GM/CA @ APS Newsletter

Spring 2021
Volume 1, Issue 1

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

INSIDE THIS ISSUE

NEW FUNDING FOR GM/CA @ APS!

Two NIH Institutes, the National Institute of General Medical Sciences (NIGMS, "GM") and the National Cancer Institute (NCI, "CA"), have supported GM/CA since our beginning at the turn of the century. We're pleased to announce that our NIGMS support is now through a new grant (P30 GM138396), while the NCI continues to support us through an interagency agreement (ACB-12002). When you publish results from GM/CA, please acknowledge our NIGMS and NCI support with text from here.

Our NIH sponsors need information about your grant support. Please continue to provide this information through the APS End of Experiment Form after your beam time. (*J. Smith/R. Fischetti)*

UPCOMING USER DEADLINES

APS Cycle: 2021-2 (June – August)

Proposal Submission Deadline: March 5 (please submit rapid access proposals for this cycle ASAP)

APS Cycle: 2021-3 (October – December)

Proposal Submission Deadline: July 2

APS User Deadlines Website

GM/CA @ APS Sponsors:

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

GM/CA @ APS

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Photo courtesy of Mike's wife, Anne Mulichak

STAFF PROFILE— DR. MICHAEL BECKER

This is the first in a series of highlights of GM/CA staff members.

Michael Becker is a protein crystallographer at GM/CA. He grew up in Havre de Grace, Maryland, and obtained undergraduate degrees in Biology and Chemistry from the University of Delaware. He completed his PhD in Biochemistry with Bill Parson at the University of Washington for fast spectroscopy of bacterial photosynthetic reaction centers and continued his studies of light-driven processes as a postdoc with Robert Huber at the Max-Planck-Institut für Biochemie in Martinsried, Germany, where he studied light-harvesting phycobiliproteins. He returned to the US as a postdoc with Jeff Bolin at Purdue University where he studied the nitrogen-fixing nitrogenase enzyme, and then with Cathy Lawson at Brookhaven National Laboratory for studies of an outer surface protein from the Lyme disease pathogen, *Borrelia burgdorferi*. His Brookhaven experience led to an appointment as a beamline scientist at the National Synchrotron Light Source in 1999 and his eventual move to GM/CA in 2006.

Many GM/CA users know Michael as an outstanding user host. He also plays a leading role in running the user program, and a critical role in testing improvements to beamline hardware and software so that new features work flawlessly for users. You may also know Michael as our leader of user outreach: he coordinates the announcement of important user publications on the GM/CA, APS, and Argonne websites. If you are about to publish an important paper, please contact him at mbecker@anl.gov before publication and share your institution's press release so we can help bring your work to a broader audience.

Michael has co-chaired a number of scientific workshops and sessions, and is currently organizing a workshop on COVID-19 and future One Health directions along with colleagues at SBC CAT and NE CAT.* His research interests include zoonotic, vector-borne, and animal diseases; diffraction and imaging methods; and One Health. He is also passionate about wildlife conservation, and is pictured here in the pioneering Selenkay conservancy on community-owned land near Amboseli National Park in Kenya. *(J. Smith)*

GM/CA IN COVID

As we approach the one year mark of the impact of COVID-19 on GM/CA operations, we can look back to see the year that was. On March 23, 2020, COVID-19 shut down the lab to onsite experimenters for safety precautions. Only remote users working on COVID-19 and critical pharmaceutical research projects were allowed access to the beamlines. The GM/CA beamlines were restricted to one staff member on-site per day for both beamlines. From May 21st to the end of May, the restrictions on user projects were lifted. After a brief ring shutdown, the 2nd run of the year started on June 15th, and beamline operations were upgraded to one staff member allowed on-site per beamline with minimum overlap. Pent-up demand for beam time in June, from university researchers, led to a bump in beam time requests followed by a lull in July. By the end of the run in August, the beamlines were running at near capacity in the new normal. We are now up to four staff members on-site per day, and beamline upgrades for focusing optics on 23ID-D are near completion.

Today, remote operation remains the only mode of access for users. This was only a minor imposition since 97% of the user visits were already remote at the time of the shutdown. The few on-site visits were long-time collaborations on beamline upgrades that are now on hold. Safety restrictions limit on-site access to four staff members, two per beamline. Mandatory COVID-19 restrictions - masks, social distancing, hand washing and contact tracing - remain in place. As with everyone else, the pandemic has also affected interactions within our group. Group meetings are now a weekly virtual event.

Conversations were replaced by a constant bombardment of emails and Microsoft Teams notifications. We anxiously await vaccination and the next stage of the evolving return to the new "normal".

Finally, in an attempt to regain contact with our users, we are starting a new program to communicate prior to a user group's beam time. This is an opportunity for users to talk to staff members about their upcoming beam time. This Beam Time Q & A will be held on Mondays and will serve as an opportunity for users to ask about features they may want to use, experimental design, beamline capabilities, and finally a test drive of the beamline software using the GUI. For details, reach out to Craig Ogata (ogata@anl.gov) or the designated host for your beam time. *(C. Ogata)*

SOFTWARE UPDATES

New Face to GM/CA User Interface

GM/CA's own JBluIce data acquisition program will soon be gaining new features along with a new name: PyBluIce. Some of these features require big changes in the way the program works, so we are starting an extensive test program to prepare for the roll-out of PyBluIce. In the first release, users can look forward to a larger variety of data collection tools, along with a more ergonomic interface. Following that will be new serial crystallography modes and fully automatic data collection including raster-based centering and multi-site strategy-based data collection.

(M. Hilgart)

Data Processing Pipeline Update

GM/CA JBluIce currently supports multiple data processing pipelines that can handle both simple and complex data collection schemes. The current developments focus on our transition to PyBluIce, web access, automation, and better capability to handle more challenging datasets, including data from serial crystallography experiments.

(Q. Xu)

RESEARCH HIGHLIGHTS

Synthesis of a Potent Antibiotic Follows an Unusual Chemical Pathway

Thiostrepton, a complex, bicyclic peptide natural product, exhibits strong potency against Gram-positive pathogenic bacteria by inhibiting protein translation and, notably, also targets breast cancer cells. It is a topical veterinary drug, but its use in humans requires improved absorption properties. TsrM (tryptophan 2C methyltransferase) catalyzes the committed step in the biosynthesis of the thiostrepton quinaldic acid moiety -- methylation of an L-tryptophan indole C2 atom. TsrM is annotated as a cobalamin-dependent "radical SAM" (*S*-adenosylmethionine) enzyme, however it does not reductively cleave SAM to a 5'-deoxyadenosyl 5'-radical intermediate, a hallmark of radical SAM enzymes. The groups of Squire Booker (Penn State University) and Catherine Drennan (Massachusetts Institute of Technology) investigated the TsrM catalytic activity and its structure with experiments at GM/CA@APS, LS CAT, and the ALS. The structures revealed a [4Fe-4S] cluster ligated by a glutamate and three cysteines in a canonical CXXXCXXC radical SAM motif. Structures of substrate complexes suggest an unusual mechanism, wherein a first SAM methylates the cobalamin, the carboxylate of a second SAM deprotonates the substrate tryptophan N1, and the resulting C2 carbanion accepts a methyl group from methylcobalamin. The non-radical mechanism is a "radical" departure from all other characterized radical-SAM enzymes and is an example of the many surprises yet to come from the >100,000-members of the superfamily. *(M. Becker)*

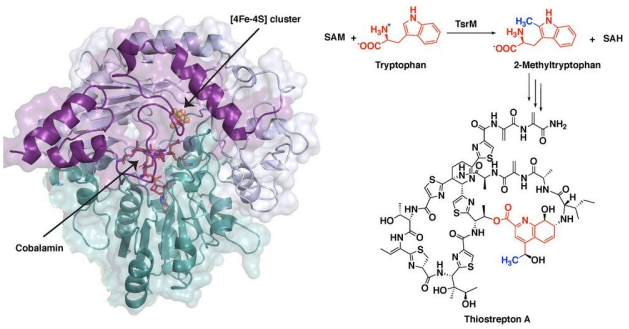

Figure: (Left) native form of TsrM, (Right) steps in thiostrepton biosynthesis.

Hayley L. Knox, Percival Yang-Ting Chen, Anthony J. Blaszczyk, Arnab Mukherjee, Tyler L. Grove, Erica L. Schwalm, Bo Wang, Catherine L. Drennan, and Squire J. Booker, "Structural basis for non-radical catalysis by TsrM, a radical SAM methylase," Nat. Chem. Biol., published on line 18 January 2021. DOI: 10.1038/s41589-020-00717-y

Llama-derived nanobodies efficiently neutralize SARS-CoV-2

While there is much focus on vaccines in preventing COVID-19 disease, the need for effective therapeutics to treat those who are infected is also essential. Monoclonal antibodies (mAbs) against the SARS-CoV-2 spike protein have shown therapeutic efficacy in helping patients avoid the worst effects of the disease, but they have drawbacks. Scale-up for large numbers of patients is complex due to the production in mammalian cells and intravenous delivery, and single mAbs are vulnerable to viral escape mutations. To address these challenges, a three-group collaboration (Cheng Zhang and Yi Shi at the University of Pittsburgh and Dina Schneidman-Duhovny of The Hebrew University of Jerusalem) immunized a llama with the recombinant receptor binding domain (RBD) of the SARS-CoV-2 spike in order to produce natural camelid antibodies, which could then be expressed as single-chain nanobodies. They identified thousands of high-affinity nanobodies, with ~350 different complementarity-determining regions. In subsequent assays, they identified three potently neutralizing nanobodies and mapped their distinct epitopes on the RBD using cross-linking mass spectrometry and integrative modelling. With data from GM/CA, they obtained a high-resolution crystal structure of one nanobody bound to the RBD. These studies led to further engineering of multivalent nanobodies that have ultra-high neutralizing potency and may also be recalcitrant to mutational escape. As nanobodies can be produced in large quantities in bacteria, are thermally stable, and may be delivered via a nebulizer, this approach shows great promise for treating COVID-19 and other diseases. *(M. Becker)*

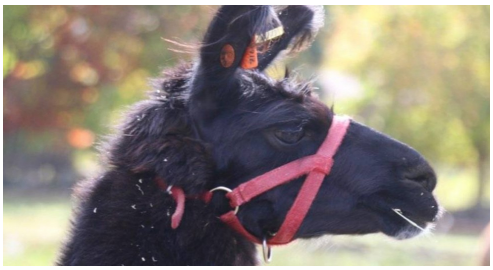

Figure: Wally, the llama (Lama glama), provided nanobodies for the study.

Yufei Xiang, Sham Nambulli, Zhengyun Xiao, Heng Liu, Zhe Sang, W. Paul Duprex, Dina Schneidman-Duhovny, Cheng Zhang, Yi Shi, "Versatile and multivalent nanobodies efficiently neutralize SARS-CoV-2," Science 370, 1479-1484 (2020). DOI: 10.1126/science.abe4747

ANNOUNCEMENTS

CCP4 School @ the APS

CCP4 and GM/CA will host a virtual workshop on June 14 - 25, 2021. The problem-solving oriented CCP4/APS workshop is the only such school in the US and has been very popular with students. Students with challenging problems in their projects are encouraged to apply by April 1st, 2021 (https://www.ccp4.ac.uk/schools/APS-2021/).

(Q. Xu)

Dynamic X-ray Crystallography Workshop

David Kissick (GM/CA), Darren Sherrel (SBC), and Rob Henning (BioCARS) have organized a full day training workshop about serial macromolecular crystallography for this year's virtual APS/CNM User Meeting. It will include scientific talks about serial crystallography results from each beamline in the morning. The afternoon will focus on practical demonstrations of sample delivery and data processing methods used at each beamline. For more information or to register you can visit the User Meeting website.

(D. Kissick)

Advances in COVID-19 Prevention and Treatment Enabled by Structural Biology Research Workshop

Michael Becker (GM/CA), Karolina Michalska (SBC CAT) and Kay Perry (NE CAT) have organized a workshop on advances in COVID-19 prevention and treatment for the virtual APS/CNM User Meeting in May. The workshop will present areas where structural biology research, including macromolecular crystallography and cryoelectron microscopy, intersects with *in vivo*, *in vitro*, and *in silico* studies of SARS-CoV-2 and COVID-19. Additional presentations will describe pandemic preparedness from a One Health perspective. The agenda and list of leading speakers is here:

COVID-19 Workshop Agenda

No-fee registration information is here:

APS User Meeting Registration

Please share!

(M. Becker)

BEAMLINE UPGRADES & MICRO-FOCUSING

The 16-year-old GM/CA beamlines are deteriorating and have several outdated components. Over the next few years, a major hardware upgrade will increase the intensity and reduce the size of the beam without compromising either the beam stability or its flexibility of use. To minimize the impact on user beam time, the upgrade is proceeding in phases. We will replace all the mirror systems, install new endstation tables and sample environments, add compound refractive lenses (CRLs) for micro-focusing, and refurbish the infrastructure of Sector 23.

In September 2020, we achieved an immediate goal to restore the mini-beam intensities to the values prior to degradation of the focusing mirrors on 23ID-D when we installed CRLs in a compact vacuum enclosure known as a transfocator. This allowed us to expand the energy range to 6 – 20 keV (from the restricted 11 – 13 keV). The entire focusing unit is truly compact; only 60 cm long, as can be seen in the photo of Steve Corcoran standing behind it. Our longer-term goal is for true micro-focusing with the CRLs: a beam as small as 1 micron in diameter over an energy range of 6 – 35 keV.

We eagerly await the installation on 23ID-D of a latest-technology focusing mirror system that will provide a small, clean focused beam. The slope errors of the new mirrors for 23ID-D are well below our 100 nm specification, which is 20-fold better than our original mirrors! In the next phase (early 2022), we plan to install a newly designed endstation table and sample environment. This will provide a high-stability base for the CRL optics and the sample while allowing rapid switching of the focusing via the mirrors or the CRLs.

We will upgrade the 23ID-B optics and endstation to be like those on 23ID-D. The 23ID-B upgrade will occur during the shutdown for the APS upgrade (see below). The x-ray beam will be slightly larger on 23ID-B because the beamline is shorter than 23ID-D. *(N. Venugopalan/R. Fischetti)*

APS UPGRADE (APS-U)

A major upgrade of the APS known as APS-U will increase the x-ray brightness by 2 – 3 orders of magnitude depending on the x-ray energy. The installation of the new storage ring and reconfiguration of the whole facility will require a complete shutdown of the APS. 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 table shows how APS-U together with our new mirrors or our new CRLs will affect the size and intensity of the x-ray beam at the sample position on 23ID-D (12 keV, 1.033 Å). The increased brightness of the APS-U will be ideal for micro-focus crystallography, we will be able to put >160 fold more photons/s on a 1-micron crystal (0.8 μm^2 area) than we can now deliver with our 10-micron mini-beam (80 μm^2 area). *(R. Fischetti)*

	Focusing with new GM/CA mirrors			Focusing with GM/CA CRLs		
Source	**Beam size at sample (μm)**	**Full beam intensity at sample (photon/s)**	**Beam intensity to a 1-μm crystal (photon/s)**	**Beam size at sample (μm)**	**Full beam intensity at sample (photon/s)**	**Beam intensity to a 1-μm crystal (photon/s)**
APS	62 x 4	3.5×10^{13}	4×10^{11}	15 x 0.5	5×10^{12}	4×10^{11}
APS-U	~7 x 4	$\sim 7 \times 10^{13}$	$\sim 4 \times 10^{12}$	~0.75 x 0.4	$\sim 1.7 \times 10^{13}$	$\sim 2 \times 10^{13}$