedicated high-core nodes available for automated spot finding and data reduction that are launched automatically from LSDC. The four other nodes, fast-core nodes, are to be used for all other computing-demanding tasks, such as MR, SAD phasing, or dedicated pipelines.

At the moment, each beamline is fitted with three workstations capable of performing some data processing. One is dedicated to data collection and two to data processing. Most crystallographic packages, including GUI versions (HKL2000, ccp4i, phenix, and other) are locally installed and can be executed by users or staff. We also installed Eiger-compatible versions of “labelit” and “best” on these workstations that allow users to calculate a strategy based on data already collected and processed. This was a collaborative effort between NSLS-II, ALS, ESRF, and EMBL. With the current hardware and software in place, spot-finder runs at about 20 FPS; automated data reduction ranges from 2 FPS to 10 FPS depending on cluster load and data complexity. As a result, we are now collaborating with data-reduction and spot-finder software authors to possibly tweak the original code for better performances, either modifying the code or implementing the code on new Intel CPU chips, called KNL.

Computing / Network Infrastructures

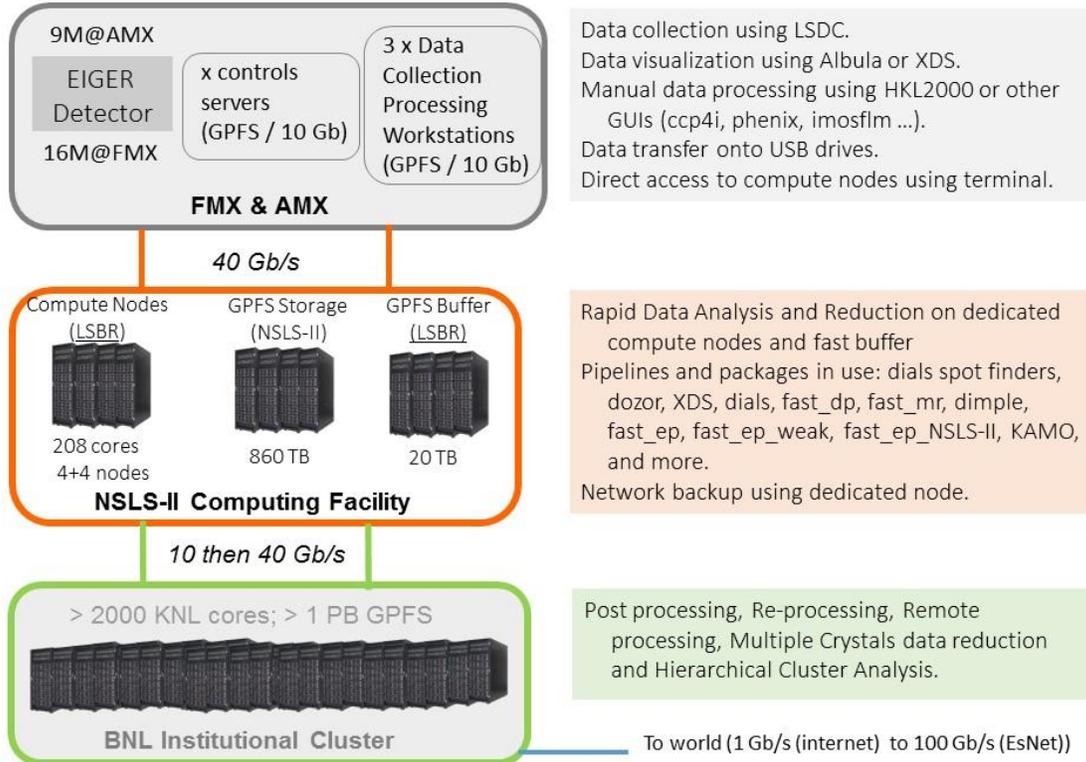


Figure 13: depict the infrastructures we have in place, mostly 2 independent facilities connected via 10 Gb/s or more; the beamline infrastructure and the NSLS-II computing facility.

AMX and FMX beamlines fitted with the automounters and the detector will be churning out data sets that one can not in any way process manually. In collaboration with colleagues from DLS and a visiting researcher from the Photon Factory, Yusuke Yamada, we modified `fast_dp` to be compatible with data from the two Eiger detectors. The resulting code, is now in use at the two beamlines and significantly reduces computing time, compared to the previous versions. We also modified the `fast_ep` pipeline to perform additional tasks, such as `dm` and model building and wrote a new pipeline called `fast_ep weak`, that apply “brute force” method for phasing of difficult structure due to weak anomalous signal. These pipelines, will be shared with users, so they will be able to reprocess data using their infrastructures. We are currently trying to optimize these pipelines further. Although we made significant progress with data processing and software optimization, it is clear that the hardware in place (compute nodes) will not be sufficient to deal with processing.

With Yusuke Yamada and Herbert Bernstein (RIT), we are also developing better software to assemble data from many partial data sets using hierarchical cluster analysis. A test-bed experiment was performed on a data set made of ~2000 small crystals, where 5 degrees were collected on each (see chapter from A. Soares).

Our goals are to :

- Run the spot finder near real time (up to 200 FPS for the E9M)
- Process an average data set (1400 frames) under 1 minute
- Obtain feedback from pipelines under 3 additional minutes.

In order to achieve these goals, we will need access to more computing and optimize the available softwares and pipelines. In order to monitor data collection, processing and the pipelines, we will need a database associated to a set of web applications allowing staff, users, PI and collaborators to monitor progress in real time. Longer term, it will be difficult for users to store data and process data at their institution; having access to a large cluster in our campus, may be the solution, to retain the data, re-process the data, run our multiple-crystals data-reduction pipeline. The challenges ahead are now clear, and in order to deal with them, we have started last year an international collaborative task, called HDRMX, where all parties (Dectris, software authors, and beamline scientists) are represented and participate to discussions and shared work to find common solution to modern facilities fitted with Eiger detectors, the better to deal with high data rates: <http://hdrmx.medsbio.org/>.

Dealing with data from multiple crystals

We have explored clustering strategies to help users make the most of serial crystallography data in cases where crystal-to-crystal nonisomorphism prevents a simple merging all of the data. We first explored situations where nonisomorphism appears when one has measured multiple clusters of data from protein crystals that were co-located on the same micromesh, but had important structural differences. The experiment was to obtain data from crystals that were co-crystallized with different ligands, and then combined onto the same micro-mesh before cryo-cooling. Up to four distinct protein+ligand pools were separately combined onto each micro-mesh. To prevent ligand diffusion, the micro-meshes were cooled less than 1s after the first crystals were added to them. As a further test, we used data that were obtained by one investigator from crystals that were physically located on separate micro-meshes, then randomly ordered into a pool of mixed data that were clustered by a different investigator with no knowledge of the correct clusters. In total, we obtained five-degree wedges of diffraction data from ~2000 crystals. Data were obtained from crystals grown using one type of test protein and one type of expressed protein.

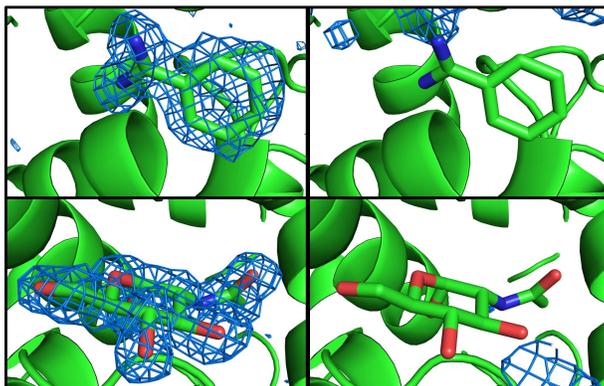


Figure 14: Representative electron density for the ligands observed in the electron density from clusters with ligands having near unity occupancy (left) and near zero occupancy (right).

Different clustering strategies were then used to disentangle the data from the distinct pools of protein+ligand crystals into clusters of data that could be merged to generate electron density maps. A successful clustering attempt resulted in each cluster yielding electron density that clearly corresponded to one of the protein+ligand categories used. Simple cases where there were the unit cell parameters for each crystal+ligand pair were very different ($>1\%$) were readily disentangled using the software package *KAMO*, which makes use of clustering algorithms in *BLEND*. Figure 14 above, shows examples of clusters with ligands having near unity occupancy (left) and near zero occupancy (right) from easily

clustered data. Difficult cases where the unit cell parameters were very similar required two-stage clustering using cell parameters to form initial clusters, followed by intensity algorithms to further partition data into the correct crystal+ligand clusters.

9 LSDC Data Acquisition Software

LSDC is the Python-based program responsible for data acquisition and beamline control at AMX and FMX. It is a database-driven system with a client GUI and an underlying command-line server. The design of the GUI was inspired by the layout of MxCUBE at ESRF (see Figure 15). As of January 2017 the database is a distributed MongoDB-based system using Amostra, Confrak, and Analysisstore written by the NSLS-II Data Acquisition Management and Analysis (DAMA) group. The EPICS-based motor control and scanning use Ophyd and BlueSky, also from the DAMA group.

LSDC has been a solid acquisition and control platform since the beginning of user operations. Flexible raster- and vector scanning were available on day 1. Since then the program has been evolving to include automated pipeline processing and integration with the robotic sample changers. As of March, hardware triggering of the detector by the hardware encoders was implemented on AMX allowing for high-speed data collection without the loss of frames at the beginning of a run.

Users began collecting data with the automounter on FMX interactively at the beginning of March wherein each sample was mounted from the “Mount” button of LSDC and then aligned with click-to-center and evaluated. As described in the previous section, all data are being stored on centralized storage made available through GPFS. Shared NSLS-II cluster nodes are used for raster spot finding with Dials and automatic processing with a version of fastDP customized in-house to work on the HDF files produced by our EIGER detectors.

We are now working on adding the ability to do automated queue-based operations. This will include integrating automatic loop centering, crystal finding, etc. We will also add automatic strategy determination with EDNA. When automated queue-based operations are in place, we’ll be able to provide for unattended operation, and then remote operations over NX. Many other additions and refinements will be required as we add new hardware (eg. secondary goniometer) and begin to exploit fully the microbeam capabilities of the beamlines and brightness of the synchrotron.

Controls Staffing Challenges

When the advisory committee met a year ago we had two high-level software developers: John Skinner working on LSDC and associated AMX/FMX software, and Hugo Slepicka working mostly on the control and DAQ software for LIX, as well as helping with some of the software configuration duties on AMX and FMX. Hugo left BNL at the end of November to take up a position at SLAC, and we do not have the resources to replace him. This has left us challenged to provide a GUI for data acquisition at LIX. Hugo was also taking on the responsibility of providing a web-based front-end to our MongoDB-based experiment database. This would provide the functionality of the PXDB front-end that we had at NSLS, or the EXI and SynchWeb interfaces to ISPyB in Europe.

This is not optional software. We must provide a convenient way for our users to keep track of and evaluate their experiments. Our underlying MongoDB database is an excellent foundation and is currently storing sample information, experiment metadata, and results. While this information can be retrieved fairly easily from a Python shell by someone who understands the database API, the results are Python dictionaries that are usually too complex to present any useful information. We have two routes we can go here: 1) write a layer of code that will interface to the REST layer of EXI or SynchWeb, or 2) develop our own web-based front-end. Although duplication of effort (reinventing the wheel) should always be avoided, developing our own front-end rather than adopting one from a different synchrotron

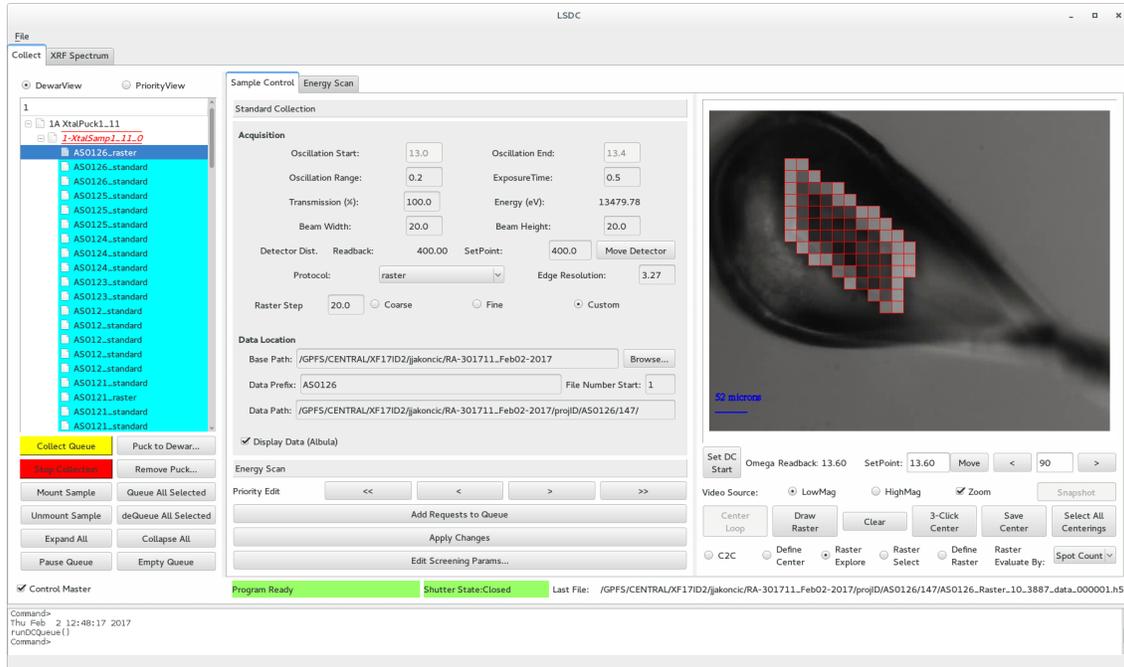


Figure 15: LSCD showing the heat-map results of a raster scan

would facilitate providing an interface that could be of more general use to other NSLS-II beamlines, and beyond. In the end, the effort involved with in-house development might be less than if we try to modify and extend an existing program and shoehorn a layer of code underneath it. Developing an interface of more general use might allow us to leverage some effort of the NSLS-II controls group, which would be highly desirable given the current LSBR software development staffing situation.

10 Other LSBR Activities

User program support at SSRL an overview

The LSBR user program for macromolecular crystallography users can be divided into two phases: (1) the NSLS User-Transition Program for NSLS MX Users that operated between October 2014 and July 2016; (2) the ramp up of the LSBR User program on the two macromolecular crystallography beam lines AMX, FMX and the scattering beam line LIX. The User transition program was centered at the Stanford Synchrotron Radiation Lightsource (SSRL) Structural Molecular Biology (SMB) beam line 14-1, a bending magnet beamline. In FY16 it operated between December 2015 and July 2016. The MX User program started in August 2016 while the LIX User program started earlier in May 2016.

The transition User program was made available for NSLS users with reviewed and active proposals at the end of NSLS operations. Researchers were requested to submit a title and an abstract to apply for beam time being granted remote access to 50% of the time available at beam line, BL-14-1. The goals of this program were to continue to provide a user friendly environment for researchers and training to the next generation of scientists, offering: 1) fast and efficient access to synchrotron beam time; 2) one-on-one assistance to researchers; 3) expert training for the next generation of structural biologists.

The significance of the transition program was to enable continued access to resources for scientists at the onset of their careers. The impact of the investment is not only reflected in the increasing numbers of structures being deposited in the protein data bank and in the number of publications

addressing basic questions related to disease mechanisms but also in the training and educational opportunities it has created for the scientific community.

The program supported by one LSBR staff member, who communicated with users, coordinated proposal submission, discussed safety issues, provided training, information on beam time availability, coordinated sample shipment, etc. Researchers once granted a proposal number scheduled their own beam time on a shared calendar that reflected the available beam time for the program. Before samples arrived at the beam line researchers were requested to complete a form that was used to complete the safety forms and to inform the nature of the experiment.

During the two years of operation, a total of 53 proposals were submitted by researchers who published the results of their research in approximately 20 peer reviewed journals, with ~ 30% reported in journals considered of high impact (Table 1). The overall impact factor for publications from research developed within the transition program is ~ 6.1. These numbers are expected to increase over the next fiscal year; typically a group takes 2-3 years to report their studies. As a result of the studies carried out over this transition program about 50 structures were deposited in the protein data bank, and further 62 are under refinement (Table 2).

Most researchers taking advantage of the NLS User Transition program were from groups at academic institutions supported by NIH RO1 grants; few were new faculty establishing their groups and even fewer were from non-academic institutions. Over the duration of the transition program as SSRL we were able to support about 191 unique investigators profited from the access to beam time through the Facility. Of these ~55% were PhD students (Table 3), with several profiting of the one-on-one training offered remotely during beam time support. By far not a complete representation, 7 PhD thesis were reported over the duration of the program .

	Publications
Published	20
Submitted	6
In preparation	10

Table 1: Publications reported as the result of survey conducted between June and August 2016. 30% of the users responded

	Structures
PDB released	50
PDB On Hold (HPUB)	12
Structures being Refined	62

Table 2: Publications reported as the result of survey conducted between June and August 2016. 30% of the users responded.

	Users
Researchers	
Faculty	16
Post Doctoral fellows	~25
PhD students	~46

Table 3: Researchers accessing the beam line according to the positions in their career.Usage figure for in FY16 (December 2015 through July 2016

Outreach and Educational activity

Outreach and educational activities address different needs. Several are developed in collaboration with the NSLS-II User Administration or the BNL Office of Educational Programs. A summary of the activities is shown in Table 1.

Crystallization workshop

Following the collaborative spirit that was the underline of the transition program at the SSRL we jointly organized a Crystallization course that followed the guidelines established by earlier courses developed at the NSLS. The Crystallization: Focus on micro and nano crystals and high throughput methods 2016 took place at the Stanford Synchrotron Radiation Lightsource (SSRL). Hosted for the Structural Molecular Biology (SMB) this is the first time that this course is offered at the West Coast. The purpose of the Crystallization focus on course series is to provide participants with hands-on experience of a variety of crystal growth methods.

Exploring life science with a new light

At the NSLS several high school teachers had profited in the development of programs with their students as part of the InSync program, an effort to connect forefront science with education. Following in the footsteps of this former program NSLS-II in conjunction with LSBR and the OEP decided to offer this one week workshop to science and research teachers. Teachers received 40 in class credits. The goal was that the teachers would submitted projects that they would develop with their students throughout the school year. Three proposals were submitted, two received beam time on NSLS-II spectroscopy beam lines. A group of four teachers develop modules of protein crystallization at their schools with their students. One teacher remained throughout the summer and in weekly contact throughout the year. His proposal was also granted beam time on a spectroscopy beam line to study Elemental Analysis of Polytene Chromosomes. The group of for teachers developing crystal growth projects will pursue this years training course.

Graduate courses

Participation in hands-on segments of graduate courses has been in demand these last few month. Typically the PI of a proposal who is responsible for a course at his/her institution asks if he/she can bring his student class to collect on a few standard crystals as part of the students course work. The beam time is usually part of his beam time application. The visit is typically organized in conjunction with the NSLS-II user office and students are able to collect data on one of the LSBR MX beam lines. The purpose of this one shift hand-on program is that the students become familiar with X-ray crystallography methods, and able to recommend the method to their PIs if in a non-crystallography Laboratory.

The growth of the user program.

The access programs to our structural biology beamlines have been developed to allow for a process that minimizes the bureaucratic overhead, helps flexibility of scheduling such that the scientists are in charge of the priorities on which experiment happens when. All while ensuring transparent peer-review of the research programs Three principle modes of access are available:

- **Block Allocation Groups** (BAGs) are intended for groups of researchers that want to combine their beam time requests into a single proposal in order to permit greater flexibility in beam time

allocation and scheduling. BAG proposals may be motivated by shared scientific interest, geographical location, affiliation, or other synergistic reasons. The term of a BAG proposal is 2 years (6 beam time cycles).

- **Rapid Access proposals** are a subset of General User proposals and are valid for one year (three beam time cycles). Rapid access proposals go through an expedited peer review process. Peer reviewed Rapid Access proposals are allocated beamtime on an as-available basis.
- **General User (GU)** proposals are valid for one year (3 beam time cycles). To request beam time, the user (PI) must submit a Beam Time Request (BTR) against their GU proposal. GU proposals are peer-reviewed by the NSLS-II Proposal Review Panel (PRP), and allocated by the Beam Time Allocation Committee.

The numbers of proposals for the two crystallography beamlines (for the moment we treat them as equivalent) and the scattering beamline over the last four NSLS-II cycles is described in Table 4.

	Review Cycle	Total Proposals	shifts requested	shifts allocated	subscription rate
FMX/AMX	2016-2	33	90.3	30	3.01
	2016-3	19	74	55	1.35
	2017-1	49	214	106	2.02
	2017-2	46	182	113	1.61
LIX	2016-2	6	24	15	1.60
	2016-3	11	50	48	1.04
	2017-1	18	58	66	0.88
	2017-2	16	72	72	1.00

Table 4: The total number of proposals and shifts requested for the crystallography beamlines and LiX over the last four NSLS-II operation cycles

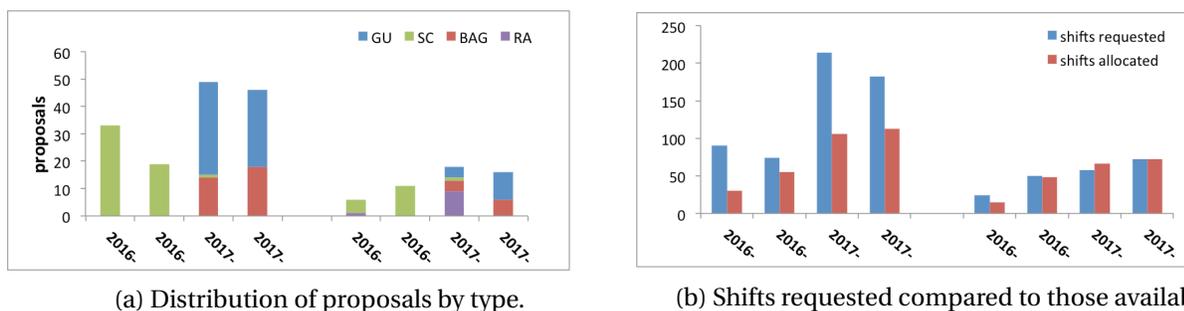


Figure 16: A summary of the proposals received for the three structural biology beamlines supported by LSBR. (a) The distribution of the groups accepted for experiments at the structural biology beamlines. Blue : BAG applications received and approved; Green: General user program applications accepted. (b) subscription rates on the beamlines for each of the last four cycles

The geographic distribution of the user communities home base reflects the early stages of our beamlines - the brave or those close by are the scientists most likely to come to the beamlines, this situation is reflected in the fact that three quarters of our user activity has been from New York and the North East of USA more generally. However as one of our out reach activities as been aimed at broadening the appeal of the NSLS-II beamlines so that we achieve a broader impact for the structural biology community, we are heartened to see the interest in the LSBR beamlines spreads across the country,

Figure 17. An important further activity will be for us to extend the range of our community through further outreach combined with excellence in technology and service.

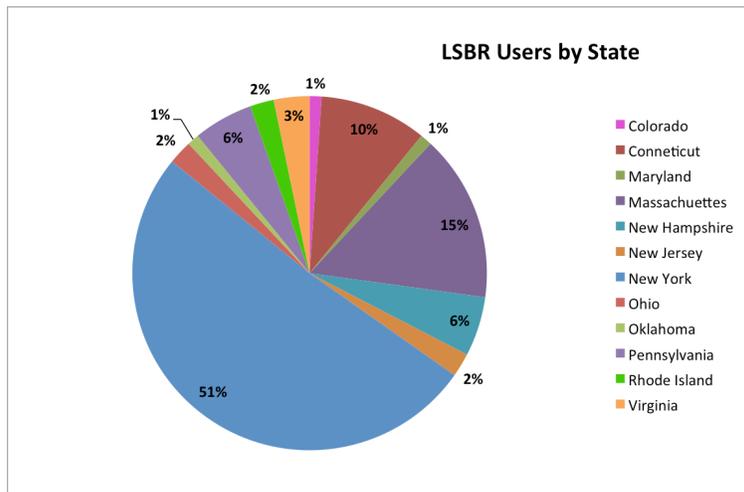


Figure 17: Distribution of LSBR beamline users by the US state of their home institutions.