GM/CA @ APS Newsletter

Spring 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 crystallography

RESEARCH HIGHLIGHT

" Structures of the σ2 receptor reveal an intramembrane ligand-binding pocket relevant to neuropathic pain, psychiatric diseases, and cancer imaging"

The σ2 receptor is an integral membrane protein that is expressed at high levels in the central nervous system and in proliferating tumor cells. Certain receptor ligands are in clinical trials or development for treating Alzheimer's disease and schizophrenia, and σ2 has been proposed as a target for cancer imaging and therapy. It differs from its cousin, the σ1 receptor, in distribution and pharmacological profile, and -- as it turns out -- in structure as well. The groups of Andrew Kruse at Harvard University and of Brian Schoichet and Allan Basbaum at the University of California, San Francisco, combined efforts with collaborators at the University of Michigan (Ann Arbor), the University of North Carolina (Chapel Hill), and Taras Shevchenko National University of Kyiv (Ukraine), to determine the first structures of the σ2 receptor and to investigate its ligand-binding properties. In contrast to the σ1 receptor, which has a β barrel cupin fold, the σ2 receptor is a four-helix bundle, which forms a dimer rather than a trimer. The ligand-binding pocket of σ2 is located inside the transmembrane region, favoring ligands with significant hydrophobic character. The ligand binding pockets of σ1 and σ2 are similar. The σ2 receptor is promiscuous with regard to binding a broad range of ligands, which gave the investigators opportunities to discover novel ligands of σ2-selective chemotype, as well to test computational ligand-docking procedures. In the tour de force effort, the investigators conducted a large-scale docking screen of 490 million virtual molecules, and synthesized 484 compounds. Of these, 31 compounds had affinities better than 50 nM, and three were optimized to achieve affinities ranging from 3 to 48 nM, with up to 250-fold selectivity versus the σ1 receptor. All three ligands decreased mechanical hypersensitivity in animal tests, suggesting a role of the σ2 receptor in neuropathic pain. This collective effort enables further investigations into the biological roles of the σ receptors, as well as design of advanced therapeutics. *(M. Becker)*
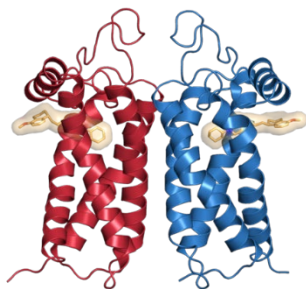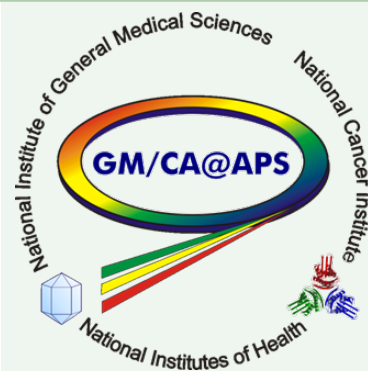

Fig 1. Crystal structure of a σ2 receptor dimer, where the membrane bilayer boundaries extend between the helical regions and the loop regions, and the PB28 ligand is shown binding to the receptor within the transmembrane region. (Figure courtesy of A. Alon, A. Kruse.)

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STAFF PROFILE— MARK HILGART

Mark Hilgart has been a software developer at GM/CA since 2006. His focus is delivering to GM/CA users reliable, structural-biologist-oriented data acquisition software supporting the latest techniques. Mark directs his efforts to solving problems that will further this goal, whether it be how to write more agile software, how to handle data more quickly, or what type of user interface is most productive for structural biologists. Mark's long-term studies of different aspects of MX acquisition software have influenced the design of the newly released PyBluIce, which will enable it to achieve some of the most difficult experimental requirements. (*M. Hilgart)*

GM/CA IN COVID

A new day has dawned at Argonne National Laboratory. In early February, lab director Paul Kearns announced the move from limited operations to inviting all employees back to the Argonne campus starting on the last day of February. The waning of the infection rate of the Omicron BA.1 SARS-CoV2 variant and the high vaccination rate, >95%, of employees paved the road to re-opening. Of course, there are mandatory restrictions such as proof of vaccination or a negative COVID-19 PCR test within 72 hours of entry to the lab, but masks and social distancing are optional except in some defined situations. Up to three experimenters are permitted on-site for your beam time, but ESAFs should be submitted 14 days in advance. The rules for on-site access are changing in a favorable direction at a fast enough rate, so it is necessary for you to consult the APS User Info webpage prior to arrival (https://www.aps.anl.gov/Users-Information/Getting-Started/Argonne-Site-Access) along with the Current COVID and site access information in the same page.

It's been two years since the pandemic forced us into remote operations. We look forward to communicating with some of you in person, but understand the convenience of remote access. Changes in software and hardware are marching on (see Software/Beamline Update sections below). Visit while you can, the Omicron BA.2 variant is lurking in the background and the possibility of another SARS-CoV2 mutant is a possibility, so don't throw away your masks, we're still holding onto our proximity sensors. We're about one year away from the APS Upgrade that is currently scheduled to begin in April 2023.

As a closing reminder, an alternative to interacting with GM/CA staff on-site, we are providing a virtual Beam Time Q & A opportunity prior to your beam time in order for you to ask about beamline features you may want to use, experimental design, beamline capabilities, and finally a test drive of the beamline controls and data analysis software. This includes a new and improved GUI (see Software Update below). Contact your host well ahead of time to schedule your session. *(C. Ogata)*

GM/CA & National School on Neutron and X-ray Scattering

GM/CA will participate for the first time in the 24th annual National School on Neutron and X-ray Scattering (NX School), which will be held jointly between Argonne National Laboratory and Oak Ridge National Laboratory, in July.
(https://www.anl.gov/education/national-school-on-neutron-and-xray-scattering).
Two half-day sessions on "X-ray Crystallography of Proteins" will be presented.

GM/CA Connection to Finalist for Inaugural Michelson Philanthropies & Science Prize for Immunology

Scott Biering was recently announced as a Finalist for the inaugural Michelson Philanthropies & Science Prize for Immunology (https://www.michelsonmedicalresearch.org/michelson-prizes-winners). He is currently a postdoctoral scholar in the laboratory of Eva Harris at the University of California, Berkeley, and is also a close collaborator of Janet Smith's group at the University of Michigan. Scott's award-winning essay, and a joint publication including key structural contributions from David Akey and colleagues in Janet's group, are cited below. Congratulations Scott!

Scott B. Biering, "One antibody to treat them all," Science 375, 830-A (2022). DOI:10.1126./science.abn9651

Scott B. Biering, David L. Akey, Marcus P. Wong, W. Clay Brown, Nicholas T.N. Lo, Henry Puerta-Guardo, FranciellTramontini Gomes de Sousa, Chunling Wang, Jamie R. Konwerski, Diego A. Espinosa, Nicholas J. Bockhaus, Dustin R. Glasner, Jeffrey Li, Sophie F. Blanc, Evan Y. Juan, Stephen J. Elledge, Michael J. Mina, P. Robert Beatty, Janet L. Smith, Eva Harris, "Structural basis for antibody inhibition of flavivirus NS1–triggered endothelial dysfunction," Science 371, 194-200 (2021). DOI: 10.1126/science.abc047

GM/CA STAFF NEWS

Michael Becker was recently elected to the Board of Directors of the Society for Science at User Research Facilities (SSURF) (https://ssurf.org/).

UPCOMING USER DEADLINES

APS Cycle: 2022-2 (June - September)
Proposal Submission Deadline has passed and scheduling efforts are underway (please contact us/submit rapid access proposals for this cycle ASAP)

APS Cycle: 2022-3 (October - December)
Proposal Submission Deadline: July 1

APS User Deadlines Website

RESEARCH HIGHLIGHTS CONTINUED

Circadian timekeeping: a balance between cooperation and competition

To synchronize with the day-night cycle on Earth, most organisms contain an internal circadian clock. The photosynthetic cyanobacterium, *Synechococcus elongatus*, serves as a model system for circadian-clock investigations at the molecular level. The core components of the circadian clock are the proteins KaiA, KaiB, and KaiC, which generate an autonomous ~24-hour rhythm of KaiC phosphorylation when assembled in vitro. However, an assembly of only core components does not transduce biological signals. To better understand the molecular mechanisms that couple the clock oscillator to signal transduction, the groups of Carrie Partch at the University of California, Santa Cruz, and Andy LiWang at the University of California, Merced, reconstituted an extended *in vitro* clock (IVC). The IVC contains a DNA duplex that carries a clock-controlled promoter, a master transcription factor (RpaA), and sensor histidine kinases (SasA and/or CikA). SasA is a KaiC-dependent kinase that phosphorylates RpaA to stimulate DNA binding and activate gene expression at dusk; CikA dephosphorylates RpaA when it associates with the Kai complex during the night. As monitored via fluorescent probes, the IVC autonomously oscillates for several days, rhythmically activating the interaction of the transcription factors with the DNA promoter element. The crystal structure of a complex between SasA and a KaiC domain illuminated the beauty of the system in molecular detail. The thioredoxin-like domain of SasA binds to KaiC in a form of molecular mimicry, imitating the binding of the active, fold-switched form of KaiB to KaiC in the core oscillator. Further biochemical studies revealed that SasA recruits KaiB molecules to KaiC in a heterotropic form of cooperativity, where KaiB eventually out-competes SasA for binding. And so the IVC cycles around and around, enabling future experiments towards an even deeper understanding of living and synthetic systems. *(M. Becker)*
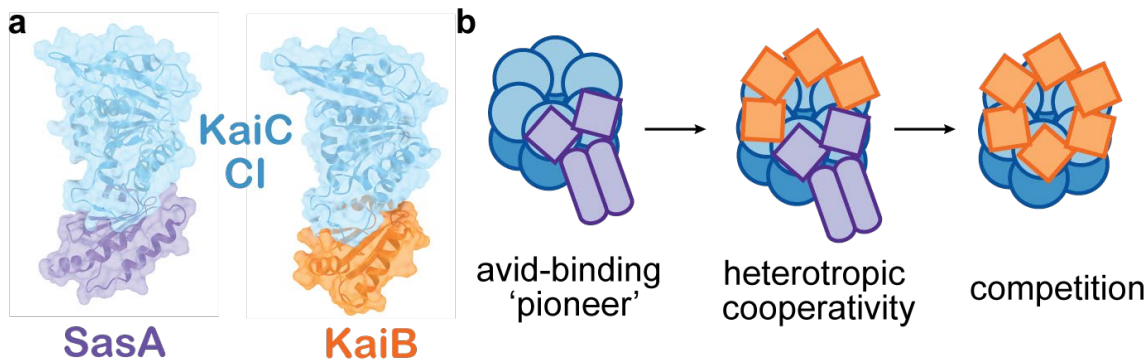

Fig. 2. (a) Crystal structures of the cyanobacterial clock proteins SasA and KaiB in complex with the CI domain of KaiC. Their analogous binding modes set the stage for a dynamic relationship characterized by a balance between cooperativity and competition. (b) SasA binds to KaiC non-cooperatively but stimulates cooperative association of KaiB monomers, which ultimately leads to the eviction of SasA from the KaiC hexamer. (Figure and legend courtesy of J. Swan, C. Partch.)

Archana G. Chavan, Jeffrey A. Swan, Joel Heisler, Cigdem Sancar, Dustin C. Ernst, Mingxu Fang, Joseph G. Palacios, Rebecca K. Spangler, Clive R. Bagshaw, Sarvind Tripathi, Priya Crosby, Susan S. Golden, Carrie L. Partch, Andy LiWang, "Reconstitution of an intact clock reveals mechanisms of circadian timekeeping," Science 374 (6564), eabd4453 (2021). DOI: 10.1126/science.abd4453

CCP4 School @ the APS

The virtual CCP4/APS School 2022 will be held on June 13-24, 2022 with an additional day of bioinformatics lectures on June 7, 2022. The deadline for applications closed on Apr 15th, 2022. We received many applications from highly motivated students with interesting projects at institutions around the world. In addition to traditional crystallographic topics, the 2022 School encompasses new developments in areas such as cryo-EM and protein modeling (e.g. AlphaFold). The schedule is available online at the CCP4/APS school website (https://www.ccp4.ac.uk/schools/APS-2022/program.php). To widen the audience, we will allow a limited number of attendees to audit the school lectures, and hope to attract senior researchers who may want to get familiar with latest developments. If you are interested, please contact Kristin Ahrens (kahrens@anl.gov) for arrangements. *(Q. Xu)*

APS UPGRADE (APS-U)

The current APS storage ring will be shut down in 12 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 April 2023d, 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 and increase the intensity by over 100-fold for mini-beams (5-20 micron) and provide ~1×10^{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 email Bob Fischetti (rfischetti@anl.gov) so we can help bring them to fruition. *(R. Fischetti)*

GM/CA SOFTWARE UPDATE

The spring run at GM/CA has seen sustained software testing of PyBluIce from the user's perspective to ensure that every interaction with it is as familiar and productive as possible. Many of the changes are user-experience refinements based on prolonged testing and discussion among the GM/CA group. The updated design more clearly shows important details and reorganizes controls to reduce clutter. Changes throughout the program make it even more familiar to JBluIce users, including color themes, widget styles, tab layouts, and newly ported JBluIce-style widgets.

Some of the few remaining features to be ported from JBluIce are now available. Collect resume, table sorting, and more beam size display options are all functions restored from the JBluIce feature set. Additional features make switching between JBluIce and PyBluIce simple. With this level of refinement complete, we are starting development on the next-generation PyBluIce features, which include serial data collection, HDRMX (high data rate crystallography) and new types of automation.

The data analysis web server is also currently being reimplemented to add more features and to improve access for current beamline users and for those who want to evaluate and process data they collected previously. This would enable more features in the future, such as integration with Globus and the Argonne Leadership Computing Facility. *(M. Hilgart & Q. Xu)*

APS NEWS

In January, Laurent Chapon became associate lab director for Photon Sciences and APS director. He joined Argonne from the Diamond Light Source where he was director of physical sciences. Former APS director Stephen Streiffer has been appointed vice president for SLAC at Stanford.
https://www.aps.anl.gov/APS-News/2021-10-07/laurent-chapon-to-lead-argonnes-advanced-photon-source/2021-10-07/laurent

BEAMLINE UPGRADES & MICRO-FOCUSING

The old "bimorph" focusing mirrors that served us well for many years were removed from the 23ID-D hutch in December of 2021. The design of the new focusing mirror system, which is optimized for the APS-U, required reconfiguration of components on either side of the mirror, so everything in the upstream section of the hutch was removed. We took advantage of this opportunity to create a new, wider "duck-under" and made new support stands for the monochromatic beam slits, two beam position monitors and the attenuator foils.

The new system has horizontal and vertical focusing mirrors (HFM and VFM) arranged in a Kirkpatrick-Baez geometry. The mirrors were installed in their benders, each bender has two actuators to shape the mirror to the ideal elliptical shape to focus the x-ray beam. The mirrors in the bender were characterized and a response function was generated using the Long Trace Profiler (LTP) in the APS metrology lab. This should allow us to use a simple set of equations that relate the mirror focal position and the two bender actuators. The HFM was measured to have a slope error of ~75 nrad after mounting in the bender. This is much better than our specification, and almost 30 times better than the old bimorph mirrors back in 2003! The HFM was installed and surveyed into place in the vacuum chamber. Everything took longer due to COVID restrictions at the time. Characterization and adjustment of VFM is more complicated than the HFM. The VFM sags under its own weight so the bender has four gravity compensators that need to be adjusted to remove the sag. The RMS height error of the mirrors was specified at <2 nm so the adjustments are extremely challenging! Unfortunately, the VFM metrology was not completed when we needed to reassemble the rest of the beamline for scheduled users in the beginning of March. For the 2022-1 run the compound refractive lenses (CRLs) provided the focused beam. The VFM will be installed during the May shutdown, and both the mirrors will be commissioned in June. 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. With the APS-U, the new mirrors should focus the beam to <5 microns (FWHM) in both directions. These are exciting times!
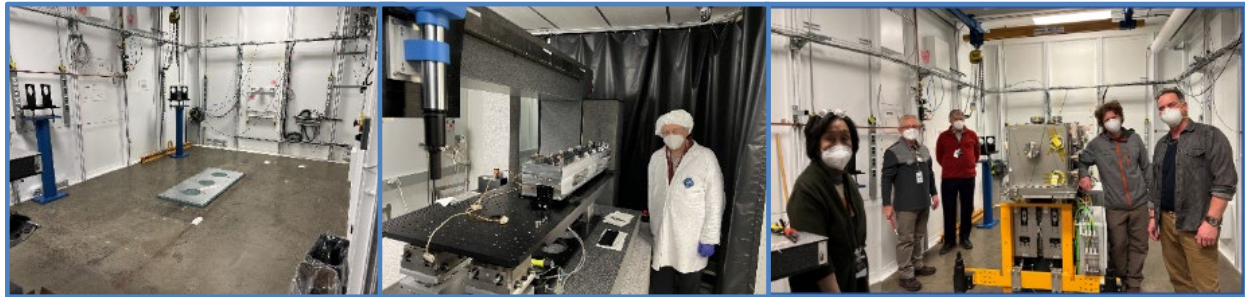
Preparation and installation of the new Kirkpartick-Baez mirror system in 23ID-D. Left: Everything was removed from the upstream section of the hutch and a new base plate for the mirrors was grouted to the floor. Middle: Oleg Makarov with the vertical focusing mirror being characterized on the Long Trace Profiler (LTP) in the metrology laboratory. Right: The mirror system in the hutch (from Left to Right: Shenglan Xu, Bob Fischetti, Oleg Makarov, David Kissick, and Dale Ferguson). *(R. Fischetti & N. Venugopalan)*