GM/CA @ APS Newsletter

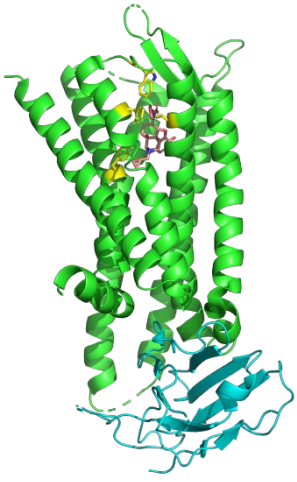
Winter 2023

The National Institute of General Medical Sciences and National Cancer Institute Structural Biology Facility at the Advanced Photon Source operates a national user facility for structural biology with synchrotron beamlines specializing in intense, tunable micro-beams for macromolecular crystallography.

Research Highlight

"Seeking pain relief without the risk of addiction"

The κ-opioid receptor (KOR) is an integral membrane protein with the familiar seven transmembrane helices of a class A G protein-coupled receptor (GPCR). KOR agonists with a G protein-biased preference have the potential to be non-addictive analgesics (pain relief) with minimal gastrointestinal side effects, as well as antipruritics (anti-itching) with minimal respiratory side effects. However, an understanding of the molecular mechanisms of biased ligand binding at KOR and how binding selectively induces specific signaling pathways was elusive. Together with Tao Che's group at Washington University in St. Louis, Ron Dror's group at Stanford University addressed these outstanding questions. Their findings were published in Nature Communications (El Daibaini et al. (2023) Nat. Comm. 14, 1338). Using the GM/CA@APS minibeam capabilities to collect and merge data from many radiation-sensitive crystals, they determined the crystal structure of KOR bound to the G protein-biased agonist nalfurafine, which is the first clinically-approved KOR agonist. Remarkable molecular interactions were further investigated and validated using atomic-level molecular dynamics (MD) simulations and functional assays to understand the molecular determinants of KOR signaling bias for different KOR agonists with distinct bias preferences. This collective effort provides insight into the structural and mechanistic understanding of KOR agonists and will facilitate the structure-based drug design to develop more effective and safer pain therapies. *(T. Che)*



Crystal structure of the κ-opioid receptor bound to its first clinically used agonist, nalfurafine. The KOR (green)-nalfurafine (salmon) complex is further stabilized by a llama-derived single chain antibody, nanobody 39 (cyan) and an N-terminal stabilizing fusion partner (BRIL, not shown). KOR amino acids that contact nalfurafine and were shown by mutagenesis to have roles in cAMP inhibition and arrestin recruitment are rendered as yellow sticks. (Image courtesy of the T. Che Group)

Amal El Daibani, Joseph M. Paggi, Kuglae Kim, Yianni D. Laloudakis,Petr Popov, Sarah M. Bernhard, Brian E. Krumm, Reid H.J. Olsen, Jeffrey Diberto, F. Ivy Carroll, Vsevolod Katritch, Bernhard Wünsch, Ron O. Dror, Tao Che, "Molecular mechanism of biased signaling at the kappa opioid receptor," Nat. Commun. 14, 1338 (2023). DOI: 10.1038/s41467-023-37041-7.)

GM/CA @ APS Sponsors:

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Argonne National Laboratory
Advanced Photon Source
9700 S. Cass Avenue
Building 436D
Lemont, Illinois 60439

Phone: 630-252-0662
Email: gmcauserinfo@anl.gov

Staff Profile — Nagarajan Venugopalan

Nagarajan Venugopalan (Naga) is a protein crystallographer at GM/CA. He grew up in the city of Chennai (formerly Madras), Tamil Nadu, India. Chennai is a very humid coastal city in the southern part of India where the climate is hot, hotter or hottest, with temperature about 80-100F most of the year and the lowest temperature reaching +60F in "Winter". He obtained his undergraduate degree in Physics from University of Madras, his Master of Science and Master of Philosophy degrees in Biophysics at University of Madras Department of Biophysics, a research facility founded by Prof. G.N. Ramachandran of Ramachandran map fame. In 1995, he travelled to Tokyo University of Agriculture and Technology, Japan on a MONBUSHO scholarship for his Ph.D. to work on the structural studies of collagen model peptides. He obtained the JSPS post-doctoral fellowship to work on structural studies of PCB degrading enzymes at Nagaoka University of Technology, Japan. In 2001, he joined Janet Smith's lab at Purdue University as a postdoctoral research associate to work on structural studies of CTP synthetase and moved with the Smith lab to University of Michigan for a short period.

Naga was one of the first users at GM/CA as it was being developed. He has long been fascinated with synchrotrons and his vast data collection experience, in both Japan and the USA, led to an appointment as protein crystallographer at GM/CA in 2006. During his time at GM/CA, Naga has supported a large number of users and has played a critical role in hardware and software development at the beamlines. He was responsible for improvements to the GM/CA on-axis sample visualization system, which has been in use for the past 14 years. He worked closely with Sudhir Babu Pothineni in the development of the sample, screening, data collection and multi-crystal strategy tabs in JBluIce. He was extensively involved in the development and implementation of the award-winning GM/CA mini-beam collimators (R&D 100 award, Hard X-ray Uni-body Quad collimator for Structural Biology, 2010) and in the development of early versions of long beamstops to reduce background radiation on the detector. He has received an Argonne Pace Setter Award (2017) and an Impact Argonne Award (2020) for his work at GM/CA.

Naga is a huge sports enthusiast, plays badminton regularly, and follows multiple sports during his free time. (*N. Venugopalan*)

Argonne Lab's highest honor to GM/CA Group Leader Bob Fischetti

On December 15, Bob Fischetti was named an Argonne Distinguished Fellow for his exceptional record of scientific and engineering achievement in developing the GM/CA beamlines for frontier structural biology and for his service in advancing the core Argonne mission.

Congratulations Bob!

More information about this award can be found here: Five researchers named Argonne Distinguished Fellows for 2023 | Argonne National Laboratory (anl.gov)

As we exited from COVID, we plunged into the Dark. How will we re-emerge?

On the morning of April 17, 2023, the APS went dark. It was with mixed emotions that I watched the beam turned off after our last user group. For approximately 20 years it was one of the premier sources in the world along with the ESRF and SPring8. In recent years, the smaller diameter, lower energy, 3rd generation sources have established themselves as worthy successors. They will carry the load as the new ESRF ramps up while the APS and SPring8 go into the shop for makeovers. The dark period has begun. For us, it is filled with anticipation of a new source that will be 2-3 orders of magnitude brighter than its predecessor. The new source will be connected to upgraded GM/CA beamlines equipped with new detectors, focusing optics, and improved automation, all integrated into controls driven through a new graphical user interface. For now, we are working hard to complete the GM/CA upgrade so that it all comes together seamlessly and on schedule. We will keep you posted on the progress as the time gets closer.

It is an exciting time for structural biology in general. The new source brings the anticipation of the use of serial synchrotron crystallographic data collection for ordinary and time-resolved experiments. The success of the community in building structure databases combined with massive sequence data have advanced structure prediction methods to the point of its use in molecular replacement structure solutions. Cryo-EM continues to improve in resolution and has become dominant for the structure determination of large complexes.

We are in the dark for now, but in the summer of 2024 we plan for GM/CA to rejoin the structural biology community, contributing to the next phase of macromolecular crystallography. In the meantime, if you need/want to visit us in person during the construction phase contact Kristin (kahrens@anl.gov). It would be great to see you. Time is passing quickly, we'll be seeing you soon. *(C. Ogata)*

GM/CA S10 Grant Application for New Detector

In our last newsletter, we wrote that we had submitted an NIH S10 shared instrumentation proposal for a next-generation high-speed pixel array detector (PAD) to replace the nearly decade-old Pilatus detector on beamline 23ID-D and that our proposal had scored very well in review. Since then, the funds were awarded and the grant became active in May.

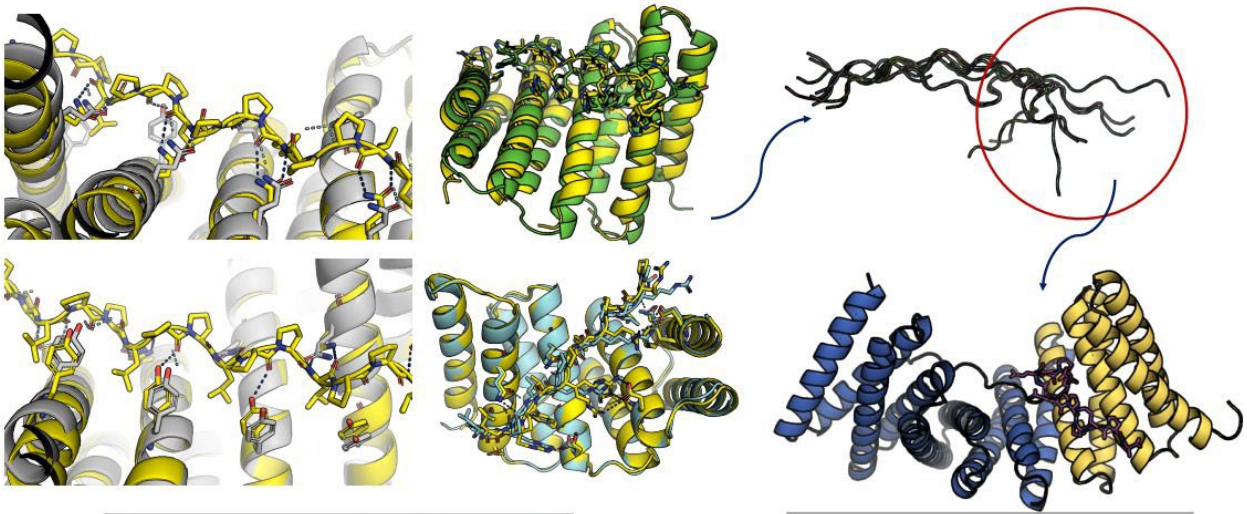
A purchase order was issued for an Eiger2 XE 16M detector with CdTe (cadmium telluride) X-ray sensors that have a significantly higher quantum efficiency (QE, a measure of the detector's sensitivity to X-rays) compared to the Si-sensor of our current Pilatus detector. The CdTe sensors have a QE of over 90% at 12 keV and remain high for energies well above 35 keV (the high energy limit of 23ID-D). The impressive specifications for the new detector include over 16 million pixels (4148×4362, W×H) that measure 75×75 microns, a count rate of 107 photons/sec/pixel, an energy range of 8 – 100 keV, and a continuous frame rate of 560 Hz at 16-bit precision (700 Hz at 8-bit).

The detector is scheduled for delivery in September 2024 and will be installed on 23ID-D. Software integration should be straightforward since we are already running Eiger2 protocols with our older Eiger detector on 23ID-B. Thanks to supplemental funding from NCI, we are upgrading our computing, networking, and storage capacity to handle the maximum high-frame rates simultaneously from the new Eiger2 (560 Hz) and the existing Eiger (133 Hz). The combination of the high-brightness APS-U source, the new focusing optics on the GM/CA beamlines, and the new Eiger2 detector will create exciting new scientific opportunities! *(R. Fischetti)*

Research Highlight

"De novo design of modular peptide-binding proteins by superhelical matching"

A recent study from Nature addresses a longstanding challenge in the field of structural biology: designing sequence-specific peptide-binding proteins with high specificity and binding affinity. Such designed proteins have the potential to significantly impact proteomics and synthetic biology by enabling targeted interactions with specific peptide sequences. Along with collaborators at several other institutions, David Baker's laboratory at the University of Washington sought to create custom proteins with repeating units that bind peptides with repeating sequences. Their methodology was based on a one-to-one correspondence between the repeating units of the designed protein and those of the peptide. The authors used a process called geometric hashing to identify suitable protein backbones and peptide-docking arrangements that would facilitate bidentate hydrogen bonds between the side chains of the designed protein and the backbone of the target peptide. This required a precise geometric match between the superhelices traced out by the repeating units on the designed protein and repeating units on the peptide. The remainder of the protein sequence was then optimized for folding and peptide binding. The proteins were designed to bind six different tripeptide-repeat sequences in polyproline II conformations. The designed proteins showed excellent stability and bound four to six tandem repeats of their tripeptide targets with affinities ranging from low nanomolar to picomolar both in vitro and in living cells. In addition to targeting repeating peptides, the researchers engineered two designed proteins to target non-repetitive endogenous disordered sequences in human proteins. The crystal structures of these protein-peptide complexes, determined in collaboration with Damian Ekiert's and Gira Bhabha's groups at the NYU School of Medicine, revealed repeating interactions between the protein and peptide as designed, including ladders of hydrogen bonds from protein side chains to peptide backbones. The design approach has many potential applications, for example targeting specific disease-associated repeat-expansion proteins or the intrinsically disordered regions of transcription factors. (*K. Wu, D. Baker)*



Crystal structures overlaid with design models showing matching interactions

Extending the interaction to target non-repetitive sequences in nature

Kejia Wu, Hua Bai, Ya-Ting Chang, Rachel Redler, Kerrie E. McNally, William Sheffler, T.J. Brunette, Derrick R. Hicks, Tomos E. Morgan, Tim J. Stevens, Adam Broerman, Inna Goreshnik, Michelle DeWitt, Cameron M. Chow, Yihang Shen, Lance Stewart, Emmanuel Derivery, Daniel Adriano Silva, Gira Bhabha, Damian C. Ekiert, David Baker, "De novo design of modular peptide-binding proteins by superhelical matching," Nature 616, 581-589 (2023). DOI: 10.1038/s41586-023-05909-9

2023 CCP4 School at the APS

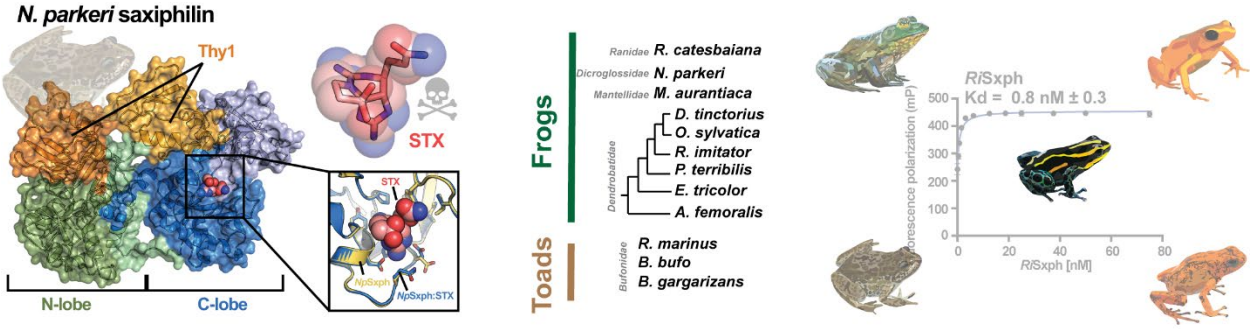
We held a successful on-site 2023 CCP4/APS Crystallographic School from March 27th to Apr 3rd, a few weeks before the APS upgrade began. Twenty students and 26 instructors (including 9 remote instructors) from US and Europe, and GM/CA staff participated in the School. During the intensive 8-day school, students attended lectures in the morning, and practiced on their own projects in the afternoon and evening, working closely with topical experts. In addition to problems and data brought by students, multiple datasets were collected at the two GM/CA beamlines, and several structures were solved. We also preserved some positive features and technologies adopted for the 2021 and 2022 virtual Schools, e.g. advance virtual preparation day, virtual lectures, and online community communication channels. We plan to hold another school when the upgraded APS resumes user operations, likely in the latter half of 2024. *(Q. Xu)*

Research Highlight

"Defining how a "toxin sponge" protein from frogs and toads captures a deadly neurotoxin"

Saxitoxin (STX), a neurotoxin made by cyanobacteria and dinoflagellates found in red tides, is so poisonous that it is the only marine toxin to have been declared a chemical weapon. STX exerts its toxic effects by blocking voltage-gated sodium channels in neurons. Shellfish accumulate STX and can remain contaminated for weeks to years after a red tide. People who eat contaminated shellfish can become sick and even die from paralytic shellfish poisoning. This threat to the food supply is becoming more common as the frequency of red tides increases due to climate change.

Certain animals, particularly frogs, are resistant to STX poisoning. In frogs, this resistance is thought to be due to their ability to produce saxiphilin (Sxph), a protein that acts as a "toxin sponge" because it can bind and sequester STX away from sodium channels. Sxph was first identified in the American bullfrog Rana catesbeiana (RcSxph) and its structure was determined by the groups of Daniel Minor at the University of California, San Francisco and Justin Du Bois at Stanford through work performed at the beamline 23-ID-B at the Advanced Photon Source. Now, the same groups used their structural knowledge in a mutational analysis of the STX binding site to define a "binding code" for Sxph affinity for STX. Using this code, the team, together with Lauren O'Connell's lab, also at Stanford, identified 10 new Spxh proteins in diverse frog and toad species. When they made and tested four of these new Sxphs, they found that all four bind to STX. Remarkably, two of the new Sxphs bound STX more tightly than did RcSxph. They used the APS to determine structures for the tight binder NpSxph, from the High Himalaya frog, Nanorana parkeri.
The mutational studies of Minor and colleagues also identified an amino acid substitution that enhanced binding to STX. Using X-ray diffraction data collected at APS, the team defined the three-dimensional structures of the modified RcSxph alone and as an STX complex, providing further detail about how the substitution enhanced STX binding. Remarkably, the ability of NpSxph to sequester STX more tightly than RcSxph comes from an amino acid change at the site identified as an affinity-enhancer in the RcSxph mutational studies. Hence, the work shows that the team uncovered some of nature's STX binding rules. These advances in understanding the fundamental rules for STX recognition could lead to the design of biological tools that can detect or neutralize STX and related toxins, and yield new ways to combat paralytic shellfish poisoning. One remaining intriguing question is why these amphibians from lineages separated by ~140 million years of evolution all produce similar "toxin sponges" even though they are not known to encounter STX on a regular basis. One possibility is that STX, which can be found in fresh water, is more widespread in the environment than currently understood. *(D. Minor)*



Left: Structure of *Nanorana parkeri* NpSxph, bound to saxiphilin (STX). N-lobe (green), C-lobe (blue), and thyroglobulin (Thy1) domains (orange) are indicated. *N. parkeri* is shown in the background. STX structure (upper right), and comparison (lower right) of the NpSxph binding pocket from X-ray structures of NpSxph alone (yellow) and the NpSxph:STX complex (blue). The image of the binding site is rotated ~90° counterclockwise relative to the NpSxph:STX image. **Center**: Sxph bearing frogs and toads. **Right**: Frogs having biochemically characterized Sxphs. Clockwise from upper left, *R. catesbeiana*, *M. aurantiaca*, *O. sylvatica*, and *N. parkeri*, Center shows *R. imitator* with an exemplar STX binding curve for its Sxph, RiSxph. (Image courtesy of D. Minor)

Zhou Chen, Sandra Zakrzewska, Holly S. Hajare, Aurora Alvarez-Buylla, Fayal Abderemane-Ali, Maximiliana Bogan, Dave Ramirez, Lauren A. O'Connell, J. Du Bois, Daniel L. Minor Jr., "Definition of a saxitoxin (STX) binding code enables discovery and characterization of the anuran saxiphilin family," Proc. Natl. Acad. Sci. USA 119 (44), e2210114119 (2022). DOI: 10.1073/pnas.2210114119

APS Dark Period and APS-Upgrade Era

As of mid-December, we are eight months into the year-long shutdown to replace the APS with a new, upgraded storage ring known as APS-U. The final section of the new storage ring was installed on December 12, 2023 (see photos below). Tests and check-out of the new ring will continue in January. The DOE will conduct an Accelerator Readiness Review in mid-February to allow the ring to start up. Another review is scheduled for sometime around May before the beam current can be ramped up. Since this is a new accelerator, all beamlines must undergo radiation surveys to validate the shielding.

When the APS shut down, one-half of the nation's synchrotron capacity for macromolecular crystallography was temporarily taken offline. We worked with our colleagues at other synchrotrons to help APS users make connections and learn about the capabilities and processes at beamlines at other facilities. The beamlines at NSLS-II, SSRL, ALS, and CHESS have all seen an increase in the number of proposals and beam time requests. NSLS-II had a Block Allocation Group (BAG) mode of access that allowed multiple groups to use beamtime more efficiently. SSRL added the BAG access mode to help address their oversubscription rate. We encourage you to contact beamline staff at the other facilities to learn about their capabilities, sample mount requirements, and beamtime access modes.

APS partnered with the Diamond Light Source (DLS) in the U.K., whereby DLS set aside 12.5% of the beam time on four of their high-throughput beamlines. APS has solicited proposals for beamtime at DLS and sent them out for peer review. So far, 31 APS General Users groups have been allocated 145 shifts at DLS, and both LS-CAT and SER-CAT members have sent samples to DLS. In total, data have been collected from over 2200 samples at DLS. Data were collected automatically on most of the samples in the Unattended Data Collection (UDC) mode. Several user groups have reported a good experience sending their samples to DLS. Access to DLS through this mechanism may continue through most of 2024 and should ramp down as APS beamlines come back online. *(R. Fischetti)*

The APS storage ring tunnel at sector 23. **Left**: The APS storage ring and beamline front ends were removed by the end of June 2023. **Right**. The newly installed APS-U storage ring and sector 23 canted-undulator front end.

Software at GM/CA: PyBluIce User Feedback and ALCF Streaming Processing

Several GM/CA users in the 2023-1 run collected data primarily with the new PyBluIce user interface. Their feedback was overwhelmingly positive, especially about the more intuitive and responsive user interface. The extended year-long software refinement period led to a productive experience for all PyBluIce users. This will be the default user interface when users return to an upgraded APS.

To prepare for high-throughput data collection with a hotter source and faster detector, we are developing a streaming system that distributes data at gigabytes per second. This new system allows image statistics and visualization to be generated in real time at the top speed of an Eiger2 XE detector. At the same time, per-image analysis can be run on all images, while multi-image HDF files are written to disk. The capability to write an HDF version containing only images with diffraction will make processing and backups more efficient, especially for serial crystallography data. Processing of individual images and whole datasets will be supercomputer-accelerated when resources are available at the Argonne Leadership Computing Facility (ALCF). This acceleration will be used for tasks such as parameter search and sparse data processing. Improvements to the data processing pipeline include support for AlphaFold (calculated using ALCF resource or existing online databases) and visualization of structures within the user interface. *(M. Hilgart/Q. Xu)*

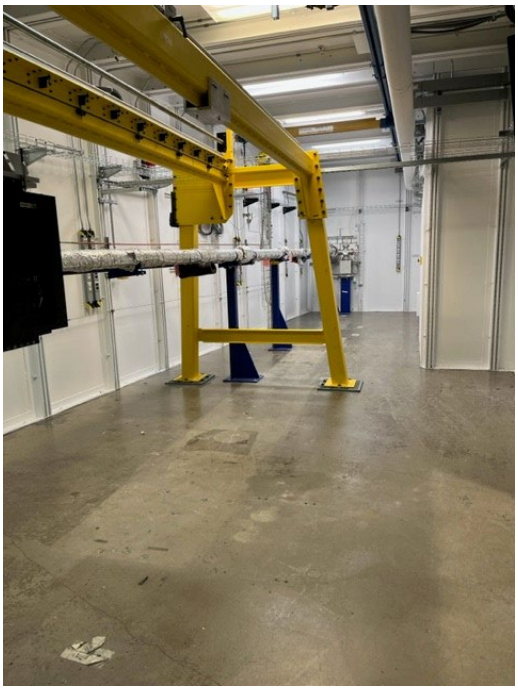
Beamline Upgrades & Micro-Focusing

Several years ago, we began planning a refurbishment of the GM/CA insertion device beamlines. Our goal is to replace old and obsolete equipment with new equipment designed to fully exploit the unique capabilities of the APS upgrade (APS-U).

The overall layout of the beamlines that we built over 20 years ago will function well with the APS-U and remains unchanged. A pair of canted undulators provides two X-ray beams that are separated by 1 mrad in the horizontal plane. We refer to these as the outboard beam for 23-ID-B and the inboard beam for 23-ID-D. To increase the separation between the beamlines, a pair of horizontal deflecting mirrors (HDMs) bounce the outboard beam 16 mrad further outboard, providing ~500 mm separation of the beamlines at the 23-ID-B endstation.

Our work towards the GM/CA upgrade began long before the APS shutdown. In January 2019, we replaced the 23-ID-A white beam slits, which served only the outboard beamline, with new canted-undulator white beam slits that service both beamlines. Next came new focusing optics for 23-ID-D. In September 2020, we installed a Compound Refractive Lens (CRL) transfocator, and in May 2022, a new Kirkpatrick-Baez Mirror (KBM) focusing system. We gained valuable insights by testing these components with the APS prior to the shutdown, allowing us to make design modifications during the shutdown to improve the performance of the focusing systems installed in 23-ID-D and the new ones for 23-ID-B.

When the APS shut down for the year-long upgrade (APS-U) in April 2023, we immediately dismantled and removed the aged components in all hutches. In 23-ID-A, we removed the horizontal deflecting mirrors (HDM), the beam stop, and the beam position monitors (BPM) so the support structures could be modified to accommodate the new HDM system. Nearly everything was removed from 23-ID-B (monochromatic beam slits, KBMs, beam delivery, BPMs, and the entire end station, leaving only the white beam transport for the inboard beam and the A-frame detector support (see photo below, left). In 23-ID-C, we removed the obsolete white beam slits, started modifying the monochromator, and are replacing the white beam stop and modifying all the other components. Everything was also removed from 23-ID-D except the new mirror system that was installed in 2022.



Left: Almost everything was removed from the 23-ID-B hutch except for the A-frame (yellow), the detector (black box at left), and the white beam transport for 23-ID-D (foil-covered pipe). **Right**: the view of 23-ID-B in December 2023, with many of the new components installed.

We are also replacing all the obsolete beamline electronics (motion control, data acquisition, and equipment protection) and upgrading the computing environment (all new drives in the data storage array with increased capacity to 1.5 PB, almost 500 TB of new solid state drives to handle the high data rate from the two Eiger detectors, and increased bandwidth to 100 GB for most of the network). Almost nothing was left untouched!

The trajectory of the X-ray beams from the APS-U will be slightly different compared to the APS, so all beamlines must be realigned. Pending a review in January 2024 of the GM/CA critical radiation safety components, our beamline components will be resurveyed. Then we can install the beampipes and bellows to complete the photon delivery systems to the endstations.

The following sections summarize the status of each of the hutches.

Hutch ID-A
The new HDMs were designed to match the characteristics of the APS-U. An important metric is the slope error of the mirror, which quantifies how much the beam shape will be distorted when the beam is reflected from the mirror. The slope errors of the mirrors, as measured in the APS metrology laboratory using the Long Trace Profiler (LTP), are 34 nrad and 46 nrad, which are 50 – 70 times better than the original mirrors!

These state-of-the-art mirrors were installed in May/June 2023. The supports for the beam stop and BPMs were modified and are ready to be surveyed into position.

Hutch ID-B
The new KBM system has a Horizontal Focusing Mirror (HFM) and a Vertical Focusing Mirror (VFM). The mirrors were installed in motorized benders that adjust the mirror curvature to an elliptical shape to focus the beam. The VFM has four gravity compensators that are adjusted to remove sagging of the mirror under its weight. Using the APS LTP, the measured slope error of the mirrors in their benders was ~50 nrad for the HFM and ~70 nrad for the VFM. The slope errors are about 2-fold better than our specifications and over 30 times better than the old bimorph mirrors that we replaced. Support stands for all the other components of the beam delivery are either being reused, have been modified, or are being replaced.

The new endstation table, now installed, supports the CRL transfocator, beam condition box (BPM, attenuators, slits, shutter, and intensity monitor), and sample environment. The high-precision air-bearing goniometer and its positioners are on the table (see photo above, right) and will be installed in January 2024 after fabrication of the pitch and yaw adjustment assemblies. A 3-axis goniometer head has been built that allows one to center single crystals on a pin mount or scan small sample chips measuring up to 12 × 12 mm. A version that allows scanning larger chips will be installed next fall. Other endstation components are being reused (sample automounter, cryo-jet, fluorescence detector, and A-frame detector support with the Eiger16 detector).

Hutch ID-C
The energy range of the double crystal monochromator was 5 – 20 keV with Si(111) crystals. We used the Si(333) reflection for energies above 20 keV; however, due to the narrow bandwidth, the intensity is low. Therefore, we developed an optics modification for 23-ID-C in order to reach 35 keV using the Si(111) reflection. This required replacing the white beam slits with a photon mask and Bremsstrahlung collimator, modifying the double crystal monochromator, fabricating a new photon stop, modifying the Bremsstrahlung stop, and increasing the height of all downstream components by 15 mm. All the components are either in hand or in the shop for fabrication or modification. We plan to reassemble the components in 23-ID-C by the end of March.

Hutch ID-D
The status of most components in 23-ID-D is the same as those in 23-ID-B. The measured KBM slope errors were 75 nrad for the HFM and 92 nrad for the VFM. The KBM system and new end station will be raised 15 mm to accommodate the changes in 23-ID-C.

Startup and commissioning
As we write this in mid-December, almost everything needed to finish rebuilding the beamlines is either in hand or has been ordered. GM/CA is currently scheduled for 1st light and shielding verification in May. We plan a phased approach to re-commissioning the beamlines. Our priority is to re-commission 23-ID-B first. We anticipate it will take three months to verify that everything is working properly (technical commissioning), and then we will invite some groups to test data collection (scientific commissioning). With the APS-U source and new high-stability endstation, the mirrors should focus the full beam to ~5 microns (FWHM) in both directions, and the CRL should provide a minimum beam size of 1 micron. We will support high-throughput single-crystal rotation data collection and Serial Synchrotron Crystallography (SSX). We anticipate it will take six months to ramp up the user program, and we look forward to welcoming you back to take advantage of the small, hot beam at GM/CA.

Much of the hardware is the same on the two beamlines, so we can apply what we learn about commissioning 23-ID-B to the technical commissioning of 23-ID-D. However, it will take an additional one to two months to characterize the beam at high energies. Initial commissioning will be performed with the Pilatus 6M detector. Once the Eiger2 XE 16M detector with CdTe sensors is operational (*see the section GM/CA S10 Grant Application for New Detector*), we plan to investigate the optimal conditions for data collection from small, weakly diffracting samples with a micron-sized beam at high energy. *(R. Fischetti & N. Venugopalan)*

Acknowledgement

When you publish results from your use of GM/CA beamlines, you are required to acknowledge the GM/CA funding sources in your publications. This requirement applies to all NIH and DOE support and is used by funding agencies to evaluate our program. Please copy and paste the following statement into your publications:

"GM/CA@APS has been funded by the National Cancer Institute (ACB-12002) and the National Institute of General Medical Sciences (AGM-12006, P30GM138396). This research used resources of the Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357."

If you used beamline 23ID-B, you are additionally required to include the NIH shared instrumentation grant S10 OD012289 for our Eiger-16M detector among the funding sources associated with your paper in the NIH manuscript submission system and add one more acknowledgement to your publication:

"The Eiger 16M detector at GM/CA-XSD was funded by NIH grant S10 OD012289."