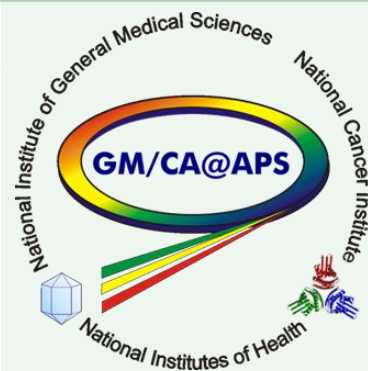# GM/CA @ APS Newsletter

Fall 2021

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 crystallography

GM/CA @ APS

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SARS-CoV-2 Research Highlight

"Structures provide insight on how SARS-CoV-2 variants evade neutralizing antibodies"

As the COVID-19 pandemic persists, SARS-CoV-2 variants have arisen that resist the neutralizing capabilities of the human immune system. These Variants of concern have included Alpha, Beta, Gamma, Delta and more recently Omicron. The Beta and Gamma variants include mutations in the receptor binding site (RBS) of the SARS-CoV-2 spike protein at residues Glu^{484}, Lys^{417}, and Asn^{501}, for example. Ian Wilson's group of The Scripps Research Institute and collaborators have compared structures of numerous complexes of neutralizing antibodies with the receptor binding domain (RBD) of the SARS-CoV-2 spike protein to visualize the detailed interactions that are disrupted when mutations occur. Combined with other biophysical and cellular techniques, these insights help to explain why neutralizing antibodies (nAbs) are less effective against variants first identified in South Africa (B.1.351, aka Beta) and in Brazil (P.1, aka Gamma), and point to other regions of the RBD that may be less vulnerable to immune escape. *(M. Becker)*
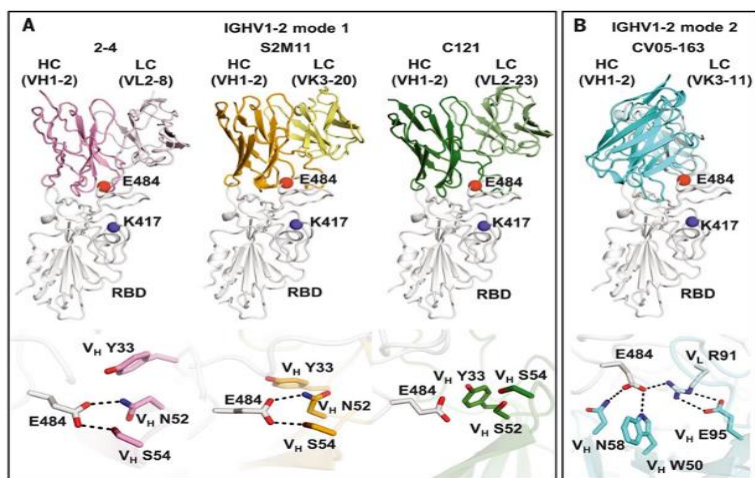

Fig. 1. Neutralizing antibody interactions with Glu484 of the spike RBD in 2 different binding modes. Antibody variable regions are shown in dark (heavy chain, HC) and light (light chain, LC) colors for antibodies with the most frequently elicited heavy-chain family (IGHV1-2). In the Gamma variant (P.1), Glu484 is replaced by Lys.

STAFF PROFILE— SHENGLAN XU

Shenglan Xu is a principal beamline engineer at GM/CA with over 30 years' experience designing and developing synchrotron x-ray instruments for insertion devices and for macromolecular crystallography experiments. She has made major contributions to the design, construction and commissioning of the GM/CA beamlines. Shenglan has a record of proud service to the APS (see photo) and has been with GM/CA since 2002, one of our staff members with longest tenure in the group. She joined the APS in 1991 and, before joining GM/CA, was involved in the design and development of insertion devices and the associated vacuum systems. Shenglan played a major role in the design and development of the soft x-ray nanoprobe at 2ID-B and was involved in the development of the fluorescence microprobe and microdiffraction microscope at beamline 2ID-D and 2ID-E.

Shenglan's physics and engineering expertise has been critical to the design and fabrication of many beamline and endstation components at GM/CA. For the experimental endstations, she designed and developed the YAG diagnostic devices and several generations of the award-winning GM/CA mini-beam collimators (R&D100 Award, "Hard X-ray Uni-Body Quad Collimator for Structural Biology", 2010). The latest version of this device is in use at GM/CA and at many beamlines around the APS ring. Shenglan works closely with other GM/CA staff on many projects such as the design of the optics upgrade, fabrication and installation of the new transfocator with compound refractive lenses (CRLs), and our high-capacity automounters. She also designed several components for our implementation of the SONICC crystal visualization system and for serial crystallography at GM/CA. Presently, she plays a significant role in the beamline optics upgrade, which will exploit the capabilities of the APS-U. Shenglan's contributions to this multi-faceted project include providing optical simulation analysis, conducting design reviews with the vendor, and preparing layout designs to integrate the optical and endstation components at both ID lines. She has been co-author of 49 peer-reviewed publications, most related to instrumentation development, and also holds a patent ("Pin base sensor for high-throughput macromolecular crystallography", US Patent 8,223,921, 2012. O. Makarov, S. Xu and R. F. Fischetti). She also received an Argonne Pacesetter Award ("Design construction and installation of vacuum chambers for the APS storage ring", 1995) and an Impact Argonne Award (2020). *(J. Smith)*

GM/CA IN COVID

Another year has passed, and SARS CoV-2 variants, Delta and more recently Omicron, have reminded us that the virus is still with us. On the other hand, in the past 12 months we have seen a series of amazing biomedical research achievements: mRNA vaccines from Pfizer/BioNTech and Moderna and a protein vaccine from J&J that reduce the risk of SARS-CoV-2 infection, effective antibody cocktail treatments for COVID-19, and more recently vaccine boosters. On the near horizon are oral pharmaceuticals from Merck/Ridgeback (Molnupiravir) and Pfizer (Paxlovid) that may provide early treatment for mild to moderate cases of the disease. A strong foundation of structural biology underlies most/all of these preventatives and treatments.

Given the progress in vaccination and treatments and in everyone's behavior, restrictions on our on-site presence has eased somewhat allowing us to have a greater number of people on-site to support user science and beamline development. Mandatory vaccines have joined the list of requirements along with masks, social distancing, proximity sensors and video meetings. Almost all beamline use has been remote. At this time, on-site experiment access for users is only available upon request, On-Site User Request Form | Advanced Photon Source (anl.gov). Requests should be submitted well in advance of your beam time and should be well justified. Approval from APS management is not guaranteed.

As an alternative to interacting with GM/CA staff on-site, we are providing a Tuesday Morning Beam Time Q & A as an opportunity for users to ask about beamline features they may want to use, experimental design, beamline capabilities, and finally a test drive of the beamline controls and data analysis software. including a new and improved GUI (see Software Update elsewhere in this Newsletter). For details on the Beam Time Q & A, reach out to Craig Ogata (ogata@anl.gov) or the designated host for your beam time.

We hope that in the coming year, the restrictions of the "new normal" will be reduced enough that we will see some of you in person again - well before the planned APS upgrade shuts down the source in April of 2023. *(C. Ogata)*

UPCOMING USER DEADLINES

APS Cycle: 2022-1 (February – April)
Proposal Submission Deadline has passed and scheduling efforts are underway (please contact us/submit rapid access proposals for this cycle ASAP)

APS Cycle: 2022-2 (June – September)
Proposal Submission Deadline: March 4

APS User Deadlines Website

RESEARCH HIGHLIGHTS CONTINUED

First Look at a Catalytically-Relevant Protease Structure involved in Childhood Leukemia, Glioblastoma, and other forms of Cancer

Taspase1 is an N-terminal nucleophile hydrolase that initiates oncogenesis by cleaving human mixed-lineage leukemia (MLL) nuclear protein. It is overexpressed in primary human cancers, and is involved in coordinating cell proliferation, invasion, and metastasis in a range of cancers, including childhood leukemia, glioblastoma, and colon and breast cancers. It is synthesized as a proenzyme (zymogen), which is auto-cleaved into N-terminal (α) and C-terminal (β) subunits that remain associated as $\alpha_2\beta_2$ heterotetramers; the newly-freed N-terminal Thr^{234} of the β subunit serves as the catalytic nucleophile. In crystals of a catalytically-relevant heterotetramer, a large disordered region at the C-teminus of the α subunit, i.e. the sequence immediately preceding Thr^{234} before cleavage, made it difficult to understand the heterotetramer assembly. Jose Martin-Garcia and Petra Fromme's group at Arizona State University showed that the disordered region is essential to full taspase catalytic activity and then employed the clever idea to create circularly permuted versions of the protein, where short linkers were used to swap the coding sequences and fuse the β subunit to the N-terminus of α. They solved a structure in which the formerly disordered C-terminus of the α subunit is ordered as a long α-helix. The structure provided insights that may assist in designing inhibitors to Taspase1 to help battle diverse cancers. *(M. Becker)*

Nirupa Nagaratnam, Silvia L. Delker, Rebecca Jernigan, Thomas E. Edwards, Janey Snider, Darren Thifault, Dewight Williams, Brent L. Nannenga, Mary Stofega, Lidia Sambucetti, James J. Hsieh, Andrew J. Flint, Petra Fromme, Jose M. Martin-Garcia, "Structural insights into the function of the catalytically active human Taspase1," Structure 29 (8), 873-885 (2021). DOI: 10.1016/j.str.2021.03.008
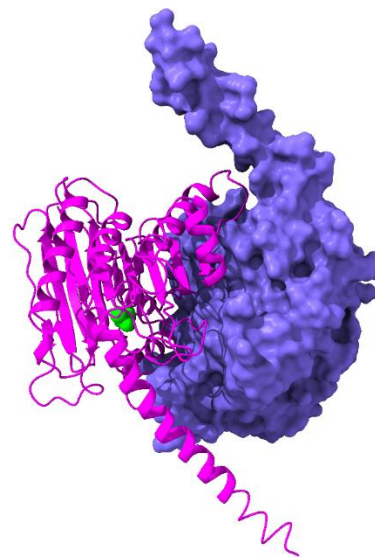
Fig.2. Crystal structure of the fully activated Taspase1. Cartoon and surface representation of the hetero-tetramer of Taspase1. The newly discovered element, the long fragment, adopts a helical conformation and points away from the dimer. The nucleohphile residue Thr234 at the catalytic site is shown in one of the monomers as green spheres

CCP4 School @ the APS

CCP4 and GM/CA successfully held the 13th - and first virtual - CCP4/APS School in Macromolecular Crystallography (June 14-25, 2021). This followed a one-year pandemic hiatus. The intensive two-week course involved 28 students from the US and Canada and 30 lecturers and instructors from Europe and the US. The students learned and practiced techniques for data collection, data processing and structure solution and validation using data they collected at a GM/CA beamline or their previously collected data. They also had help from experts in tackling challenging problems with their projects. Although we would love to see an on-site 14th CCP4/APS School in 2022, it is currently unclear whether this will be feasible. Please watch the CCP4 bulletin board ccp4bb (http://www.jiscmail.ac.uk/lists/ccp4bb.html) for announcements or query ccp4school@anl.gov directly if you are interested in attending the 2022 School. *(Q. Xu)*

APS UPGRADE (APS-U)

The current APS storage ring will be shut down in 16 months. The APS storage ring will be removed and replaced with a new state-of-the-art storage ring, the APS-U. The removal and installation are planned to take 12 months and there will not be any x-rays during that period. The shutdown is currently scheduled to begin in mid- 2022, with operations resuming at the upgraded APS one year later. However, this schedule is strongly dependent on vendor deliveries and on-site assembly work over the next years, both of which have seen delays due to the COVID-19 pandemic. APS leaders continue to evaluate the schedule in light of those effects and will provide updates as early as possible. You can find the latest information at https://www.aps.anl.gov/APS-Upgrade.

The APS-U upgrade will change the shape of the APS x-ray beam (like the cross-section of a pancake) to a nearly circular cross-section and will vastly increase the beam brightness by confining all photons to the smaller cross-section. The new focusing optics that we are installing will efficiently collect the x-rays from the new source an increase the intensity by over 100-fold for mini-beams (5-20 micron) and provide $\sim 1\times10^{13}$ photon/sec in to a 1-micron beam! This will provide some exciting new opportunities that will enhance our serial crystallography capabilities and enable time-resolved measurements. If you have a project in mind that would benefit from these new capabilities please let us know so we can help bring them to fruition. *(R. Fischetti)*

GM/CA SOFTWARE UPGRADE

GM/CA is proud to announce the release to users of a new Graphical User Interface (GUI) for beamline controls, data acquisition and cluster data processing with the same familiar interface of JBluIce. The new program, named PyBluIce and implemented in Python, is the result of 2 years of intensive development by GM/CA developers Mark Hilgart and Qingping Xu with feedback from staff crystallographers.

PyBluIce will replace the Java-based JBluIce GUI, which we have provided to GM/CA users since 2011. (In the first 2022 run, users will see icons for both GUIs.) While PyBluIce implements all the functionality of JBluIce with the same look and feel, it includes a completely new, modular data collection server that will soon be used to implement features supporting serial crystallography, High Data Rate Macromolecular Crystallography (HDRMX) and higher levels of automation. Although this upgrade is primarily to enable those next generation features yet to come, this first version includes some long-requested features by GM/CA crystallographers and developers that were made possible by the rewrite.
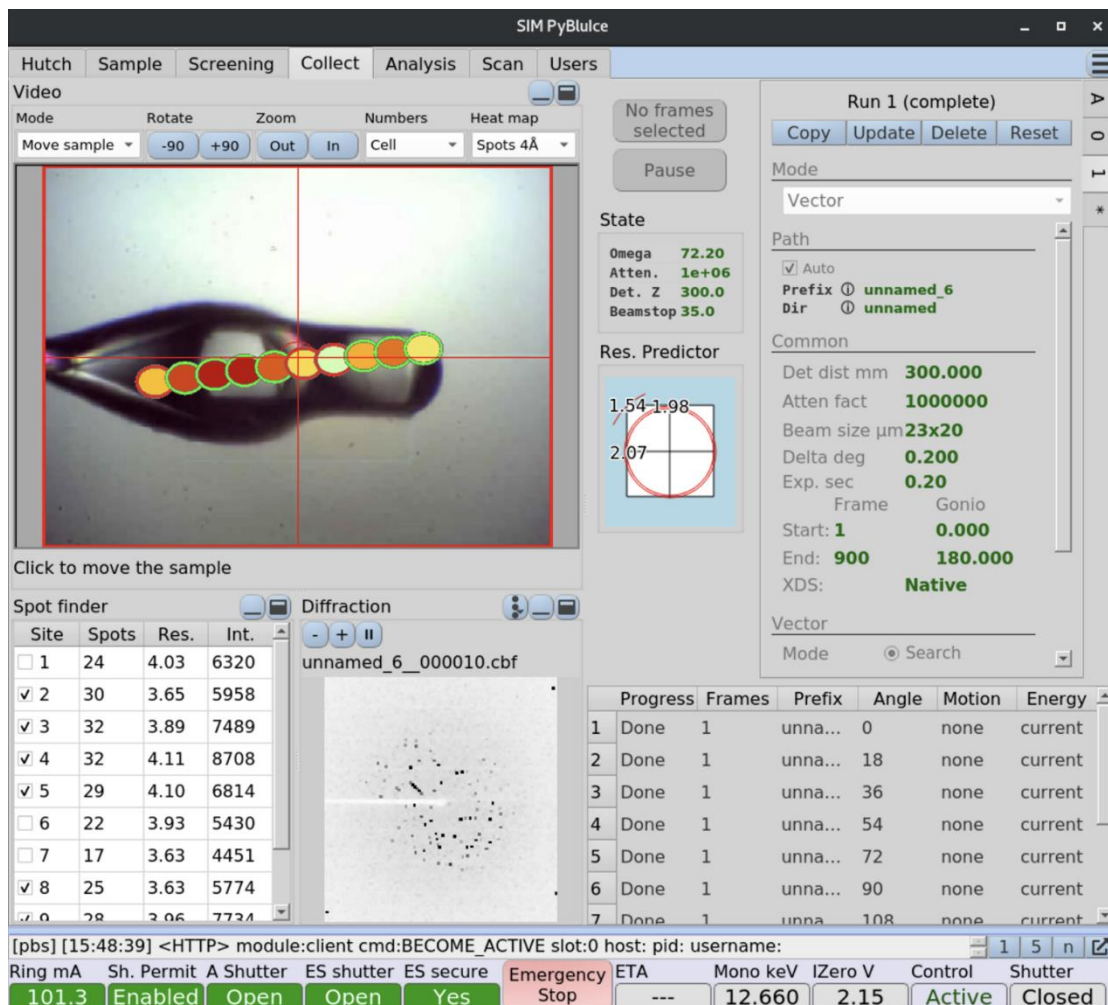
Our initial release contains the following enhancements:

Automation
- Beam center, detector distance, and energy calibration using small molecule standard

Helical
- Helical range avoidance for deselected sites
- Vector search heat map

Site collect
- Import of collect sites from the raster mesh filter or by selecting cells from the heatmap
- Scriptable import for various types of sample mounts
- Capability for collect frames to be spanned across all sites or repeated on each site

Raster
- Mesh (filter) select redesigned for the new site mode
- New grid view for search areas larger than the camera field of view

Data viewing
- Adxv socket connection for improved load times when following data collection
- Resizeable image, data and camera views throughout the application

Feature Highlight: Helical Range Avoidance

Helical collections are easily planned with PyBluIce to only use portions of the vector that diffract well. An initial search generates a heat map (see screenshot) and images that can be visually inspected by users. Then by clicking "off" the sites that are not high quality, the remaining sites are assembled into sub-vectors and frames are distributed among them with equal spacing.

Feature Highlight: Site Collect

PyBluIce's new site collect mode gives users more options to manage and collect across 3D sites defined on a sample. From raster, sites may be added from grid cells individually or all at once with the mesh filter. The site list may be pruned or added to using optical centering. For mounts with a known pattern of collect sites and fiducial markers, sites may be imported with a script. Then once a site list is created, a data collection run can be spanned across all sites or repeated on each site, with optional inverse beam and multi-energy collection modes.

In addition to the new PyBluIce GUI, a data processing web server was implemented by Qingping Xu. Users now have two routes to access the data processing pipelines: using a web browser independent of data acquisition and through PyBluIce, where the interface is similar to the familiar interface of JBluIce. *(M. Hilgart)*

BEAMLINE UPGRADES & MICRO-FOCUSING

A long-term GM/CA goal has been to replace our aging x-ray focusing systems with state-of-the art optics that create a true micro-focus beam for study of ever more challenging samples. Last Spring, we reported that a Compound Refractive Lens (CRL) transfocator was installed on 23ID-D, restoring mini-beam intensities to values before the current focusing mirrors degraded. We are now commissioning the transfocator for its intended purpose of providing a ~1-micron beam over a wide energy range (5 – 35 keV) and have achieved a 2-3 micron beam over that energy range. We believe the minimum beam size is limited by the vibrational stability of the positioning table now supporting the transfocator. This temporary table allowed us to restore the mini-beam intensities and gain valuable experience commissioning the micro-focus capability while a new high-stability table is fabricated. The APS source has a very wide horizontal dimension of ~650-micron FWHM. To create a micron-sized beam, one uses a slit to define a smaller source size, albeit with a significant intensity reduction. Thus the micro-beam intensity is low compared to the mini-beams (5/10/20 micron) and decreases with increasing energy. Nevertheless we collected good diffraction data from some small lysozyme crystals at energies up to 35 keV. A key component of the high energy data collection was a borrowed pixel array detector with high sensitivity over the wide energy range (Dectris Eiger2 4M with CdTe sensors). With the small horizontal source size of the upgraded APS (see APS Upgrade section), we will not need a slit and can image the undulator source directly, resulting in significant increases in the micro- and mini-beam intensities. This will enable data collection from smaller, more weakly diffracting crystals than are useful today.

The new mirror focusing system for 23ID-D has arrived (see picture). The system has horizontal and vertical focusing mirrors arranged in a Kirkpatrick-Baez geometry. (The configuration is named for its designers, one of whom was the singer Joan Baez's father.) Axilon AG designed the mirror system to our specifications and incorporated mirrors fabricated by the JTEC Corporation. The mirrors are mounted in benders that curve each mirror to the proper elliptical shape to focus the beam. Initial metrology results indicate that, when bent, the mirrors have slope errors of <100 nrad, which is ~20 times better than the mirrors they will replace! The lower the slope error the better the mirror will image the source without distortion and produce a hotter focused beam. We plan to install the system in January 2022 and commission it in February. The new mirrors should focus the APS source to ~60 × <5 microns (H×V, FWHM) and deliver a hot beam with over 10^{13} photons/sec. The intensity of the mini-beams will also increase compared to the CRL focusing now in place. The mirrors will also be used with the CRLs to position both the horizontal and vertical foci at the sample position, resulting in a smaller beam size and also increased rejection of higher harmonic x-rays compared to using the CRLs alone. With the APS-U, the new mirrors should focus the beam to <5 micron (FWHM) in both directions. This will result in a 5-micron mini-beam with greater intensity than the current full beam.

The new Kirkpatric-Baez mirror system being unpacked by Naga Venugopalan (left) and Dale Ferguson (right). Naga and Dale are leaning on the mirror tank, NOT the mirrors. The beam entrance and exit ports are visible on the left and right sides of the tank, as are vacuum ports on the top and the granite plinth below. The granite will provide a stable base for the mirrors and tank. *(R. Fischetti)*