

GM/CA @ APS Newsletter

Fall 2022

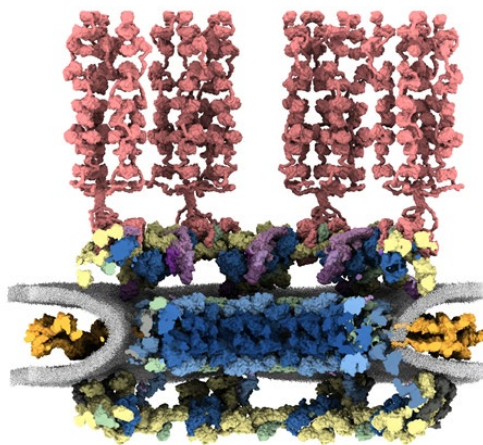
The National Institute of General Medical Sciences and National Cancer Institute Structural Biology Facility at the Advanced Photon Source operates a national user facility for structural biology with synchrotron beamlines specializing in intense, tunable micro-beams for macromolecular crystallography.

Research Highlight

“Building the nuclear pore complex”

In humans, the nuclear pore complex (NPC) is an ~120 MDa assembly built from ~1,000 copies of 34 different proteins, termed nucleoporins. Its architecture consists of a symmetric core that is asymmetrically decorated by uniquely nuclear or cytoplasmic nucleoporins on the respective sides of the nuclear envelope. The NPC’s primary function is to mediate the selective transport of macromolecules through its central transport channel, which can accommodate entire ribosomes. In an iris-like fashion, the central transport channel dilates and constricts by at least 20 nm in response to membrane tension, revealing the NPC as the cell’s largest mechanosensitive channel.

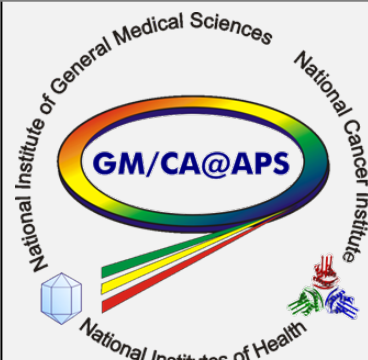
Previous work by André Hoelz’s group at Caltech elucidated the key building blocks of the NPC symmetric core and how they fit into the overall architecture. However, the molecular interactions that hold these blocks together while also allowing the NPC to dilate and constrict, and how asymmetric nucleoporins attach to the symmetric core, remained elusive. Together with Martin Beck’s group at the Max Planck Institute in Frankfurt, Germany (Mosalaganti et al. & Beck (2022) Science 376, 1176), the Hoelz group addressed both of these outstanding questions. Their findings were published in a focused issue of Science. *(Research Highlight continued on*



Cut-away image of the NPC embedded in the nuclear envelope (gray) between the cytoplasm (above) and nucleoplasm (below). The 60-nm cytoplasmic filaments, anchored via specific subcomplexes to the cytoplasmic side of the NPC, are docking sites for transport factors. The new structures from crystallography and cryo-ET placed all NPC components, including also the transport channel (blue), intermembrane space (orange) and nucleoplasm subunits. (Image courtesy of the A. Hoelz Group)

GM/CA @ APS Sponsors:

National Institute of General Medical Sciences (NIGMS) and National Cancer Institute (NCI) of the National Institutes of Health (NIH)



page 2)

INSIDE THIS ISSUE

Staff Profile.....	2
GM/CA S10 Grant.....	3
Research Highlights (cont.).....	3
GM/CA in COVID.....	4
APS Upgrade.....	4
Software Upgrade.....	5
Beamline Upgrades.....	5

GM/CA @ APS

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Research Highlight continued

The Hoelz group used extensive biochemical reconstitution and mutational mapping to establish the NPC's subcomplexes and then solved >30 crystal and single-particle cryo-EM structures. The Beck group then interpreted an ~12-Å cryo electron tomographic (cryo ET) map of the intact human NPC by quantitative docking of the subcomplex structures into the map to generate a near-atomic composite structure of the human NPC. A series of *in vivo* experiments carried out in yeast and human cells validated and revealed the physiological relevance of many atomic details.

The study showed how mostly unstructured “linker” proteins simultaneously bind multiple scaffold surfaces and coalesce large folded “scaffold” protein components into stable subcomplexes that ultimately assemble into the NPC. In the ~12 Å composite structure, the human NPC has a constricted central transport channel, whereas a similarly obtained composite structure from a ~37-Å cryo ET map shows the human NPC in a dilated state, leading to the first movie of the reversible constriction and dilation of the NPC. Owing to the linker-scaffold architecture, the NPC segments can move along with the nuclear pore membrane in response to membrane tension, which also creates lateral channels for the transit of membrane proteins between the outer and inner nuclear membranes.

Eight pentameric bundles of the protein NUP358, each of which flexibly projects into the cytoplasm to form the characteristic ~60-nm filaments that provide binding sites for transport factors, and sixteen copies of an evolutionarily conserved ~540-kDa heterohexameric subcomplex dubbed the cytoplasmic filament nucleoporin complex, are anchored to the NPC's cytoplasmic face. These NPC components establish the directionality of nucleocytoplasmic transport and mediate the irreversible remodeling of mRNA ribonucleoprotein particles (mRNPs) as they emerge from the nucleus. Besides being a hotspot for virulence factor targeting, the NPC cytoplasmic components are associated with several human pathologies. Providing residue-level detail and an atlas of RNA binding interfaces, these studies are a roadmap for elucidating the mechanism of the final key steps in mRNA export.

These advances, pending experimental validation of AlphaFold predictions of the few missing structural elements, complete the initial visualization of the near-atomic snapshot of the yeast and human NPCs, opening the door for further studies aimed at understanding NPC dynamics in various nucleocytoplasmic transport and gene regulation events relevant to health and disease. (*A. Hoelz Group*)

Bley CJ,* Nie S,* Mobbs GW,* Petrovic S,* Gres AT,* Liu X,* Mukherjee S, Harvey S, Huber FM, Lin DH, Brown B, Tang AW, Rundlet EJ, Correia AR, Chen S, Regmi SG, Stevens TA, Jette CA, Dasso M, Patke A, Palazzo AF, Kossiakoff AA, Hoelz A (2022) Architecture of the cytoplasmic face of the nuclear pore. *Science*. 376(6598):eabm9129.

Petrovic S, Samanta D,* Perriches T,* Bley CJ, Thierbach K, Brown B, Nie S, Mobbs GW, Stevens TA, Liu X, Tomaleri GP, Schaus L, Hoelz A (2022) Architecture of the linker-scaffold in the nuclear pore. *Science*. 376(6598):eabm9798.

PyBluIce: Workflow Streamlining, Tutorials and New Feature Development

Over the past six months, GM/CA staff used PyBluIce to study our own collection of protein samples. By paying attention to the crystals and not the program, we found minor changes here and there that would remove friction or roadblocks from our workflows. Today's PyBluIce incorporates these changes to make working with the program more productive.

We're updating our video tutorials and documentation for PyBluIce and will post these to our website soon. They will detail what's new and what's changed since JBluIce.

Development has started on the first major new feature for PyBluIce: 3D Raster. We chose this feature because many of you will be able to benefit from the improved efficiency and data quality it will enable. Watch this space for more details soon. (*M. Hilgart*)

Staff Profile – Craig Ogata



Craig Ogata is a Protein Crystallographer in the GM/CA group. As the temperature in the Chicago area drops below freezing and the first snow arrives, he reminisces fondly about growing up in Honolulu, Hawaii, where putting on a sweatshirt in the early mornings of the winter month(s) was the winter attire. He left Hawaii to go to the Mainland, continental United States, for his education. He began his protein crystallographic career as a graduate student in the Chemistry Department at UC Berkeley in Sung-Hou Kim's lab. His thesis work was the structure determination of a sweet tasting protein, monellin. This was followed by a leap to the east coast joining the Columbia University laboratory of Wayne Hendrickson as a postdoc. These were the early days of MAD phasing. While there, he demonstrated the use of MAD phasing based on a substituted brominated base to solve a drug-nucleic acid complex using a detector based on imaging plates, which needed to be manually scanned on a prototype reader. He also collaborated with Marvin Hackert's group to solve the structure of *Urechis* hemoglobin using its intrinsically bound iron. Wayne Hendrickson then sentenced him to 10 years of hard labor at the HHMI beamline X4 at the old NSLS. This was the first dedicated MAD beamline, contributing to the acceptance and popularity of the MAD method through the publication of many high-profile structures.

Craig eventually joined the GM/CA group at the APS. During his time at GM/CA, Craig, together with Oleg Makarov and Shenglan Xu, designed, built and implemented a high capacity modified cartesian Berkeley automounter. He worked with Mark Hilgart to design and implement the Vector Collect method using the mini-beam collimators. His interest in the use of small beams led him to work on serial synchrotron crystallographic (SSX) approaches to data collection.

All in all, he's come a long way from his first summer job as a pineapple picker on the island of Lanai. To this day, he claims that his childhood friends always have a surfboard ready for his return trips to the islands. (*C. Ogata*)

GM/CA S10 Grant Application for New Detector

In May 2022, we 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. Several GM/CA users provided strong scientific justifications for a new detector, and the proposal was scored very well. We plan to purchase a detector with CdTe (cadmium telluride) X-ray sensor that has 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. Both the Si and CdTe sensors have a QE of over 90% at 12 keV. However, the efficiency of the CdTe sensor remains high for energies well above 35 keV (the high energy limit of 23-ID-D), while the QE of the Si sensor falls off dramatically.

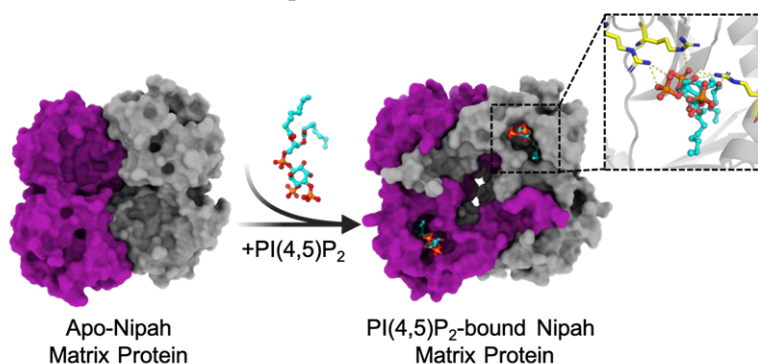
Why does this matter? Greater efficiency means one can reduce the number of X-rays incident on the sample to get the same diffracted signal and, therefore, reduce radiation damage.

We previously demonstrated that radiation damage from 18.5 keV X-rays was reduced within the footprint of the beam as the beam size was decreased from 16 microns to 1 micron. In the summer of 2021, we borrowed an Eiger2 4M CdTe detector from Dectris and demonstrated that radiation damage could be reduced by using both higher energy X-rays (up to 35 keV) and smaller beam sizes (down to 2.5 microns). The APS-U will provide a 2-3 orders of magnitude increase in brightness extended to high X-ray energies. Combining the APS-U and new focusing optics recently installed on 23ID-D, and a 4-5 fold increase in frame rate of the new detector creates a game-changing opportunity for the structural biology community. We plan to provide a small, intense beam of up to 35 keV to maximize the amount of useful information extracted from a single crystal. The new detector with a CdTe sensor will be a key component of this new capability.

Research Highlight

“Measles and Nipah virus assembly: Specific lipid binding drives matrix polymerization”

Paramyxoviruses are collectively among the most infectious and deadly viruses known and have the potential to trigger a devastating pandemic. Notable paramyxoviruses include Measles virus, which infects >7 million people and causes >100,000 deaths annually, and Nipah virus, which has a case fatality rate of up to 90%. Despite the threats paramyxoviruses pose to public health, we have no therapies to manage or control outbreaks of severe disease caused by these viruses. Paramyxovirus infections spread when new virus particles assemble and “bud” from the plasma membrane of infected cells. Virus matrix proteins coordinate this budding by binding the inner leaflet of the plasma membrane and self-assembling to form a matrix lattice. This lattice bridges surface glycoproteins and internal ribonucleocapsid complexes containing the RNA genome to form virion particles. Matrix formation is a promising target for antivirals, but advancement in this area was hindered by an insufficient understanding of the molecular determinants of viral assembly. Erica Ollmann Saphire's group at the La Jolla Institute for Immunology and Robert Stahelin's group at Purdue University, together with an international team of collaborators led by postdoctoral fellow Michael Norris (now at the University of Toronto), demonstrated for the first time that specific binding of measles and Nipah virus matrix proteins to the plasma membrane lipid, PI(4,5)P₂, mediates protein interactions with host membranes. They further showed that dimerized matrix proteins helically assemble on membranes to form long filaments only when PI(4,5)P₂ is present. This result suggested that interactions with PI(4,5)P₂ induce protein assembly that favors matrix polymerization. To gain detailed molecular insight into matrix-PI(4,5)P₂ interactions, they determined high-resolution crystal structures of both measles and Nipah virus matrix proteins alone as well as Nipah matrix in complex with a soluble form of PI(4,5)P₂. This structure, collected on GM/CA beamline 23ID-B, is the first crystal structure ever determined for any viral matrix protein in complex with a lipid ligand. This detailed view revealed not only a discrete PI(4,5)P₂-binding pocket located in the C-terminal domain basic patch of Nipah matrix protein, but also that binding of PI(4,5)P₂ drives major rearrangements in conformation and surface charge that together facilitate matrix layer polymerization, membrane deformation, and virion assembly. These studies, published as a cover story in *Science Advances*, established a framework for paramyxovirus assembly and revealed how future therapies might stop these viruses in their tracks. (M. J. Norris, E. Ollmann Saphire)



Crystal structures of the apo (left) and PI(4,5)P₂-bound (right) forms of the Nipah virus matrix protein dimer shown as surfaces with purple and gray monomers. PI(4,5)P₂ (ball-&-stick) binding induces an extensive conformational rearrangement to drive matrix polymerization, membrane curvature, and virus particle assembly. The inset shows details of PI(4,5)P₂ binding in the C-terminal domain of the Nipah matrix protein with atomic colors: cyan C for PI(4,5)P₂, yellow C for matrix, orange P, blue N and red O. (Image courtesy of M. J. Norris; E. Ollmann Saphire)

Michael J. Norris, Monica L. Husby, William B. Kiesses, Jieyun Yin, Roopashi Saxena, Linda J. Rennick, Anja Heiner, Stephanie S. Harkins, Rudramani Pokhrel, Sharon L. Schendel, Kathryn M. Hastie, Sara Landeras-Bueno, Zhe Li Salie, Benhur Lee, Prem P. Chapagain, Andrea Maisner, W. Paul Duprex, Robert V. Stahelin, Erica Ollmann Saphire, “Measles and Nipah virus assembly: Specific lipid binding drives matrix polymerization”, *Sci Adv.* 2022 Jul 22;8(29):eabn1440. doi: [10.1126/sciadv.abn1440](https://doi.org/10.1126/sciadv.abn1440).

CCP4 School @ the APS

The 15th annual CCP4/APS Crystallographic School will resume on-site at Argonne in March 27 to April 3, 2023. This will be the first on-site school since 2019. The date is scheduled to accommodate the APS upgrade, which will start in mid-April 2023. The School provides great one-on-one problem-solving and training opportunities for students facing challenging problems in their projects. A limited number of seats is available, so students interested in attending in person are encouraged to apply early. Additional information is available at the school website (<https://www.ccp4.ac.uk/schools/APS-school/>) (Q. Xu)

GM/CA in COVID

Now in the penultimate run before the April 2023 APS shutdown, we are all anticipating the arrival and impact of the new source that will be delivered in approximately 18 months. The APS-U promises enhanced stability, a 2-3-fold increase in brightness, and a round beam leading to an even greater brightness at the sample crystal. GM/CA has started preparation for the new source by replacing the focusing optics in the 23ID-D beamline with new, state-of-the-art optics (a pair of JTEC mirrors with mechanical benders). On the horizon is the release of the new PyBluIce GUI. PyBluIce will replace JBluIce and is undergoing final commissioning and user testing.

In anticipation of the release of the new PyBluIce GUI, we are increasing the frequency of our Beam Time Q & A sessions in order to introduce you to PyBluIce before your scheduled beam time. After your ESAFs are submitted, Kristin Ahrens will contact you in regards to the schedule of the training sessions.

This is a good time for old and new users to experience an on-site visit to the GM/CA beamlines. This is an opportune time to refresh knowledge of beamline operations. (And you'll be able to tell your colleagues stories about how it was at the old APS!) COVID-19 restrictions have been relaxed, however you should always consult the APS webpage for changes in current restrictions (<https://www.aps.anl.gov/Users-Information/Getting-Started/Argonne-Site-Access>). We look forward to seeing you in person. (C. Ogata)

2022 Lasker Award to Timothy Springer

GM/CA congratulates Timothy Springer of Boston Children's Hospital and Harvard Medical School upon his receiving the [2022 Albert Lasker Basic Medical Research Award](#). Co-awardees are Richard Hynes of MIT and Erkki Ruoslahti of the Sanford Burnham Prebys Medical Discovery Institute. The award recognizes their independent discoveries concerning the integrin proteins, which are key mediators of cell-matrix and cell-cell adhesion in physiology and disease. Springer and his group are long-time users of GM/CA beamlines. They have solved structures of many important integrin complexes, for example the integrin $\alpha V\beta 6$ bound to a transforming growth factor- $\beta 1$ precursor (pro-TGF- $\beta 1$) dimer (Dong et al. & Springer (2017) [Nature](#) 542, 55). The Albert Lasker Award, one of the most prestigious honors in biomedical research, is bestowed by the Lasker Foundation whose mission is to improve health by accelerating support for medical research through recognition of research excellence, advocacy, and education.

APS Upgrade (APS-U)

At 8 AM on April 17, 2023, user experiments at the APS will end, and shortly after that, a year-long process will begin to install a new, upgraded storage ring, known as APS-U. In Spring 2024, upon completion of the installation and commissioning of the new storage ring, APS-U will deliver a smaller, hotter beam to all beamlines. At GM/CA, we will use the dark period to implement several upgrades and improvements to our beamlines (see "Upgrades and Micro-Focusing" elsewhere in this Newsletter). Commissioning of our upgraded beamlines will begin as soon as we receive X-rays from APS-U. We anticipate taking three months to re-commission the beamlines before ramping up the user program. It will be an exciting time, and we look forward to welcoming you back to take advantage of the small, hot beam at GM/CA.

Combining the small APS-U source and new optics, GM/CA will provide a focused 5-micron beam with at least 5-times more intensity to the sample than today's full beam, and a micro-focused (~1-micron) beam with about the same intensity as today's full beam. These hot beams will enhance our serial crystallography capabilities with crystals in a stream (viscous-jet) or a fixed mount. Other improvements include a new endstation with greater stability for smaller crystals and beams, an improved data processing and analysis pipeline, and a new high-efficiency pixel array detector to replace the aging Pilatus.

But until then, the APS dark period will temporarily remove one-half of the nation's synchrotron capacity for macromolecular crystallography. Thus, there will be strong competition for beam time at other synchrotrons: [NSLS-II](#), [SSRL](#), [ALS](#), [CHESS](#), and international sources. If you have not already made plans to use other facilities, we encourage you to contact beamline staff at the links above to learn about their capabilities, sample mount requirements, and beamtime access modes. Some sources support the Block Allocation Group (BAG) beamtime access mode, where several research groups apply together and split the beamtime amongst themselves. BAGs may provide groups with more frequent access and reduce the administrative burden on the facilities from the influx of APS users.

Automounting systems differ among beamlines. However, most beamlines accept universal pucks (UniPucks) and SPINE pin bases. Please check with the beamline staff at the specific beamline you plan to use.

We will use this Newsletter and the GM/CA website (<https://www.gmca.aps.anl.gov/>) to inform you of our progress during the dark period and plans to ramp up user access at GM/CA. The APS-U webpage (<https://www.aps.anl.gov/APS-Upgrade>) will provide information about the status of the storage ring replacement. If you have questions, [please reach out to any GM/CA staff member](#). (R. Fischetti & J. Smith)

Upcoming User Deadlines

Current APS Cycle: 2022-3 (October – December)

Last Day of Beam: December 19

Please contact us if you need time before the end of the year

Last APS Cycle Before the APS-U: 2023-1 (February - April)

Proposal Submission Deadline has passed and scheduling efforts are underway (please contact us/submit rapid access proposals for this cycle ASAP)

Cycle Start Date: January 31, 2023

Cycle End Date: April 17, 2023

[APS User Deadlines Website](#)

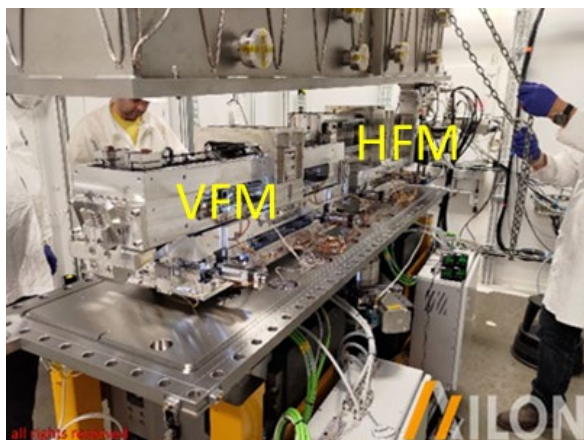
GM/CA Data Analysis Update

The data analysis pipelines at GM/CA currently support structure determination that can be completed in a short time on the GM/CA computers, such as by anomalous diffraction (automatic if anomalous signal is detected) or molecular replacement using a probe (from a PDB file defined in the screening spreadsheet). Developments underway will incorporate more powerful molecular replacement pipelines that use models from latest prediction algorithms, such as AlphaFold2, on supercomputers at the Argonne Leadership Computing Facility (ALCF). (Q. Xu)

Beamline Upgrades & Micro-Focusing

Last spring, we reported the removal of our aging x-ray focusing mirrors at beamline 23ID-D, reconfiguring the components surrounding the new mirror tank, surveying, and installing the Horizontal Focusing Mirror (HFM) into its vacuum chamber. Before installation, the HFM was characterized using the Long Trace Profiler (LTP) in the APS metrology lab. The measured slope error was ~ 75 nrad when mounted in the bender, surpassing our specifications and ~ 30 times better than the old bimorph mirrors. The Vertical Focusing Mirror (VFM) characterization in the bender was still in progress, so we closed the mirror tank without the VFM and provided a focused beam using only the CRL translocator during the 2022-1 run.

Characterizing the VFM was more difficult and time-consuming due to the need to adjust four gravity compensators. The motorized benders are adjusted to curve the mirror into an elliptical shape to focus the beam, while the manual gravity compensators are adjusted to remove the sagging of the mirror under its own weight. The VFM was measured to have a slope error of 80 nrad and an RMS height error of < 2 nm after mounting in benders and adjusting the gravity compensators, well below the GM/CA specifications. During the APS shutdown in May, the VFM installation and surveying were done in the presence of Andreas Schacht from AXILON (mirror system vendor). The new mirrors were commissioned at the beginning of 2022-2 run, and with the APS source, we achieved a hot beam with a minimal focal size of 55×5 microns (H \times V, FWHM) and over 10¹³ photons/sec. The intensity through the mini-beam collimators is ten times greater than when using only the CRLs and two times greater than when using the old bimorph mirrors. With the APS-U, the new mirrors should focus the beam to < 5 microns (FWHM) in both directions resulting in a 5-micron beam with greater intensity than the current full beam. We have shown that when using only the Compound Refractive lens (CRL) translocator, the minimum beam size is 2-3 microns over the 5-35keV energy range. Commissioning is in progress to utilize both the mirrors and the CRL translocator to achieve an intense 1-micron beam, fulfilling GM/CA's long-term goal of providing a true micro-focus beam. With the APS-U, the intensity of the 1-micron beam will increase by up to two orders of magnitude, enabling the study of ever more challenging samples. (R. Fischetti & N. Venugopalan)



Left: The HFM and VFM can be seen in their benders on the support structure. The lid of the vacuum chamber is being lowered into place. Right: Andreas Schacht (right) and Oleg Makarov (left) are working on the mirror controls system.

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 ODO12289 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 ODO12289."