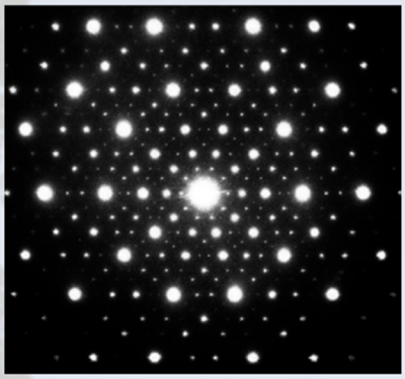
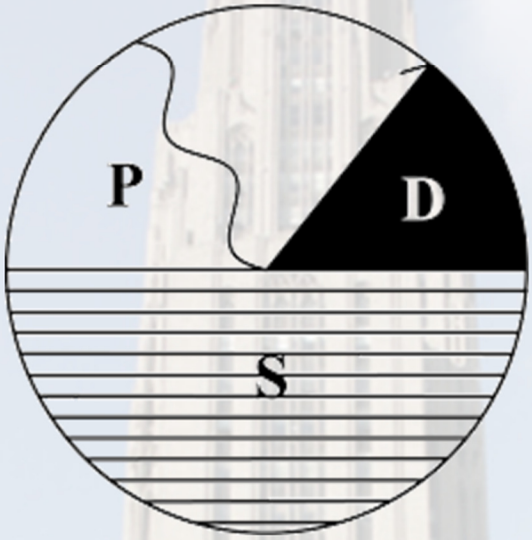
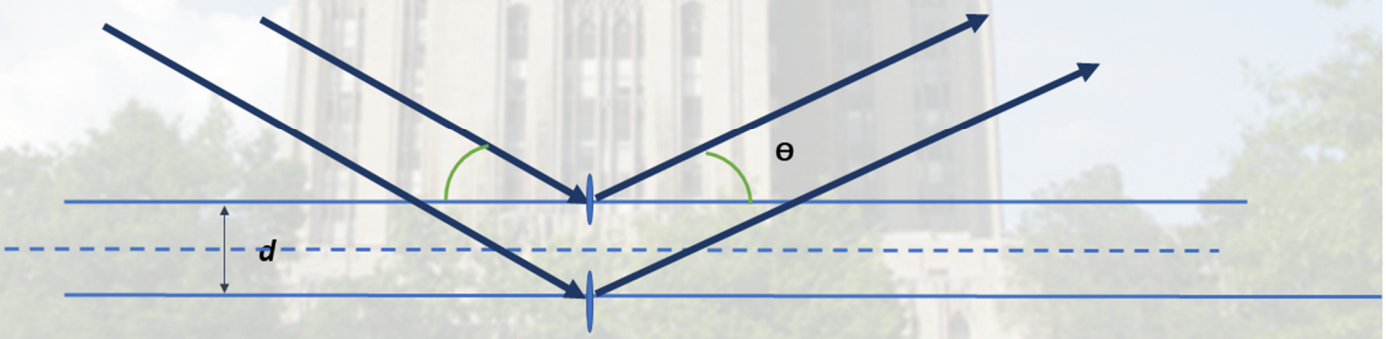
The 80th Annual Pittsburgh Diffraction Conference

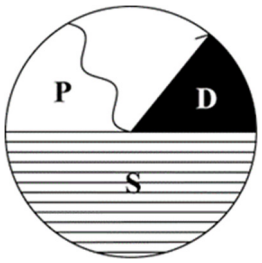
at the Wyndham University Center, Pittsburgh
October 15-17, 2023





Program and Abstract Book





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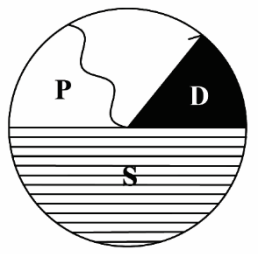


The Pittsburgh Diffraction Society

The Pittsburgh Diffraction Society (PDS) is a not-for-profit 501c3 organization which **promotes *fundamental and applied diffraction and crystallographic research and the exchange of ideas and information concerning such research.*** The society was founded *by Professor Surain S. Sidhu* who organized the first Pittsburgh Diffraction Conference (PDC) on Saturday January 16, 1943 in Lecture Room 105, Thaw Hall, University of Pittsburgh which was entitled "Conference on the Uses of X-rays, X-ray Diffraction Cameras, Electron Diffraction Cameras and Electron Microscopes." Informal remarks were given by *Surain S. Sidhu* (University of Pittsburgh), *Earl Gulbransen* (Westinghouse Research Laboratories) and *Charles S. Barrett* (Carnegie Institute of Technology), followed by discussion. The second PDC, November 3 and 4, 1944 had eleven presentations covering many diverse topics such as X-ray and electron diffraction techniques, X-ray diffraction studies of bread (no typo!), and preferred orientation in metallic systems. A banquet was held at the Webster Hall Hotel for $2.24 pp with gratuity included. By 1947 the PDC expanded to 203 registrants including *Dorothy Crowfoot Hodgkin* (Oxford University) who presented a paper entitled "The X-ray Crystallographic Investigation of the Structure of Penicillin." In 1948, *Sir Lawrence Bragg*, Cambridge University, presented on "X-ray Structure of Proteins and Other Organic Molecules". In 1999, *Herbert A. Hauptman* presented at the annual PDC hosted by the Ohio State University. The PDC has been a continuous forum to disseminate advances in crystallography and diffraction and is currently the oldest Crystallographic society in the United States. The Pittsburgh Diffraction Society (PDS) has sponsored the annual PDC, returning to Pittsburgh, Pennsylvania area every five years. The societies founder *Professor Sidhu* is honored and remembered through the **Sidhu Award**, which is given to an outstanding scientist who is within six years of having earned a Ph.D. or its equivalent. Other awards sponsored by the PDS to support and encourage young scientists include the **George Jeffrey Award**, which provides travel assistance for students attending the triennial Congress of the International Union of Crystallography, and the **Chung Soo Yoo Award** given to the best student presenter at the annual Pittsburgh Diffraction Conference.

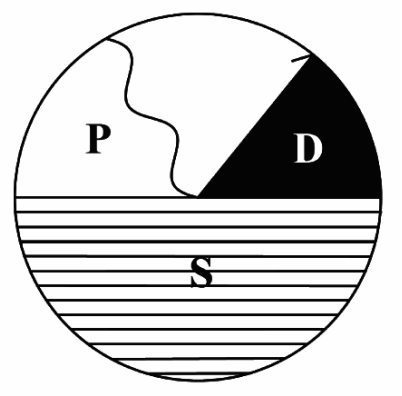
Chung Soo Yoo Award

Shozo Takagi's Wedding. **Chung Soo Yoo** is on the very right.
(Photo provided by Helen Berman)

Dr. Chung Soo Yoo, Adjunct Associate Professor in the Department of Medicinal Chemistry and Research Associate in the Department of Crystallography of the University of Pittsburgh, was killed in the Korean Airlines Flight 007 disaster of 31 August 1983. ***Dr. Yoo*** came to the U.S. from Korea in 1965; he obtained his M.S. Degree in Chemistry at Rice University in 1967 and his Ph. D. in Crystallography at the University of Pittsburgh in 1971, and became a U.S. citizen. He was a member of the Biocrystallography Laboratory of the Veterans Administration Medical Center in Pittsburgh.

Dr. Yoo was one of the most likeable crystallographers among students and colleagues in Pittsburgh, and was always very enthusiastic about the Pittsburgh Diffraction Conference.

The **Chung Soo Yoo Award**, established by the Pittsburgh Diffraction Society to honor ***Dr. Yoo's*** memory, is given to a graduate student presenting the best poster at the annual Pittsburgh Diffraction Conference and carries a cash prize of $400.



The PDS Award Funds

Over the years, the Pittsburgh Diffraction Society has created and bestowed awards to scientists and students involved in the many facets of diffraction study of matter. The first of these is the **Sidhu Award**, which recognizes the work of a young scientist who has made outstanding contributions to diffraction science within six years of earning a Ph.D. The second of these is the **Chung Soo Yoo Award**, which is given to the graduate student with the best poster presentation at a Pittsburgh Diffraction Conference. The most recent of these awards is the **George A. Jeffrey Award** given to meritorious graduate students who desire support to attend the triennial meeting of the International Union of Crystallography. The **Bryan M. Craven Scholarship** provides assistance for a foreign student to travel to the United States to participate in the Summer Course of the American Crystallographic Association, ACA. Strong preference is given to students from New Zealand or Australia.

The four awards were established with generous gifts from family and friends of *Surain S. Sidhu*, *Chung Soo Yoo*, *George Jeffrey* and *Bryan M. Craven*. We are seeking help to secure a more solid financial footing for the three PDS award funds. Please consider making a generous donation to the Pittsburgh Diffraction Society targeting one or more of the award funds.

Checks should be sent to the PDS Treasurer, ***Dr. Matthias Zeller***, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907 (zeller4@purdue.edu)

The PDS is a 501c3 organization and All donations are tax deductible in the USA; check with your tax consultant in foreign countries.

Location and Venue:

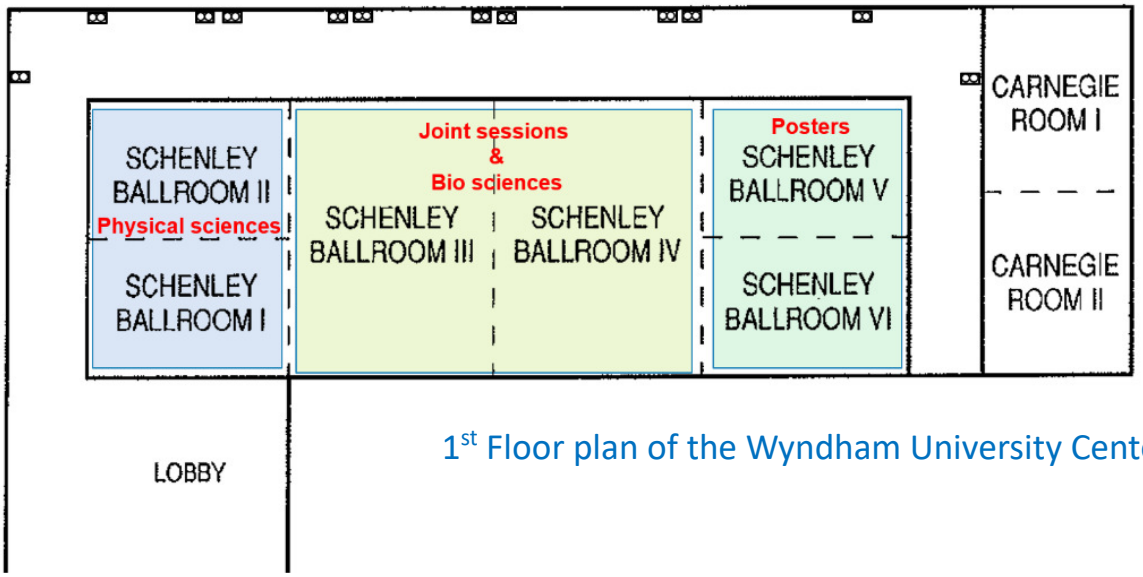
Wyndham Pittsburgh University Center

100 Lytton Avenue, Pittsburgh, PA

The Wyndam is located at the Heart of the Pittsburgh's beautiful **Oakland Campus** nestled in the hills right in between the **University of Pittsburgh** and **Carnegie Mellon University**, **Schenley Park**, **Phipps Conservatory and Botanical Gardens** and the **Carnegie Museums of Art** and **Natural History**

Registration:

Starting 1 pm Sunday Oct 15th, in the Lobby in front of the Schenley and Carnegie meeting rooms on the 1st floor of the Wyndam (to the left of the hotel registration desk).



1st Floor plan of the Wyndham University Center

Conference Rooms:

Talks and sessions will take place in Schenley Ballrooms I/II (physical sciences, B-sessions) and III/IV (joint and biological sessions, A-sessions).

Poster Sessions:

Posters will be on display for the entire conference and can be set up in Schenley Ballrooms V/VI as well as in the hallways around the meeting rooms and in the front Lobby. Poster boards (48 x 36 in) will be provided.

Student presenters are eligible for one of two **Chung Soo Yoo Awards** which carry a **$400 cash price**. To be eligible please sign up during registration and be at your poster during the poster session on Sunday at 6-7 pm and Monday at 5.40-7:00 pm.

Breaks:

Light breakfast and coffee/cookies as well as Reception provided with registration.

Exhibition:

Please join our sponsors at their booths set up in the Lobby and the Hallways around the meeting rooms. Don't forget to get your Raffle ticket signed for a chance to win a **$250 amazon gift card** sponsored by **Art Robbins Instruments**.

Agenda:

Time	Schenley Ballrooms III/IV
Sat, Oct 14, 8:30 am	**Phenix Workshop** Presented and facilitated by Phenix Developers Dorothee Liebschner (LBNL) Tom Terwilliger (New Mexico Consortium) Pavel Afonine (LBNL) Christopher Williams (Duke University)
Sat, Oct 14, 12 noon	**Lunch Break**
Sat, Oct 14, 1 pm	**Phenix Workshop** (continued)
Sat, Oct 14, 5:00 pm	End of Phenix Workshop

Time	Schenley Ballrooms III/IV	Schenley Ballrooms I/II
Sun, Oct 15, 1-4:30 pm	**Registration - Coffee, Poster set-up**	
Sun, Oct 15, 2 pm	**Opening Remarks**	
Sun, Oct 15, 2:15 pm	**Keynote Lecture Erica Ollmann Saphire** (La Jolla Institute for Immunology) "Antibodies against Emerging Infections: A Global Collaboration"	
Sun, Oct 15, 3:10 pm	**Keynote Lecture Chong-Yu Ruan** (Michigan State University) "Femtosecond electron crystallography and microscopy: recent advances and applications"	
Sun, Oct 15, 4:05 pm	**Coffee Break**	
Sun, Oct 15, 4:30 pm	**YOUNG INVESTIGATOR PLATFORM** **Tyra Douglas** (ANL) **Jordan Kelly** (Duquesne) **Vanessa Moresco** (UC Riverside) **Ari Selzer** (Pitt) **Sylwia Pawledzio** (ORNL) **Giancarlo Gonzalez-Areizaga** (Pitt)	
Sun, Oct 15, 5:30 pm	**Recollections of Pittsburgh Diffraction Conferences Past: 1977 – 2023** Part 1: **William Furey** (University of Pittsburgh) Part 2: **John P. Rose** (University of Georgia)	
Sun, Oct 15, 6:00 pm	**Poster viewing / judging**	
Sun, Oct 15, 7:00 pm	**Reception (pasta bar, drink tickets and cash bar) -**	
Sun, Oct 15, 8:00 pm	**Karaoke**	
Sun, Oct 15, 10 pm	End of 1st Day	

Time	Schenley Ballrooms III/IV	Schenley Ballrooms I/II
Mon, Oct 16, 7:30 am	**Breakfast**	
Mon, Oct 16, 8:30 am	**Keynote Lecture Andrew Kruse** (Harvard Medical School) "Structural basis for antibody fragment modulation of a G protein-coupled receptor" Intro: Crissy L. Tarver	
Mon, Oct 16, 9:25 am	**Coffee Break/Poster viewing/Vendors**	
Mon, Oct 16, 9:45 am - 11:45 am	**Session 1A** **Structure-based drug design** Chairs: Eddie Snell, Simone Brixius-Anderko	**Session 1B** **Latest developments at light sources** Chairs: Uta Ruett, Kevin Stone
	Focco van den Akker (Case Western Reserve) "Exploring the inhibition of the soluble lytic transglycosylase Cj0843c of Campylobacter jejuni via targeting different sites with different scaffolds"	**Daniel Olds** (Brookhaven National Laboratory) "The present and future vision for advanced automation at synchrotron powder diffraction beamlines at NSLS-II"
	Rachel Harding (University of Toronto) "Target 2035: the quest to develop a pharmacological modulator for every human protein"	**Yu-Sheng Chen** (University of Chicago) "Novel Techniques Following APS-U at ChemMatCARS for Small Molecule Advanced Crystallography"
		Pascal Hofer (Dectris) "PILATUS4 - the new large area high framerate photon counting detector for high energy experiments"
	Tori Drago (ORNL) "Neutron Crystallographic studies of serine hydroxymethyltransferase for cancer research"	**Kevin Stone** (SLAC) "Latest Developments at the SSRL Materials Science Scattering Beamlines with Illustrative Examples"
	Crissy L. Tarver (Stanford) "Structure-Based Drug Design of Small Molecules for Viral and Cancer Treatments"	**James Weng** (Argonne National Laboratory) "Compressed sensing-based data collection strategies"
		Joseph Ferrara (Rigaku) "X-ray and Electron Diffraction: Complementary Methods for Structural Science"
Mon, Oct 16, 11:45 am	**Lunch Break /Poster viewing / Vendor presentations**	
	11.50-12.05 **Nik Balog** (Art Robbins Instruments) "High-Throughput Automated Crystallization System (HACS)"	**11.50-12.05** **Cora Lind-Kovacs (Toledo)** "AAAPD: Global Round Robin for High Resolution Powder Diffraction Beamlines"
Mon, Oct 16, 1:15 pm - 3:15 pm	**Session 2A** **Synergy of Computation and Crystallography for Structural Biology** Chair: Denise Okafor	**Session 2B** **Spins, phonons, and disordered states in complex materials** Chair: Jacob Ruff
	David Koes (Pitt) "Deep Learning for Structure-Based Drug Discovery: AlphaFold and Beyond"	**Ziming Shao** (Cornell University) "Real-space imaging of periodic nanotextures in thin films via phasing of diffraction data"
	Margaret Johnson (Johns Hopkins) "Learning assembly kinetics for multi-	**Jian Liu** (Univ. Tennessee Knoxville) "Elasto-spin modulation of

	subunit protein complexes"	antiferromagnetic ordering in Sr_2IrO_4"
	Maria Kurnikova (CMU) "Gating Mechanisms and Ion Selectivity in Tetrameric Ion Channels inferred from Molecular Modeling aided by Machine Learning analysis"	**Pat Clancy** (McMaster University) "Searching for Kitaev Magnetism in Low-Dimensional Iridates"
	Jerome Baudry (UAH) "Protein Dynamics in Structure-Based Drug Design: Leveraging the Conformational Selection Process"	**Purnima Ghale** (CHESS) "Diffuse crystallography for phonons"
	Lucy Forrest (NIH) "Exploring Symmetry and Structural Patterns in the Evolving Landscape of Membrane Protein Structures"	
Mon, Oct 16, 3:15 pm	**Coffee Break/Poster viewing/Vendors**	
Mon, Oct 16, 3:40 pm - 5:40 pm	**Session 3A** **Crystallography meets CryoEM** Chair: Angeline Lyon	**Session 3B: Investigating Materials' Structures and Properties using X-ray Diffraction Microscopy** Chair: Yueheng Zhang
	Qiuyan Chen (IUSM) "ACKR3–arrestin2/3 complexes reveal molecular consequences of GRK-dependent barcoding"	**Rachel Lim** (Lawrence Livermore NL) "Understanding the Micromechanics of α-Ti Using Far-Field High Energy X-Ray Diffraction Microscopy"
	Nami Tajima (Case) "Structural Insights into NMDA Receptor Pharmacology"	**Seunghee Oh** (UMich) "Advancements in In-Situ X-ray Diffraction for Characterizing Mechanical and Compositional Developments in Metals"
	Krishna Chinthalapudi (OSU) "Decoding Myosin Function: Insights from Integrative Structural Biology"	**Joseph Aroh** (NIST) "The Solidification Behavior of Stainless Steels during Additive Manufacturing Revealed by In Situ Synchrotron X-ray Diffraction"
	Jonathan Coleman (Pitt) "Structure and function of a synaptic vesicle transporter"	**Yueheng Zhang** (CMU) "Progress in Understanding Strain dynamics and Dislocation Interactions Across Grain Boundaries via Bragg Coherent Diffraction Imaging"
Mon, Oct 16, 5:40 - 7:00 pm	**Poster viewing / judging**	
Mon, Oct 16, 7:30 pm	**Banquet Dinner (separate registration) & some fun activity**	
Mon, Oct 16, 10:30 pm	End of 2nd Day	

Time	**Schenley Ballrooms III/IV**	**Schenley Ballrooms I/II**
Tue, Oct 17, 7:30 am	**Breakfast**	
Tue, Oct 17, 8:30 am	**Keynote Lecture Daniel Shoemaker** (University of Illinois Urbana-Champaign) "Inorganic quantum crystallography: Discovering materials in the computer and in the lab" Intro: Jennifer Aitken	

Tue, Oct 17, 9:25 am	**Coffee Break/Poster viewing/Vendors**	
Tue, Oct 17, **9:45 am – 11:45 am**	**Session 4A** **High Pressure in Macromolecular Crystallography** Chair: Jeney Wierman	**Session 4B** **Structure-property relationships in Quantum and Layered Materials** Chairs: Jennifer Aitken, Efrain Rodriguez
	Jeney Wierman (MacCHESS) "Under Pressure: Why we should study biology in extreme environments"	**Xiaoyan Tan** (George Mason University) "Designing non-centrosymmetric magnetic oxides from centrosymmetric materials"
	Laurel Leber (U Toledo) "High Pressure X-ray Diffraction for Visualization of Transient Intermediates in the Tryptophan Synthase Mechanism"	**Luis De Jesús Báez** (University of Buffalo) "Transforming Layered Materials: New Looks to Old Faces"
	Richard Gillilan (CHESS) "Hiding in the crowd: how hydrostatic pressure reveals hidden states and sensitive structures"	**Xin Gui** (University of Pittsburgh) "Magnetic Order and Crystal Structure of a New High-Temperature Phase of MnBi"
	Gabrielle Illava (CMU) "Novel structural characterization of oxygen sensing Fumarate and Nitrate Reduction transcription factor using in-line anoxic small-angle X-ray scattering"	**William Ratcliff** (National Institute of Standards and Technology) "Semi and self-supervised approaches to space group and Bravais lattice determination"
Tue, Oct 17, 11:45 am	**Lunch Break / Poster Viewing / Vendor presentations**	
	11.50-12.05 **Jean-Luc Brousseau** (Anton Paar) "Examples of X-ray to probe ensemble average molecular behavior"	
	12.05-12.20 **Kimberley Blair** (Formulatrix) "Introducing NT8 v4: Efficient and Reproducible Protein Crystallization Experiments"	
Tue, Oct 17, **1:15 pm – 3:20 pm**	**Session 5A: General Interest and cool techniques** Chairs: John Alvarado, Andy Hinck	**Session 5B: Materials under High Pressure – Using Diffraction Techniques to understand Synthesis Pathways, Phase Transitions and Phase Behaviors** Chair: Bianca Haberl
	Gabby Budziszewski (HWI) "High-throughput crystallographic screening in the age of modern structural biology"	**Antonio dos Santos** (ORNL) "Opportunities and challenges for high-pressure neutron research on barocalorics"
	Guillermo Calero (Pitt) "Watching RAS Break a Phosphate Bond"	**Sachith Dissanayake** (University of Rochester) "Investigating magnetic phase transitions at high pressure using neutron diffraction"
	Mike Martynowycz (UCLA) "*MicroED and its Impact on Structural Biology*"	**Stella Chariton** (University of Chicago) "Multigrain crystallography at

		extremes"
	John Jeff Alvarado (Pitt) "*Unique Conformations of the Acute Myeloid Leukemia-Associated Src-family kinase, Fgr, Induced by ATP-Site Inhibitors"*	**Samuel Dunning** (Carnegie Institute) "How Phase Changes Impact Nanothread Polymerization"
		Collin Broholm (Johns Hopkins) "High pressure studies of quantum materials with neutron scattering"
Tue, Oct 17, 3:15 am	**Business Meeting** **Treasurer's Report** **Poster awards** **Next Conference** **Outlook into the Future**	
Tue, Oct 17, 4:15 am	**Closing Remarks**	
Tue, Oct 17, 4:30 pm	**End of Conference**	

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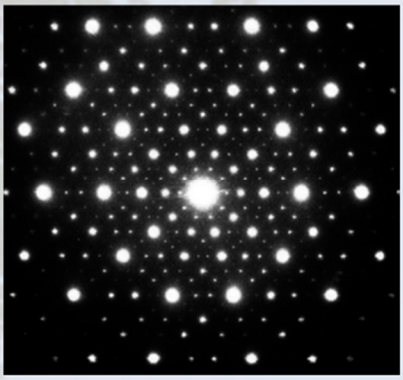

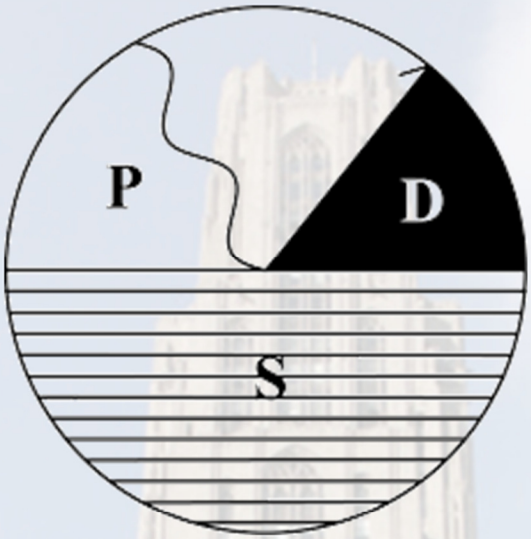

Abstracts

KEYNOTE SPEAKERS

Erica Ollmann Saphire
La Jolla Institute for Immunology

Chon-Yu Ruan
Michigan State University

Andrew Kruse
Harvard Medical School

Daniel P. Shoemaker
University of Illinois Urbana-Champaign

Keynote Lecture 1

Antibodies against Emerging Infections: A Global Collaboration

Erica Ollmann Saphire

La Jolla Institute for Immunology, La Jolla, CA

Contacts:
Saphire lab: Michelle Moss Lawrence, mmoss@lji.org

Antibodies provide a critical line of defense against infectious diseases and are a primary goal of protective vaccines. Antibodies themselves can also serve as therapeutics or as design templates to develop or improve needed vaccines. Understanding the structure, reactivity, breadth, and protective activities of antibodies is key to both therapeutic and vaccine design. We have galvanized global consortia to understand which antibodies are most effective against viruses like Ebola, Lassa, and SARS-CoV-2. These consortia analyzed which antibodies demonstrate potent neutralization, which inspire Fc-mediated effector functions, which exhibit breadth across strains, and which yield durable neutralization despite emergence of highly mutated variants, and why. Results from the Bill and Melinda Gates Foundation-, GHR- and NIAID-funded Coronavirus Immunotherapeutics Consortium (CoVIC) that studied 400 candidate therapeutic antibodies against SARS-CoV-2 afforded fine epitope mapping on the spike protein; these distinctions forecast antibody activities. Structures of CoVIC IgGs in complex with the spike explain why some antibodies retain potency even though the footprint they target is highly mutated, like in the Omicron Variants. The geometry of their IgG recognition allows bivalent binding that confers avidity to preserve neutralization activity. Results of another project, the Viral Immunotherapeutic Consortium (VIC), mapped first-in-class antibody therapies for Ebola Virus Disease (Inmazeb) and Lassa Fever (Arevirumab), explained their mechanism of action and why a cocktail approach that combines complementary functions endows resistance to escape and opens multiple avenues of in vivo protection. For Lassa virus in particular, these studies help explain why neutralizing antibodies against this virus are so rare and provide specific design strategies to develop antigens to find and elicit more potently neutralizing antibodies.

Keynote Lecture 2

Femtosecond Electron Crystallography and Microscopy: Recent Advances and Applications

Chong-Yu Ruan

Department of Physics and Astronomy, Michigan State University, East Lansing, MI 48824, USA

Contact: ruanc@msu.edu

In the last two decades, the rapid development of time-resolved scattering methodologies utilizing femtosecond X-ray pulses through either table-top or free electron laser (FEL) systems, as well as the versatile electron-based femtosecond scattering and microscopy setups, have led to a new area of investigating structural dynamics. We will first review the emerging scientific opportunities from specialized lower energy (10-30 keV) ultrafast electron diffraction systems fashioned to investigate gas-phase molecules, surfaces, nanostructures and interfaces, taking advantage of the short penetration depth and high scattering strength of electrons. Femtosecond electron crystallography remains a niche area due to the unavailability of femtosecond hard X-ray sources to regular users. The high energy beam (80-200 keV) can effectively penetrate sub-micrometer sized small crystals, equally producing sharp Bragg peaks, synergistic to femtosecond X-ray crystallography. Meanwhile, the high-energy beam also produces a large Ewald sphere, giving access to a great number of Bragg peaks in the patterns even from a singular specimen tilt angle. Since the ultrafast crystallography approach typically does not suffer greatly from the pump-probe wave front mismatch issues, it is more straightforward to obtain high temporal resolution and is currently gaining momentum as an alternative to FEL for material research. The ultrafast coherent crystallography approach can simultaneously sample the relevant multiscale dynamics and is expected to play an essential role in understanding the emergent phenomena in quantum materials.

Finally, injecting ultrafast time resolution into the typical TEM modalities will not only allow ultrafast imaging and diffraction patterns to be obtained for studying material processes, the delicate control of timing between the pump and probe

pulses can create a new contrast mechanism at various stages of the material's responses, which can allow imaging and spectroscopy in ways not available in the CW case. Here, we will discuss the uses of dynamical contrast to enhance the imaging capabilities as well understanding of the material processes not available from crystallography investigation. These multimodal operations could be performed without changing the basic optical pump and the electron illumination geometry, so the information retrieved from different dynamical modalities hence are entirely correlated to piece together information not available from a special-purpose ultrafast probe. To this end, we will discuss the latest ultrafast electron microscopy technologies supported by new development in generating high-brightness electron bunches, and the RF-optics-based techniques to overcome the space-charge to reach sub-100 fs resolution, enabling multi-modality observations based on the optimization of merit-based brightness.

Young Investigator Platform 1

Exploration of Reaction Pathways during Rapid Joule Synthesis Through Time–Resolved In–Situ High–Energy Powder X–ray Diffraction

Tyra C. Douglas,[1] Andrey A. Yakovenko,[1] Charles A. Kurtz,[1] Bryan L. Monk,[1] Guy Jennings,[1] James Weng,[1] Olof Gutowski,[2] Fernando Igoa,[2] Oleh Ivashko,[2] and Uta Ruett[1]

[1]X-ray Science Division, Advanced Photon Source, Argonne National Laboratory, Lemont, IL 60439
[2]Deutsches Elektronen-Synchrotron DESY, Hamburg, Germany 22607

Contact: tdouglas@anl.gov

High-temperature (>1000 K) techniques with sub-second reaction rates, like rapid Joule synthesis, offer a unique approach for fast and energy-efficient manufacturing of materials with outstanding mechanical, electrical, thermal, and magnetic properties to advance battery, thermoelectric device, catalyst, and ultra-high-temperature thermal protection technologies. However, harnessing the full potential of these methods requires a comprehensive fundamental understanding of the thermodynamic and kinetic pathways. The study of atomic level ordering can provide these insights, but the reaction rates challenge the observation of the phase progression through direct methods like in-situ x-ray diffraction. The characterization of these reactions has the following requirements:

1. High-energy X-rays to penetrate into the bulk of materials.
2. A single photon counting detector for high-energy x-rays with a high frame rate and low signal-to-noise ratio to capture all the phase transformations during the fast-kinetical processes.
3. The high flux of a synchrotron dedicated to high-energy x-rays.
4. Strategies to efficiently analyze the large (>15,000 frames) data sets obtained.

An apparatus for time-resolved in-situ rapid Joule synthesis and a data collection strategy were developed. Leveraging the high-energy x-rays and other capabilities at APS beamline 11-ID-C and PETRA P21.1, the phase transformations and reaction kinetics resulting from heating metal nitrate salts (Zn, Cu, Co) were investigated.

Young Investigator Platform 2

Elucidating the effect of polymorphism and cation disorder on the nonlinear optical properties of the Cu_2ZnGeS_4 diamond-like semiconductor

Jordan C. Kelly, Zachary T. Messegee, Yan Xin, Seung Han Shin, Jeong Bin Cho, Jun Sang Cho, Kyung Seok Seo, Jong Hoon Lim, Thomas E. Proffen, Qiang Zhang, Janae Chase, Joon I. Jang, Xiaoyan Tan, and Jennifer A. Aitken

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Diamond-like Cu_2ZnGeS_4 polymorphs have been studied over the years for applications in solar cells and photochemical catalysis. Although much work has been published, the single crystal structures and the nonlinear optical (NLO) properties of the polymorphs have not been determined. Our work includes the comprehensive characterization of the structures, preparation of phase-pure α- and β-polymorphs, and assessment of NLO properties. X-ray single crystal diffraction, X-ray powder diffraction (XRPD), neutron diffraction, electron diffraction, and high-angle annular dark field scanning transmission electron microscopy (HAADF-STEM) were utilized to definitely ascertain the structures. The structures obtained from single crystal X-ray diffraction data agreed with the previously published structure types, wurtz-stannite ($Pmn2_1$) for the high-temperature α-phase and stannite ($I\text{-}42m$) for the low-temperature β-phase. Neutron diffraction was needed to verify these structures, due to the isoelectronic nature of the metal cations. Furthermore, electron diffraction and HAADF-STEM imaging support the existence of a third polymorph, γ, prepared at high temperature with a wurtz-stannite-like structure but having metal cation disorder. The disorder was exacerbated when the reactants were subjected to an ice-water quench, as opposed to a controlled slow cooling. For the NLO results, the high-temperature α-phase had the largest second harmonic generation response of the three polymorphs with a $\chi^{(2)}$ value of 17.3 pm/V, while the quenched γ-phase and the low-temperature β-phase had values of 5.04 and 0.5 pm/V, respectively. Finally, the quenched γ-phase possessed the largest laser-induced damage threshold of 1.0 GW/cm^2 with the high-temperature α-phase and the β-phase coming in lower at 0.5 and 0.3 GW/cm^2, on par or better than some commercial standards. These results indicate polymorphs can possess drastically

different NLO properties, and that different heat treatment, including cooling procedures, should be investigated when preparing new NLO materials.

Young Investigator Platform 3

Structural and Mechanistic Characterization of Protective Broad Spectrum Non-Neutralizing Antibodies Targeting Crimean-Congo Hemorrhagic Fever

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Crimean-Congo hemorrhagic fever virus (CCHFV) causes a debilitating hemorrhagic fever with a mortality rate as high as 40%. With recent spread to new countries and no approved vaccine or therapeutics available, CCHFV is viewed as a priority public health threat by the WHO. Recently, a set of human and mouse non-neutralizing monoclonal antibodies (mAb) targeting CCHFV glycoprotein GP38 showed to confer post-exposure protection. Here, we reveal the broad cross protection properties of these mAbs as well as initial insights into how they confer this protection. Starting with a collection of anti-GP38 mAbs gathered from Turkey survivors (CC5) and mice (13G8), mAbs were screened against strains across the six clades of CCHFV as well as Aigai Virus using BioLayer Interferometry (BLI) as well as a novel CCHFV cell spread assay. X-ray crystallography was utilized to perform fine epitope mapping

of two antigenic sites on clinically relevant Hoti GP38 with mutagenesis used to probe mAb resiliency and broad-spectrum potential. *In vivo* efficacy studies were also performed.Through the collection of mAbs, five antigenic sites were identified. The epitope of Site 1 and Site 4 mAbs were mapped using 13G8/CC5-17 and CC5-20 respectively. The conserved nature of these sites, along with biochemical data revealed that these mAbs could productively engage 80%-100% of known CCHFV species and be resilient to epitope drift. Through *in vivo* and cell spread assays revealed that affinity was not the sole determinate for protection and highlights other factors involved in protection. This study highlights that non-neutralizing mAbs targeting GP38 can serve as a broad-spectrum option for pre-and-post treatment for CCHF. Also, it reveals key considerations in developing mAbs that target nairovirus GP38s as well as those seeking to leverage the non-neutralizing pathways responsible for protection.

Young Investigator Platform 4

Small Molecule Allosteric Modulators of the AML-associated Src-family Kinase, Hck

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Hck and Fgr are members of the Src family of non-receptor tyrosine kinases expressed in myeloid hematopoietic cells where high-level expression can drive the formation of acute myeloid leukemia (AML). While ATP competitive inhibitors of Hck and Fgr show promise for AML therapy, their ultimate clinical efficacy is likely to be limited by the emergence of resistance mutations. Combination therapy targeting a single protein with allosteric and ATP-site inhibitors dramatically reduced resistance potential in various cancers and may have potential for AML as well. Our group has identified two small molecules with a shared pyrimidine diamine core (PDA1 & PDA2) that have potential as allosteric Hck inhibitors. Surface plasmon resonance (SPR) and NMR spectroscopy indicate that these compounds recognize a shared binding site involving the PPII-helix binding surface of the regulatory SH3 domain. *In vitro* kinase activity assays and hydrogen-deuterium exchange mass spectrometry (HDX-MS) reveal that despite the shared binding site, the compounds have opposite effects on overall kinase activity and dynamics. PDA1 stabilized overall Hck dynamics and did not affect kinase activity, while PDA2 disrupted the closed conformation of the kinase and stimulated kinase activity. To test PDA1's efficacy in cells, we transformed human TF-1 myeloid leukemia cells with a kinase-active chimeric protein that fuses Hck to the coiled-coil (cc) domain

of Bcr. Treatment of TF-1/cc-Hck cells with PDA1 resulted in growth suppression which was significantly reduced with the introduction of PDA1-resistant mutants, providing evidence for on-target activity. To determine the structural basis of PDA1 interaction with Hck, X-ray crystallography was used to map the binding site. While this effort yielded a high-resolution crystal structure of near-full-length Hck, electron density for PDA1 was not observed which may reflect the relatively low affinity of this compound and competition with the SH2-kinase linker at the SH3 domain. However, the crystal structure of the kinase domain, which was bound to the ATP-site inhibitor A-419259, revealed an extended structure of the activation loop for the first time. *In silico* docking and molecular dynamics simulations suggest that PDA1, but not PDA2, stabilizes the closed, inactive, conformation of Hck by bridging the SH3 and kinase domains. Ongoing studies are exploring the structural basis of pyrimidine diamine interaction with Hck and the potential for the combination of PDA1 with ATP-site inhibitors to reduce the potential for acquired resistance.

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Application of quantum crystallography to study relativistic effects

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Keywords: quantum crystallography, relativistic effects, Hirshfeld atom refinement

During the last 50 years, relativistic quantum chemistry has undergone significant development and methodological progress. Nowadays, it is well-known that a relativistic quantum formalism is necessary for the study of compounds with heavy elements [1].
However, studies of the chemical properties of compounds and crystals containing heavy elements are challenging for both theoreticians and experimentalists. The Schrödinger equation is no longer applicable and experimental studies require special facilities and conditions to be considered. A perspective method is quantum crystallography which relies on the XRD data to describe crystal structure in unprecedented detail. Hirshfeld atom refinement (HAR) [2] is a method that uses tailor-made aspherical atomic scattering factors, obtained from the quantum mechanical calculations which can be done at different levels of theory, to refine atomic positions and their ADPs in the standard least-square refinement. It has been shown that HAR overcomes all the shortcomings of the Independent Atom Model (IAM), yielding more accurate hydrogen atom positions and enabling the refinement of hydrogen atom ADPs [3]. Furthermore, HAR was successfully applied to small and big, light and heavy molecules [3]. During the last few years, new

software for HAR was extensively developed and nowadays modelling of disorder, even for structures with heavy elements, is possible. On the other hand, intensities of the diffracted beam are affected not only by relativistic effects but also by absorption, anharmonic motion, anomalous dispersion, and many other effects which highly influence electron density distribution in the crystal and, in consequence, derived properties. Thus, studies of the relativistic effects with HAR require the collection of outstanding quality X-ray diffraction data sets in terms of resolution, absorption corrections, and error model.

Here, we present the results of relativistic Hirshfeld atom refinements [4] carried out as implemented in Tonto [5] or NoSpherA2 [6] for high-resolution X-ray diffraction data sets. The outcome of DFT-based refinements with the nonrelativistic and quasi-relativistic approaches will be compared, including an analysis of the nature of the Me–X bonds in Au crystal [7], the role of modelling disorder [8], and a description of aurophilic interactions [9].

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Young Investigator Platform 6

Conformation-dependent Inhibition of the AML-associated Src-family kinase Fgr by ATP-site inhibitors

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Development of acute myeloid leukemia (AML) is often associated with overexpression of non-receptor protein-tyrosine kinases, including three members of the Src family: Hck, Lyn and Fgr. Our group is actively investigating small molecule Src-family kinase inhibitors with significant anti-AML efficacy. These inhibitors include the pyrrolopyrimidine A-419259 and the *N*-phenylbenzamide TL02-59; the latter compound potently inhibits Fgr and Lyn *in vitro* and reverses bone marrow engraftment of the human AML cell line MV4-11 in a mouse model of AML. We recently solved X-ray crystal structures of near-full-length Fgr, consisting of the SH3, SH2, and kinase domains plus the tyrosine-phosphorylated tail, in complex with each inhibitor. A-419259 bound to the Fgr ATP-site with the regulatory SH3 and SH2 domains packed against the back of the kinase domain, resulting in a closed conformation observed in previous structures of Hck with this inhibitor. However, while TL02-59 also bound to the Fgr ATP-site, it induced allosteric displacement of the SH3 and SH2 domains from their regulatory positions, resulting in an open conformation. To explore the effect of allosteric domain displacement on the TL02-59 inhibitory mechanism, Fgr mutants were generated with enhanced SH3 domain interaction with the SH2-kinase linker (high affinity linker or 'HAL' mutants). Fluorescence polarization assays confirmed enhanced intramolecular SH3:linker interaction, thus favoring the closed conformation. X-ray crystallography of an Fgr SH3-SH2-linker protein with the HAL substitution showed that the orientation of the SH3 and SH2 domains is virtually identical to that observed in the structure of the near-full-length kinase, suggesting

that the high affinity linker does not impact the overall closed kinase conformation. To test the effect of HAL substitutions on Fgr sensitivity to TL02-59, we created an active form of Fgr by fusing it to the coiled-coil (CC) domain of the breakpoint cluster region protein (Bcr). Expression of CC-Fgr (wild-type and HAL mutants) transformed TF-1 cells into a cytokine independent phenotype and rendered them sensitive to Fgr inhibition by TL02-59. Stabilizing the closed conformation by introduction of the HAL mutations enhanced TL02-59 potency in these cells, suggesting that TL02-59 prefers a single Fgr kinase conformation. Ongoing work is directed toward identification of small molecules that mimic the effect of HAL substitutions on Fgr. Combining allosteric modulators that lock a single Fgr conformation are predicted to synergize with TL02-59 and suppress the evolution of resistance mutations, a common limitation of many clinical ATP-site kinase inhibitors currently in use for AML and other cancers.

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Recollections of Pittsburgh Diffraction Conferences Past: 1977 – 2023 Part 1

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I joined the University of Pittsburgh as a Post Doc in late 1977 with an appointment in the Department of Crystallography, after obtaining a Ph.D. in Physical Chemistry/Crystallography. I quickly learned about the Pittsburgh Diffraction Conference history first via conversations with faculty members, and later through my own participation at the events. This was an exciting time to be a crystallographer, with applications to proteins just developing and the Protein Data Bank starting to take off.

One of my earliest meeting presentations was at a PDC over 40 years ago (a poster I recently discovered I still have!), and I recall meeting many movers and shakers in the field. I later chaired one of the conferences, had presentations at some others, and over the year have heard many exciting talks at them. I will discuss what I've learned or witnessed regarding the PDC, it's place in the history of crystallography, and what the meetings and field in general were like in this period. Some of my memorable recollections of PDC's past will be discussed, hopefully conveying the state of crystallography at the time.

Recollections of Pittsburgh Diffraction Conferences Past: 1977 – 2023 (Part 2)

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I attended my first Pittsburgh Diffraction Conference in 1978. I was in my third year of Graduate school at Rutgers doing small molecule crystallography when Bill Furey (a recent Rutgers Ph.D.) suggested that I come to the Pittsburgh Diffraction Conference (PDC) to meet B.C. Wang who was looking for a postdoc.

The PDC was amazing, and I remember that Keith Hodgson & George DeTitta won the Sidhu Award. Upon graduation in 1980 I joined the Wang lab working on his neurophysin project but paid through the University of Pittsburgh's Department of Crystallography. It was during this time the Conference was held yearly in Pittsburgh and I came to know its background and how each year's Conference was organized (location, publicity, sessions, speakers, housing, coffee breaks and meals). Everyone - faculty, postdocs and students came together each September to make October's PDC a reality. I ended up Chairing or Co-Chairing several PDC's in Pittsburgh and at the University of Georgia (UGA) in Athens, GA.

My talk will focus on the PDC during my years in Pittsburgh and at UGA using text and pictures, including when a poster presenter wins the Nobel Prize weeks before the PDC and an astronaut who describes his microgravity crystallization efforts aboard the space shuttle Columbia.

Keynote Lecture 3

Structural Basis for Antibody Fragment Modulation of a G Protein-Coupled Receptor

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G protein-coupled receptors (GPCRs) are key regulators of human physiology and are the targets of many small molecule research compounds and therapeutic drugs. While most of these ligands bind to their target GPCR with high affinity, selectivity is often limited at the receptor, tissue, and cellular level. Antibodies have the potential to address these limitations but their properties as GPCR ligands remain poorly characterized. Using protein engineering, pharmacological assays, and structural studies, we developed a series of heavy chain camelid antibody fragment ("nanobody") antagonists against the angiotensin II type I receptor (AT1R). We found that these nanobodies can simultaneously bind to AT1R with specific small-molecule antagonists. To determine the structural basis for this activity, we attempted crystallography and a variety of mass enhancement strategies for cryo electron microscopy, ultimately developing a high-throughput cryoEM approach to determine a series of AT1R-nanobody complex structures. The results illustrate that antibody fragments can exhibit rich and *evolvable* pharmacology, attesting to their potential as next-generation GPCR modulators.

Exploring the Inhibition of the Soluble Lytic Transglycosylase Cj0843c of *Campylobacter jejuni* via Targeting Different Sites with Different Scaffolds

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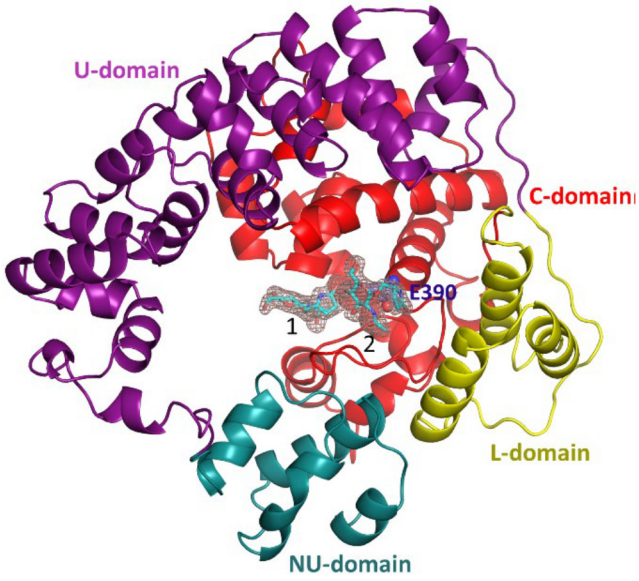
Bacterial lytic transglycosylases (LTs) contribute to peptidoglycan cell wall metabolism and are potential drug targets to potentiate β-lactam antibiotics to overcome antibiotic resistance. Only one LT inhibitor, bulgecin A discovered in 1982, has been discovered whose binding has been crystallographically validated. Since LT inhibitor development is underexplored, we probed fifteen N-acetyl-containing heterocycles in a structure-guided fashion for their ability to inhibit and bind to the *Campylobacter jejuni* LT Cj0843c. We hypothesized that starting with compounds having an N-acetyl moiety would give compounds an initial affinity since both the peptidoglycan substrate and bulgecin A use this moiety to bind to LTs. We used docking calculations and molecular dynamics simulations in the design process. Ten GlcNAc analogs were synthesized with substitutions at the C1 position, with two having an additional modification at the C4 or C6 position. Most of the compounds showed weak inhibition of Cj0843c activity. Compounds with alterations at the C4 position, replacing the -OH with a -NH2, and C6 position, the addition of a -CH3, yielded improved inhibitory efficacy. All ten GlcNAc analogs were crystallographically analyzed via soaking experiments using Cj0843c crystals and found to bind to the +1 +2 saccharide subsites with one of them, Z7285, additionally binding to the -2 -1 subsite region. We conducted a dose-response soaking experiment of Z7285 which revealed a higher affinity for the +1 subsite compared to the -2 subsite region. That the compounds unexpectedly favored binding to the +1 subsite region affected our docking prediction accuracy, due to steric clashes, as the docking focused on the -2 subsite region where bulgecin A

positions its N-acetyl moiety. We also probed other N-acetyl-containing heterocycles and found that sialidase inhibitors N-acetyl-2,3-dehydro-2-deoxyneuraminic acid and siastatin B inhibited Cj0843c weakly and crystallographically bound to the -2 -1 subsites. Analogs of the former also showed inhibition and crystallographic binding and included zanamivir amine. This latter set of heterocycles positioned their N-acetyl group in the -2 subsite with additional moieties interacting in the -1 subsite. Overall, these results could provide novel opportunities for LT inhibition via exploring different subsites and novel scaffolds. The results also increased our mechanistic understanding of Cj0843c regarding peptidoglycan GlcNAc subsite binding preferences and ligand-dependent modulation of the protonation state of the catalytic E390. Despite their low affinity, this study is the first advancement of new inhibitors for LTs that have been crystallographically confirmed since bulgecin A.



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Target 2035: The Quest to Develop a Pharmacological Modulator for Every Human Protein

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The Structural Genomics Consortium (SGC) is a global public-private partnership which aims to uncover novel human biology through structural genomics and chemical biology approaches. To interrogate protein function, the SGC develops chemical probes which are structurally validated, selective, potent and cell active compounds. Recently, the SGC and partners embarked on Target 2035, an ambitious project which aims to develop a pharmacological modulator for every protein in the human proteome by 2035. These tools can then be used to modulate and understand protein function and reveal novel therapeutic targets for tomorrow's medicines.

Session 1A-3: Structure-based drug design

Neutron Crystallographic Studies of Serine Hydroxymethyltransferase for Cancer Research

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Hydrogen (H) atoms make up ~50% of all atoms in proteins, contributing to structure and function through H-bonding, electrostatics, and van der Waal's interactions. In enzymatic catalysis, H atoms control the electrostatic environment and regulate molecular recognition as well as participate in proton transfer reactions. Because hydrogen atoms are "invisible" with conventional structural biology techniques, the overwhelming majority of protein structures used in structure-based drug design are missing critical information. Neutron crystallography reveals the positions of H atoms, and unlocks the detailed knowledge of reaction mechanism and substrate binding necessary for potent inhibitor design. Neutrons interact with the atomic nuclei, allowing hydrogen and deuterium (D) atoms to scatter neutrons on the same magnitude as carbon, oxygen, and nitrogen, allowing for detection of H/D atoms at moderate resolutions. We demonstrate the abilities of neutron crystallography through our analysis of serine hydroxymethyltransferase (SHMT), a universal PLP-dependent enzyme responsible for contributing single carbon units to one-carbon (1C) metabolism. Human mitochondrial SHMT (hSHMT2) is overexpressed in several types of cancers as a

result of metabolic reprogramming of the 1C pathway, rendering hSHMT2 an attractive target for antimetabolite chemotherapeutics. SHMT catalysis is not well understood as there are multiple proposed mechanisms for the acid-base reaction and deciphering between them requires knowledge of protonation states. By direct detection of H atom positions with neutron diffraction, we gained unique atomic-level knowledge of the SHMT active site environment that sheds new light on the catalytic mechanism of SHMT and offers insight to future drug design.

Structure-Based Drug Design of Small Molecules for Viral and Cancer Treatments

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Structures of proteins that are essential for reproduction/survival of many viruses and cancers have been determined and used in therapeutic drug research. Medications like Nirmatrelvir, which is given with Paxlovid, are now available for the treatment of Coronavirus. However, medications like Paxlovid do not strongly select for new variants and can increase blood concentrations of other medications resulting in serious drug toxicities. Our Stanford AViDD (antiviral drug discovery) group used x-ray crystallography and structure-based drug design to develop a series of small molecules that inhibit M^{PRO} activity. Additionally, we are in the process of determining the ternary complexes of a new class of molecules that bind BCL6 to transcriptional activators that play a role in oncogenesis, like BRD4. These small molecules have been found to kill large B cell lymphoma cells, including chemo-therapy resistant lines. Structure-based drug design applying *in silico* and crystallography methods will be used to optimize affinities and efficacies to design new medications.

The Present and Future Vision for Advanced Automation at Synchrotron Powder Diffraction Beamlines at NSLS-II

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Synchrotron based powder diffraction is a core characterization technique for many domains of research, particularly those requiring in situ studies of materials under change. However, the measurement speeds, data rates, and subsequent analysis required to fully utilize all measured data is often daunting for even expert users. Advances in automation and analysis pipelines employing both conventional and machine learning based approaches are required to fully leverage these techniques.

In this contribution, we will present recent efforts at the National Synchrotron Light Source II (NSLS-II) to develop both hardware and software solutions for advancing power diffraction and total scattering methods. We will demonstrate how these tools have been used on PDF beamline for user driven experiments, as well as how they are being combined to enable automated, human/AI-driven multi-beamline experimentation. Finally, we will discuss the role these tools can play in the planned high resolution powder diffraction beamline, HRD, at NSLS-II.

Novel Techniques Following APS-U at ChemMatCARS for Small Molecule Advanced Crystallography

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NSF's ChemMatCARS, situated in Chicago, IL, USA, is a national user facility founded by the National Science Foundation (NSF). It operates at the Advanced Photon Source (APS) within Argonne National Laboratory (ANL). Administered by The University of Chicago, ChemMatCARS provides wide X-ray energy ranges and a high-brilliance X-ray source, which enables cutting-edge research in various fields such as advanced small-molecule crystallography, liquid surface and interface scattering, and anomalous small-angle X-ray scattering.
For more details on the current capabilities of Advanced Crystallography at NSF's ChemMatCARS, please visit our website: https://chemmatcars.uchicago.edu/scientific-program/advanced-crystallography/

During the APS upgrade project's dark period (APS-U), ChemMatCARS is embarking on the construction of a canted beamline equipped with innovative optics, poised to enhance its advanced crystallography capabilities. This ambitious initiative introduces innovative methodologies, prominently featuring resonant diffraction. Resonant diffraction harnesses lower X-ray energies, extending down to approximately 3.5 keV, thereby granting researchers access to the entire spectrum of 3d and 4d transition metals. Furthermore, it opens the door to small molecule serial crystallography.

A presentation will be organized to introduce these pioneering techniques. ChemMatCARS eagerly invites both researchers interested in participating in the commissioning process and those keen on utilizing these state-of-the-art techniques once they reach full development.

Latest Developments at the SSRL Materials Science Scattering Beamlines with Illustrative Examples

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The Stanford Synchrotron Radiation Lightsource (SSRL) operates five hard X-ray scattering beamlines as part of the suite of instruments supported by the material science division (MSD). These encompass a range of techniques including small and wide angle scattering, powder diffraction, and thin film or single crystal diffraction. SSRL has long had an emphasis on enabling *in situ* and *operando* measurements, developing a number of experimental setups for such customized measurements. In this presentation, I will present the latest developments taking place at SSRL MSD beamlines. These have been focused on advancing two complimentary goals:

1. Enabling the development of new instrumentation, beamlines, and experimental techniques, considering and addressing gaps in the existing US-based synchrotron complex.
2. Bringing these advances to the broader scientific community by lowering the barriers to high quality, reliable instrumentation, analysis tools, and providing a platform for education.

This has resulted in the development of novel experimental capabilities as well as improved automation and remote access to SSRL instruments. This presentation will also cover the basic beamline functionality and the latest updates to beamlines optics and controls. To illustrate the opportunities afforded by the latest developments and planned future directions, several examples of recent work done at SSRL beamlines will be presented.

Compressed sensing-based data collection strategies

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With the advent of brighter light sources there are new challenges in data collection. Naively increasing exposure time can easily destroy both the sample and detector, resulting in useless measurements and expensive instrument damage. In the case of disordered materials, where material defects are the information of interest, beam damage (which creates new defects) must be avoided during measurement. Here we present a number of compressed sensing measurement strategies, which take advantage of the structured nature of data in order to provide measurements with minimal beam exposure. By leveraging modern signal processing and computing resources, these strategies not only reduce necessary collection time, but also provide less noisy measurements than conventional measurement strategies.

Deep Learning for Structure-Based Drug Discovery: AlphaFold and Beyond

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Protein structure predictions from deep learning models like AlphaFold2, despite their remarkable apparent accuracy, remain problematic for use in drug discovery, such as when used in molecular docking. We describe our experience using such models with our deep learning powered molecular docking software GNINA. We propose a new method to train deep learning protein structure prediction models using molecular dynamics force fields with the goal of generating more physically realistic structures. Our custom PyTorch loss function, OpenMM-Loss, represents the potential energy of a predicted structure. OpenMM-Loss can be applied to any all-atom representation of protein structure capable of mapping into our software package, SidechainNet. We demonstrate our method's efficacy by finetuning OpenFold. We show that subsequently predicted protein structures, both before and after a relaxation procedure, exhibit comparable accuracy while displaying lower potential energy and improved structural quality as assessed by MolProbity metrics.

Learning Assembly Kinetics for Multi-subunit Protein Complexes

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Macromolecular complexes are frequently composed of diverse subunits. While evolution may favor repeated subunits and symmetry, we show how diversity in subunits generates an expansive parameter space that naturally improves the 'expressivity' of self-assembly, much like a deeper neural network. By using automatic differentiation algorithms commonly used in deep learning, we searched these parameter spaces to identify classes of kinetic protocols that mimic biological solutions for productive self-assembly. We further demonstrate how assembly of the HIV immature lattice relies on time-dependent activation of subunits for productive assembly, combining structure-resolved reaction-diffusion simulations with theory and validation against experiment. Our results reveal how high-yield complexes that easily become kinetically trapped in incomplete intermediates can instead be steered by internal design of rate constants or external and active control of subunits to efficiently assemble, exploiting nonequilibrium control of these ubiquitous dynamical systems.

Gating Mechanisms and Ion Selectivity in Tetrameric Ion Channels inferred from Molecular Modeling aided by Machine Learning analysis

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Large families of biological ion channels are formed as tetrameric transmembrane protein complexes. Common structural features of such channels include their cone-like shape formed by long transmembrane alpha-helices, a narrow constriction formed in the wide side of the cone by shorter helical and disordered peptides typically served as an ion selectivity filter and an ability of the narrow part of the cone to widen to control open and closed states of the channel in response to chemical or environmental stimuli, i.e. gate the channel. Despite common architecture tetrameric ion channels exhibit wide variability of gating mechanisms, ion selectivity, and permeation properties. We have performed extensive molecular dynamics (MD) simulations of the structure of the Glutamate Receptor of type AMPA to shed light onto their structure-function relationships. Our models are based on the ensemble of the high resolution structures resolved by cryo-EM spectroscopy and x-ray diffraction experiments. The analysis of the extensive data-sets generated in our simulations is aided by machine learning (ML) technics.

Yelshanskaya MV, Patel DS, Kottke CM, Kurnikova MG, Sobolevsky AI. Opening of glutamate receptor channel to subconductance levels. Nature. 2022 605 (7908):172-178.

Protein Dynamics in Structure-Based Drug Design: Leveraging the Conformational Selection Process

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We demonstrate the advantage of using protein dynamics to vastly improve the performance docking in structure-based drug discovery. Usually, docking is done on a static protein experimental structure. In our lab, we use a combination of docking and molecular dynamics simulations to obtain an ensemble of protein conformations. We show that a subset of these conformations are much more likely to interact with the protein ligands than most of the other confirmations and than the static protein experimental structure. We also discuss how we are using AI/ML approaches to improve the selection of protein conformations that will be selected by the proteins' ligands.

Exploring Symmetry and Structural Patterns in the Evolving Landscape of Membrane Protein Structures

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The evolution of membrane proteins depends on the physicochemical constraints imposed by the lipid bilayer, and by extension, their folding mechanisms. Repetition of well-folded motifs following these rules is frequently observed within protein sequences and has allowed efficient creation of larger macromolecules capable of more complex functions. Such repeated elements within individual protein chains can result in structural symmetry, as does association of subunits into higher-order assemblies. Nevertheless, much remains to be learned about interplay between folding, assembly, symmetry, and evolution. Structural data obtained by crystallography or electron cryo-microscopy provides an evolutionary snapshot with which to examine these questions in greater detail. At the time of writing, almost 8000 membrane protein structures have been collated into our structure and symmetry database, EncoMPASS. I present a systematic analysis of these data and discuss the implications for folding, function, and evolution.

Real-space Imaging of Periodic Nanotextures in Thin Films via Phasing of Diffraction Data

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New properties and exotic quantum phenomena can form due to periodic nanotextures, including Moire patterns, ferroic domains, and topologically protected magnetization and polarization textures. Despite the availability of powerful tools to characterize the atomic crystal structure, the visualization of nanoscale strain-modulated structural motifs remains challenging. Here, we present nondestructive real-space imaging of periodic lattice distortions in thin epitaxial films. By leveraging the iterative phase retrieval method and unsupervised machine learning, we invert the diffuse scattering pattern from conventional X-ray reciprocal-space maps into real-space images of crystalline displacements. Our imaging in $PbTiO_3/SrTiO_3$ superlattices exhibiting checkerboard strain modulation substantiates published phase-field model calculations. Furthermore, the imaging of biaxially strained Mott insulator Ca2RuO4 reveals a strain-induced nanotexture comprised of nanometer-thin metallic-structure wires separated by nanometer-thin Mott-insulating-structure walls, as confirmed by cryogenic scanning transmission electron microscopy (cryo-STEM). The nanotexture in Ca_2RuO_4 film is induced by the metal-to-insulator transition and has not been reported in bulk crystals.

Elasto-spin Modulation of Antiferromagnetic Ordering in Sr_2IrO_4

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Sr_2IrO_4 is known to show remarkable similarities to high-Tc cuprates, such the quasi-2D antiferromagnetic Mott insulating state, despite their big difference in spin-orbital coupling (SOC). This emergent analogy is partly due to the lifting of the t2g orbital degeneracy by SOC instead of Jahn-Teller effect. On the other hand, the resulted pseudospin-half state has a strongly spin-orbit-entangled wavefunction, which could lead to novel collective phenomena beyond the cuprates. In this talk, I will discuss our recent work that utilized the elasto-x-ray scattering to perform resonant magnetic diffraction on Sr_2IrO_4 under in situ strain control in addition to temperature and magnetic field. Giant responses of the metamagnetic transition were observed due to the highly effective detwinning of the antiferromagnetic domains by the uniaxial strain. It enables efficient switching of the metamagnetic transition. In contrast, when we apply the strain in a shear configuration to the ab-plane, a new spin modulation that breaks the c-axis translation symmetry emerges. Our analysis indicates the underlying interaction is an unusual fourth-order anisotropy that is only activated under strain. Our results showcase the rich physics of 5d complex oxides and demonstrate the power of elasto-x-ray scattering in revealing their emergent phenomena.

Session 2B-3: Spins, Phonons, and Disordered States in Complex Materials

Searching for Kitaev Magnetism in Low-Dimensional Iridates

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The properties of heavy 5d transition metal oxides, such as iridates, are often remarkably different from those of their lighter 3d counterparts. In particular, the presence of strong spin-orbit coupling (SOC) can give rise to a variety of exotic quantum states, including spin-orbital Mott insulators, topological insulators, Weyl semimetals, and quantum spin liquids. In materials based on edge-sharing octahedral crystal structures, large SOC can also lead to unconventional magnetism, and a form of highly anisotropic, bond-directional Ising interaction known as the Kitaev interaction. The first, and best known, experimental realizations of Kitaev magnetism are honeycomb lattice materials: the 5d iridates A_2IrO_3 (A = Na, Li) and the 4d halide α-$RuCl_3$. These compounds have attracted considerable attention due to predictions of a Kitaev quantum spin liquid with exotic anyonic excitations. However, there has recently been growing interest in the search for Kitaev magnetism in other families of materials with different lattice geometries. In this talk, I will describe a new candidate for Kitaev magnetism in a chain-like material: the quasi-one-dimensional iridate $Ca_{5-x}Y_xIr_3O_{12}$. We have characterized the structural, magnetic, and electronic properties of this system using a combination of x-ray diffraction, resonant magnetic x-ray scattering, x-ray absorption spectroscopy, and SQUID magnetometry. These measurements reveal particularly interesting behaviour at higher Y dopings, offering a potential route towards the realization of quasi-1D Kitaev (or Kitaev-Heisenberg) chains.

Diffuse Crystallography for Phonons

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X-ray scattering has historically been a tool to determine the atomic structure of materials. As early as 1927, however, it was hoped that with enough resolution, we would be able to determine finer quantum degrees of freedom. We now have enough resolution, volume in reciprocal space, and dynamic range of measurements to discern quantum degrees of freedom. In this talk, we focus on a particular example: the means to obtain by straightforward crystallographic measurement the density matrix and band structure of phonons. We will describe the motivating intuition, experimental capabilities, and theoretical constraints, involved in the inversion of total scattering to obtain the density matrix of phonons, using only the information which is extractable from a fairly conventional single crystal scattering measurement at a modern synchrotron beamline.

ACKR3–arrestin2/3 Complexes Reveal Molecular Consequences of GRK-Dependent Barcoding

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Atypical chemokine receptor 3 (ACKR3, also known as CXCR7) is a scavenger receptor that regulates extracellular levels of the chemokine CXCL12 to maintain responsiveness of its partner, the G protein-coupled receptor (GPCR), CXCR4. ACKR3 is notable because it does not couple to G proteins and instead is completely biased towards arrestins. Our previous studies revealed that GRK2 and GRK5 install distinct distributions of phosphates (or “barcodes”) on the ACKR3 carboxy terminal tail, but how these unique barcodes drive different cellular outcomes is not understood. It is also not known if arrestin2 (Arr2) and 3 (Arr3) bind to these barcodes in distinct ways. Here we report cryo-electron microscopy structures of Arr2 and Arr3 in complex with ACKR3 phosphorylated by either GRK2 or GRK5. Unexpectedly, the finger loops of Arr2 and 3 directly insert into the

detergent/membrane instead of the transmembrane core of ACKR3, in contrast to previously reported “core” GPCR–arrestin complexes. The distance between the phosphorylation barcode and the receptor transmembrane core regulates the interaction mode of arrestin, alternating between a tighter complex for GRK5 sites and heterogenous primarily “tail only” complexes for GRK2 sites. Arr2 and 3 bind at different angles relative to the core of ACKR3, likely due to differences in membrane/micelle anchoring at their C-edge loops. Our structural investigations were facilitated by Fab7, a novel Fab that binds both Arr2 and 3 in their activated states irrespective of receptor or phosphorylation status, rendering it a potentially useful tool to aid structure determination of any native GPCR–arrestin complex. The structures provide unprecedented insight into how different phosphorylation barcodes and arrestin isoforms can globally affect the configuration of receptor–arrestin complexes. These differences may promote unique downstream intracellular interactions and cellular responses. Our structures also suggest that the 100% bias of ACKR3 for arrestins is driven by the ability of arrestins, but not G proteins, to bind GRK-phosphorylated ACKR3 even when excluded from the receptor cytoplasmic binding pocket.

Structural Insights into NMDA Receptor Pharmacology

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N-methyl-D-aspartate (NMDA) receptors are tetrameric ligand-gated ion channels which mediate the majority of excitatory neurotransmission throughout the central nervous system. NMDA receptors are also associated with a number of brain diseases, including epilepsy, intellectual disability, schizophrenia, Alzheimer's disease, autism spectrum disorder and major depression. Therefore, they are potential therapeutic targets in treating these disorders. NMDA receptors form heterotetrameric ion channels composed of the obligatory GluN1 subunits and GluN2 and/or GluN3 subunits. These subunits define the receptor functions, expression pattern, and pharmacological and channel properties (e.g., open channel probability, ligand binding affinity, and deactivation/desensitization kinetics). I present cryo-EM structures of NMDA receptor complexes. The structures reveal the subunit-specific functional modulation by small compounds and functional antibodies. Comparisons among the ionotropic glutamate receptor structures reveal the divergence in modulation mechanisms.

Decoding Myosin Function: Insights from Integrative Structural Biology

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Class-2 myosins are the major contractile proteins in eukaryotic cells. The precise function and regulation of their enzyme function determine the dynamic interaction with filamentous actin pivotal for biological processes including muscle contraction, cell adhesion, and cell migration. For smooth and nonmuscle myosin-2, the mechanochemical function depends on the assembly state of the protein which is characterized by a phosphorylation-dependent transition between a monomeric autoinhibited state in which the heads are docked against each other in a conformation known as the interacting heads motif (IHM) and a filamentous active state. In cardiac and skeletal muscle myosin-2, contractile functions of the thick filament within the sarcomere are regulated by the proportion of active and autoinhibited myosins. While the biological roles of class-2 myosins have been well characterized by previous biochemical, lower resolution EM, and physiological studies, the structural differences between autoinhibited and active myosins essential to their specific functions remain elusive. Our integrated X-ray crystallography and cryo-EM structural biology approaches now unravel these complexities, offering insights into the precise regulation of myosin function in health and disease.

Structure and Function of a Synaptic Vesicle Transporter

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The vesicular monoamine transporter 2 (VMAT2) is a proton-dependent antiporter responsible for loading monoamine neurotransmitters into synaptic vesicles. Dysregulation of VMAT2 can lead to several neuropsychiatric disorders including Parkinson's disease and schizophrenia. Furthermore, drugs such as amphetamine and MDMA are known to act on VMAT2. Despite VMAT2's importance, there remains a critical lack of mechanistic understanding, largely driven by a lack of structural information. Here we report a 3.3 Å resolution cryo-EM structure of VMAT2 complexed with TBZ, a non-competitive inhibitor used in the treatment of Huntington's chorea. We find TBZ interacts with residues in a central binding site, locking VMAT2 in an occluded conformation and providing a mechanistic basis for non-competitive inhibition. We further identify residues critical for intracellular and luminal gating, including a cluster of hydrophobic residues which are involved in a luminal gating strategy. The structure elucidates mechanisms of VMAT2 inhibition and transport, providing insights into VMAT2 architecture, function, and the design of small-molecule therapeutics.

Understanding the Micromechanics of α-Ti Using Far-Field High Energy X-Ray Diffraction Microscopy

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On the grain scale, materials are heterogeneous and anisotropic, and these non-uniformities are often the initiation sites for failure. However, most traditional materials understanding assumes a homogeneous and isotropic material to predict performance and failure. Increasing understanding of the effects of anisotropy requires experiments which supply three-dimensional, in situ data to make accurate predictions. Titanium alloys are commonly used in many biomedical and aerospace applications. However, the α-phase in this material is both thermally and mechanically anisotropic due to its hexagonal closed packed structure. This anisotropy can lead to the development of significant grain-scale stresses in polycrystals. Far-field high energy x-ray diffraction microscopy (ff-HEDM), a synchrotron-based in situ x-ray characterization technique, is employed to study the in situ micromechanical evolution of Ti-7Al under a variety of different conditions.

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Advancements in In-Situ X-ray Diffraction for Characterizing Mechanical and Compositional Developments in Metals

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In-situ X-ray diffraction is a non-destructive technique widely used for investigating continuously changing behaviors. Due to technological advancements in X-ray sources and detectors, the acquisition rate and quality of acquired X-ray diffraction data have continually improved. Specifically, fast acquisition in our experimental setup enables us to capture transient changes in lattices during rapid processes, which were not accessible with conventional techniques.

We conducted several in-situ experiments to characterize Ni-alloy 718 under laser processing. The rapid developments of thermal and mechanical strains were quantified using the in-situ experimental data. Furthermore, we investigated the phase evolution of an as-printed (martensitic) Ti-6Al-4V alloy during rapid heating. The acquired diffraction profiles temporally resolved the progress of martensite decomposition, revealing an intermediate stage as martensite, α', transitions into α+β.

Finally, this study explores the potential of a laboratory X-ray source to implement one of in-situ X-ray diffraction techniques, high-energy x-ray diffraction microscopy, which was previously only available at synchrotron facilities.

The Solidification Behavior of Stainless Steels during Additive Manufacturing Revealed by In Situ Synchrotron X-ray Diffraction

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Additive manufacturing (AM) induces severe departures from typical processing conditions, often resulting in microstructures beyond conventional metallurgical knowledge. This is problematic for the solidification pathway in stainless steels, as the sequence of phases can significantly affect the properties of the AM-fabricated component. To investigate this solidification behavior at relevant length and time scales for AM, we employed synchrotron X-ray diffraction to directly measure the evolution of phases during in situ laser melting and re-solidification under a variety of laser processing conditions. Studies were performed on several multicomponent austenitic stainless alloys to elucidate the sensitivity of subtle compositional variations on the phase pathway. In addition, surrogate Fe-Ni-Cr alloys were investigated to construct a microstructure selection map as a function of Cr/Ni ratio without the influence from minor alloying elements. A martensitic stainless steel was also examined to assess how the primary solidifying phase influences subsequent solid-state transformations.

Progress in Understanding Strain dynamics and Dislocation Interactions Across Grain Boundaries via Bragg Coherent Diffraction Imaging

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Bragg Coherent Diffraction Imaging (BCDI) has emerged as an instrumental technique in the study of mesoscale properties in materials science. Innovations in methodologies for multi-peak and multi-grain reconstruction have primarily been motivated by investigations into the interactions between dislocations and grain boundaries. Furthermore, the implementation of polycrystalline mapping technologies such as High-Energy Diffraction Microscopy (HEDM) and Laue diffraction has enriched the field considerably. Anticipated upgrades to the Advanced Photon Source (APS) are projected to substantially enhance X-ray beam intensity, thereby facilitating expeditious assessments of polycrystalline specimens and the kinetic behavior of dislocations. We report on state-of-the-art advancements in screening algorithms specifically designed for the screening crystals, twins, and dislocations. We also report the experimental measurements of the Ti-6Al-4V alloy obtained at the European Synchrotron Radiation Facility (ESRF), which had been indexed and mapped via HEDM at APS. Our result shows the advantage of multi-grain reconstruction techniques, and analyses of strain across grain boundaries in gold oligocrystals, consisting of matrix-twin-matrix 3-grain and matrix-twin-matrix-twin 4-grain configurations, supported on strontium titanate substrates.

Keynote Lecture 4

Inorganic Quantum Crystallography: Discovering Materials in the Computer and in the Lab

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For functional materials that are in a nascent stage, such as antiferromagnetic spintronics, quantum information storage, and new semiconducting compounds, it is not clear what will be the high-performance materials of tomorrow. There is a pressing need to examine the complex properties of these emerging materials, and growing single crystals is a crucial step. I will introduce cases where the chemical phase spaces of interest hold many undiscovered compounds. First, we developed a computational framework to analyze topology and symmetry of these materials. The immediate impact for this framework was the organization and prediction of quantum spin liquids and critical point compounds. Then the task of growing single crystals begins. Due to the required millimeter dimensions, they must be grown from solutions, fluxes, or vapors. This process is often hard to observe, and highly kinetically dependent, so in situ techniques can be especially valuable. Frontiers in computational synthesis and diffraction-contrast tomography will be discussed.

Under Pressure: Why we should study biology in extreme environments

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The discovery of abundant microbiota deep within rocks has challenged our previous understanding of life on Earth. As scientists explore new habitats for extreme life forms, the importance of hydrostatic pressure in biology is becoming more widely acknowledged. These findings raise fundamental questions about life's origins, the differences between extreme life and more common organisms, the minimum conditions necessary for cell survival, the maximum temperatures and pressures at which organisms can endure, and the influence of extreme environmental factors on biomacromolecules. As protein function relies on subtle changes in three-dimensional structure, changing conditions such as raising temperature or pressure can offer accessible insights into these changes and expand the knowledge of conformational landscape and protein function. This intro provides a non-technical overview, emphasizing the significance of studying how extreme environments impact biomacromolecules.

High Pressure X-ray Diffraction for Visualization of Transient Intermediates in the Tryptophan Synthase Mechanism

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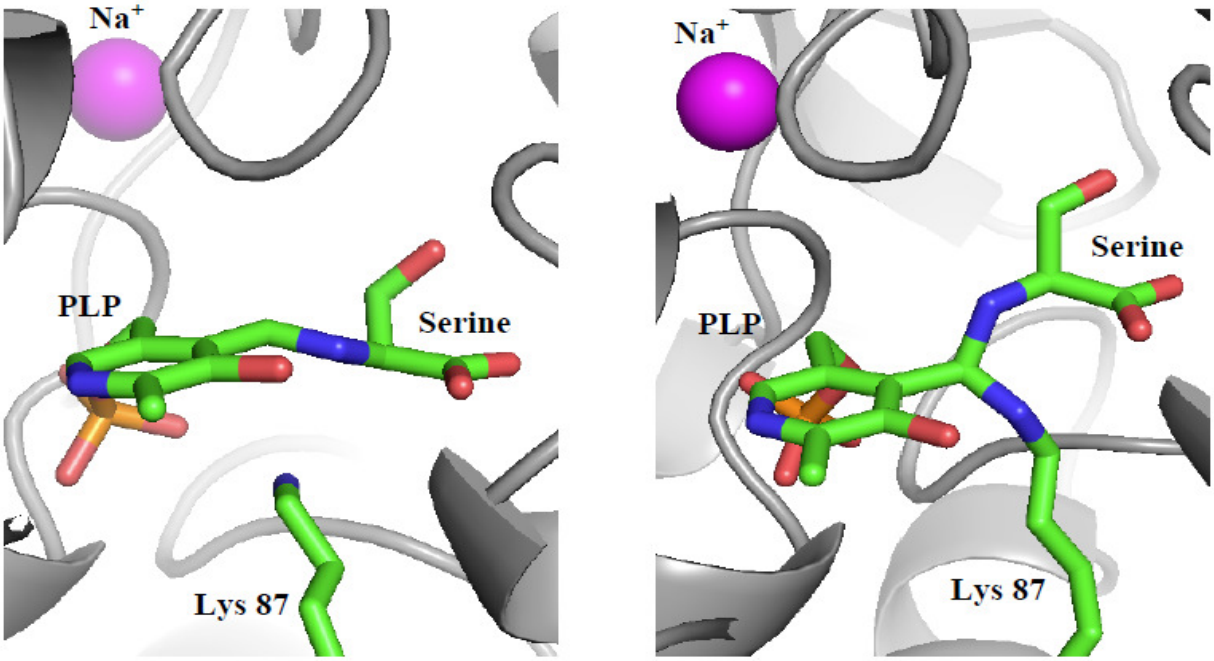
The University of Toledo

Pyridoxal 5'-phosphate (PLP) dependent enzymes are involved in a vast number of catalytic reactions including hydrolases, isomerases, ligases, lyases, oxidoreductases, and transferases. Because of this catalytic diversity there is a vast interest in better understanding their reaction mechanism. Most PLP-dependent enzymes have a similar reaction mechanism involving a Schiff's base linkage to the aldehyde group on PLP. The internal aldimine form sequesters PLP to a conserved lysine residue in the β-active site. An enzyme-specific unprotonated primary amino acid binds in the active site as a Michalis Complex which initiates bond rearrangement that passes through several transient steps culminating in a PLP-substrate external aldimine, a structure that can be visualized using inhibitors. In the first transient intermediate, PLP is bound as a gemdiamine to both the conserved lysine residue and unprotonated primary amino acid. The transient nature of the gem-diamine makes visualization challenging. Performing macromolecular x-ray crystallography at pressures of 2 to 5 kbar stabilizes transients intermediates while using real substrate. Tryptophan synthase (TS) is the prototypical Type-II PLP dependent enzyme that catalyzes the last two steps in the biosynthesis of tryptophan. TS is an abba heterodimer in which the a-reaction converts indole-3-glycerole phosphate into indole and glyceraldehyde-3-phosphate and the b-reaction links indole arriving through an internal channel to a PLP activated serine. For our initial experiments, presented here, TS crystals were subjected to several different soaking conditions followed by XRD at various pressures. XRD (ambient pressure, cryo-trapped at 100K) of TS crystals soaked with K+ or Na+, and serine produced the external-aldimine, shown on the left. At 2-5 kbar, crystals of the same soaking conditions produced the gem-diamine transient intermediate, shown on the right. TS crystals measured at ambient pressure, soaked with serine Na+, and glycerol-3-phosphate, show ordering of the αL6 loop (Arg179-Thr193) near the α-active site with glycerol-3-phosphate in the α-active

site and an internal aldimine in the β-active site. The same crystals under pressure (2 kbar) show disordering of the αL6 loop, no glycerol phosphate in the α-active site, and the gem-diamine intermediate in the β-active site. These results indicate the use of high-pressure XRD can be utilized to visualize and study transient reaction intermediates that are otherwise difficult.



Hiding in the crowd: how hydrostatic pressure reveals hidden states and sensitive structures

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Under hydrostatic pressure, biomolecules in water rearrange to minimize total molar volume. This relationship is easily visualized in lipid mesophases. Ctenophores, or comb jellies, are one of the more ancient lifeforms on our planet. When a deep-sea ctenophore is brought to the surface, its body literally melts as the pressure-adapted cellular lipids revert to the wrong phase. Researchers visiting the CHESS HP-Bio beamline have used distinct scattering signatures of lipid mesophases to understand pressure and temperature adaptation in this species. Lipid structures are differentially affected by pressure depending upon how well packed they are at the molecular level. Proteins likewise minimize their volume under pressure, though in sometimes counter-intuitive ways. Functionally important voids and cavities tend to open up rather than simply compressing, protein complexes dissociate, and individual proteins can even unfold under extreme pressure. As molecules carry out their function, they necessarily pass through states of different molar volume; intuitively, pressure alters the population of these states. Recent work on tRNA dramatically bears this out: a normally invisible “excited” conformation relevant to interaction with HIV RNA becomes visible under pressure (Wang *et al*., PNAS 120, 2023). The overarching principle suggested here for lipids, proteins, and other biomolecules, is that pressure perturbation is a novel and selective means of identifying sensitive structures and deconstructing complex interactions and multistep biomolecular processes. Though many individual pressure-sensitive biomolecules have been studied now, the process of identifying systems with measurable effect has been largely hit-or-

miss with common standard proteins showing little effect. Building upon our unique high-pressure technology, here we introduce the first high-pressure structural proteomics study. In limited proteolysis mass spectrometry (LiP-MS) , pulse digestion with proteinase K results in selective cleavage at solvent-exposed regions, forming peptide fragments that can be sequenced with LC-MS/MS. This method is sensitive to subtle structural alterations, such as those that form when proteins misfold (To *et al.* J. Am. Chem. Soc. 143, 2021), and here we apply it to map out deformations induced by hydrostatic pressure. By conducting LiP-MS at both ambient and high pressure on a model organism (*T. thermophilus*) with a purpose-built apparatus, we report a proteome-wide map of pressure-induced structural change at the molecular level. Preliminary results suggest that structural change on a whole-proteome level at modest ocean-bottom pressures (100 MPa) is rapid and more extensive than expected. This talk will present a few of the early results and possible implications but will also focus on instrumentation. We believe these maps will be a rich source of new targets for structural and biophysical studies.

Novel structural characterization of oxygen sensing Fumarate and Nitrate Reduction transcription factor using in-line anoxic small-angle X-ray scattering.

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In this talk I will describe our investigation of the oligomeric interconversions of the Fumarate and Nitrate Reduction (FNR) transcription factor, which is responsible for the transcriptional response to changing oxygen conditions in the facultative anaerobe *Escherichia coli*. Using anoxic small-angle X-ray scattering (anSAXS), we provide the first direct structural evidence for the oxygen-induced dissociation of the *E. coli* FNR dimer and its correlation with cluster composition. We further demonstrate how complex FNR-DNA interactions can be studied by investigating the promoter region of the anaerobic ribonucleotide reductase genes, *nrdDG*, which contains tandem FNR binding sites. By coupling SEC-anSAXS with full spectrum UV-Vis analysis, we show that the [4Fe-4S] cluster-containing dimeric form of FNR can bind to both sites in the *nrdDG* promoter region. The development of the first in-line anoxic SAXS system at a major national synchrotron source featuring both batch-mode and chromatography-mode capabilities, greatly expands the toolbox available for the study of complex metalloproteins and provides a foundation for future expansions into high-pressure (HP) anoxic studies. Preliminary experiments have shown that these techniques can be extended to high-pressure SAXS as well, opening the possibility for studies of life under deep-sea anoxic conditions.

Designing non-centrosymmetric magnetic oxides from centrosymmetric materials

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Materials with non-centrosymmetric (NCS) crystal structures and magnetic ordering are widely used in laser technology, access memory elements, energy conversion, and spintronics. The discovery of such materials has been challenging due to limited design strategies. Here, we report that non-centrosymmetric magnetic oxides can be achieved by modifying the crystal and electronic structure of centrosymmetric oxides. The disorder behavior and NCS crystal structure were confirmed by the Rietveld refinement using synchrotron X-ray and neutron diffraction data and convergent-beam electron diffraction. The oxidation state of transition metals was confirmed by near-edge X-ray absorption spectroscopy. The magnetic properties of prepared NCS compounds will be compared with those of related centrosymmetric compounds.

Transforming Layered Materials: New Looks to Old Faces

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The discovery and development of new materials tailored to a specific function remain as one of the grand challenges for materials' scientists. To this end, layered materials have been extensively studied due to the sundry of properties that emerge from chemical and physical modifications. Unfortunately, little is still known of the fundamental requirements to generate pronounced and tailored alterations to the electronic structure upon dimensional reduction or functionalization of their active sites. Comprehensive nanoscale characterization is essential to understand how the loss of 3D structural coherence and further modifications of the surface, induced because of ion intercalation, exfoliation to 2D sheets, or functionalization of the basal planes, alter the electronic structure of these materials which translates into physical properties. In this talk, I will discuss the use of layered VS_2 structures as seed to nucleate VOOH nanostars and the precise control of ligand concentration on Fe-based layered double hydroxides as chromophores.

Session 4B-3: Structure-property relationships in Quantum and Layered Materials

Magnetic Order and Crystal Structure of a New High-Temperature Phase of MnBi

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Permanent magnets are of great importance due to their vast applications, especially for the rare-earth-free ferromagnets due to the availability issue of rare-earth elements. MnBi has been proposed and studied as a potential permanent magnet that can be widely used. However, various challenges have been encountered due to a paramagnetic high-temperature phase (HTP) of MnBi. Past efforts have been focused on modifying the ferromagnetic low-temperature phase of MnBi. Herein, we report a new pathway that can potentially solve the existing problems in MnBi system by inducing magnetic order in a series of new materials crystallizing in HTP-MnBi-related structures.

Semi and self-supervised approaches to space group and Bravais lattice determination

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Keywords: Artificial Intelligence, Diffraction, Neural Network

During this talk, I will discuss our work [1] to use neural networks to automatically classify Bravais lattices and space-groups from neutron powder diffraction data. Our work classifies 14 Bravais lattices and 144 space groups. The novelty of our approach is to use semi-supervised and self-supervised learning to allow for training on data sets with unlabeled data as is common at user facilities. We achieve state of the art results with a semi-supervised approach. Our accuracy for our self-supervised training is comparable to that with a supervised approach.

Support for Satvik Lolla was provided by the Center for High Resolution Neutron Scattering, a partnership between the National Institute of Standards and Technology and the National Science Foundation under Agreement No. DMR-2010792.

[1] Satvik Lolla et al, Journal of Applied Crystallography 55 (2022) https://doi.org/10.1107/S1600576722006069

High-throughput Crystallographic Screening in the Age of Modern Structural Biology

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For over two decades, the National Crystallization Center has been helping structural biologists crystallize their proteins of interest using a microbatch-under-oil 1536-well plate setup. In this talk, we discuss our NIH-subsidized crystallographic screening approach, including state-of-the art robotics and imaging modalities designed to visualize even the smallest crystals and proto-crystalline material.

Watching RAS Break a Phosphate Bond

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GTPases are a ubiquitous family of enzymes that catalyze hydrolysis of guanosine triphosphate (GTP) into guanosine diphosphate (GDP). The active, GTP-bound form, of the RAS family of monomeric GTPases, comprising the three human RAS genes (Kirsten-, Harvey- and N-RAS), initiate signaling pathways that trigger cellular proliferation. RAS inactivation through GTP hydrolysis can be intrinsic or mediated by a GTPase activating protein (GAP). RAS mutations that disrupt GTP hydrolysis can lead to uncontrolled cellular proliferation and are present in 20-30% of human cancers. Recent pharmacological developments demonstrated that the once undruggable KRAS protein, can be now targeted with inhibitors that currently have full FDA approval. One of the next challenges in structural biology is advancing from a static picture to the observation of enzymes in action. Time-resolved (TR) structural studies can uncover conformational changes that reveal "hidden" transition state intermediates, and hence increasing the "druggable landscape" available for molecular modelling and inhibitor testing and development. *Albeit its significance, TR structural studies have been scarce (as they present technical challenges) and have not yet been applied to the field of drug discovery*. Using UV photolysis of caged substrates and X-ray crystallographic experiments we have, for the first time, created molecular movies of GTP hydrolysis by NRAS and its oncogenic mutants G12V, Q61L and G12C. The movies reveal transition state intermediates with distinctive cryptic pockets that could potentially bind chemical fragments. The results of our innovative experiments could expand the drug/inhibitor landscape for these clinically relevant small GTPases and thus generate more efficient therapies.

MicroED and Its Impact on Structural Biology

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Conventional X-ray crystallography often falls short when it comes to solving the structure of hard-to-crystallize membrane proteins. These challenges arise from various factors, such as limited crystal size and the necessity of lipidic mesophases for crystallization, which introduce complexities in sample handling. Microcrystal Electron Diffraction (MicroED), an emergent technique stemming from cryogenic electron microscopy (cryo-EM), offers a powerful alternative for analyzing such elusive samples. In this presentation, we will explore how MicroED overcomes traditional barriers by utilizing advances like plasma focused ion beam milling (pFIB/SEM) integrated with fluorescence light microscopy (iFLM) for precise crystal location and manipulation. Cutting-edge improvements in direct electron detection and data quality have led to unprecedented structural resolution, even permitting ab initio phasing in select cases. The session will also discuss the role of optimized sample preparation in driving the technology's success, making MicroED an increasingly indispensable tool in not just structural biology but also in accelerating drug discovery initiatives.

Unique Conformations of the Acute Myeloid Leukemia-Associated Src-family kinase, Fgr, Induced by ATP-Site Inhibitors

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The Src-family kinase Fgr is expressed primarily in myeloid hematopoietic cells where it normally contributes to control of innate immune responses. Overexpression and constitutive activation of Fgr is a common finding in acute myeloid leukemia (AML), and ATP-competitive inhibitors of Fgr show promise as antileukemic agents. These inhibitors include the pyrrolopyrimidine, A-419259, as well as the N-phenylbenzamide, TL02-59, both of which are orally active and suppress bone marrow engraftment of AML cells in mouse models. Like other members of the Src family, Fgr is comprised of an N-terminal unique region followed by regulatory SH3 and SH2 domains, the catalytic kinase domain, and a negative regulatory C-terminal tail. Previous crystal structures of Src and other family members demonstrate that intramolecular interactions between these domains regulate kinase activity. In the 'closed' conformation, the SH3 domain binds to the linker connecting the SH2 and kinase domains, while the SH2 domain engages the tyrosine phosphorylated C-terminal tail. In the present study, we solved X-ray crystal structures of near-full-length Fgr (SH3-SH2-kinase-tail) bound to A-419259 and TL02-59 and observed unique inhibitor-induced conformations. A-419259 induced a 'closed' Fgr conformation, with the SH3 and SH2 domains bound to the SH2-kinase linker and C-terminal tail, respectively, as observed previously for Src. In the crystal, the Fgr:A-419259 complex packed as an asymmetrical homodimer such that the activation loop of one monomer inserted into the active site of the other, providing a snapshot of the trans-autophosphorylation event required for

kinase activation. By contrast, TL02-59 binding induced SH2 domain displacement from the C-terminal tail and SH3 domain release from the linker. Solution studies using hydrogen-deuterium exchange mass spectrometry were consistent with the crystal structures, with A-419259 reducing and TL02-59 enhancing solvent exposure of the SH3 domain. These structures demonstrate that allosteric connections between the kinase and regulatory domains of Src-family kinases are influenced by the ligand bound to the active site, raising the possibility of allosteric inhibitor discovery.

Session 5B-1: Materials under High Pressure – Using Diffraction Techniques to understand Synthesis Pathways, Phase Transitions and Phase Behaviors

Opportunities and Challenges for High-Pressure Neutron Research on Barocalorics

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Keywords: Neutron Scattering, High Pressure, Barocalorics

Neutron scattering allows the detailed study of the crystal structure and dynamics in a wide range of materials systems. This is especially true in those containing light elements or that undergo magnetic transitions. Owing to its weak attenuation by most materials, neutron techniques are especially compatible with high pressure research. There are many studies on materials systems that depend on a fine environmental control (e.g. temperature) at low to moderate pressure and where the use of neutron beams can provide important advances [1].

Solid state caloric systems are promising alternatives for refrigeration and heat pumping applications as they may have improved efficiency and would eliminate the use of hydrofluorocarbon (HFC) refrigerants, known for extreme global warming potential.[2] A special case is the exploitation of the barocaloric effect (BCE). Materials with strong BCE undergo a phase transition associated with a large volume change. Ideally this transition temperature is strongly pressure dependent. It follows that if pressure is applied adiabatically in the vicinity of the phase transition, a large change in temperature may be observed. This can, be exploited for refrigeration or heat pumping applications. [3]

In this presentation we will discuss how neutron scattering techniques, especially those incorporating high pressure, can be leveraged for the study of new and existing BCE materials. At the SNS at ORNL, we have a suite of pressure devices that are well matched for BCE exploration and allow in-situ determination of volumetric

expansion (by tracking the unit cell parameters) across the transition, along with, in some cases, the corresponding detailed structural information. This will be illustrated with the recent study of the first giant BCE material where the transition driving the effect is a spin crossover of an Fe(II)-containing molecular crystal, where the Fe adopts two spin states: S=0→ S=2. [4]

Finally, we will briefly describe the prospect of using in-situ inelastic neutron scattering and companion computer simulations that can be directly compared, via a neutron weighted simulated approach. This workflow, optimized to match the observed neutron-weighted density of states (DOS), can be then used to estimate the enthalpy, vibrational entropy, and temperature-dependent free energy, which will assist in understand quantitatively how the spin crossover, in the present case, is driving the giant BCE effect.[5]

A portion of this research used resources at the Spallation Neutron Source, a DOE Office of Science User Facility operated by the Oak Ridge National Laboratory.

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Session 5B-2: Materials under High Pressure – Using Diffraction Techniques to understand Synthesis Pathways, Phase Transitions and Phase Behaviors

Investigating Magnetic Phase Transitions at High Pressure using Neutron Diffraction

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Tuning phase transitions in magnetic systems using control parameters such as high pressure is a highly active and dynamic field. The research conducted on quantum materials under high pressure is an area that poses significant challenges but has been making substantial progress in numerous facilities. Combining high pressure with other control parameters like magnetic fields or chemical substitutions makes it possible to achieve even more unique phases in these materials. In this talk, I will focus on MnP, which shows successive magnetic transitions at ambient pressure from ferromagnetic to helical-c magnetic phase, and magnetic ground state changes to helical-b structure with applied pressure. Magnetic transition temperature in helical-b phase decreases with increasing pressure, and superconductivity is observed near ~ 8 GPa. Several single-crystal high-pressure magnetic diffraction experiments were performed using various pressure cells at ORNL, including palm cubic anvil cells, clamp cells, and diamond anvil cells. The capabilities and limitations of each pressure cell also will be discussed. We successfully performed high-pressure neutron diffraction measurements on MnP up to 7.5 GPa to elucidate the magnetic ground state adjacent to the superconducting phase. Using experimental and theoretical results, we propose detailed exchange interactions near the superconducting phase. I will also briefly discuss high-pressure neutron diffraction studies performed at ORNL on a few other magnetic systems.

Session 5B-3: Materials under High Pressure – Using Diffraction Techniques to understand Synthesis Pathways, Phase Transitions and Phase Behaviors

Multigrain Crystallography at Extremes

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Experiments in the laser heated diamond anvil cell (LHDAC) can present a great variety of challenges, from achieving multi-megabar pressures to sustaining high temperatures for novel material synthesis. Recent advances in the DAC design and supporting equipment aim to alleviate such challenges and drive the science front of the high-pressure research in previously unapproachable domains. The application of single-crystal X-ray diffraction (SCXRD) techniques to study multigrain/multiphase samples at extreme conditions is an excellent example of how a well-established method can be "modernized" to assist in higher quality result evaluations and increased depth of information that conventional powder diffraction in the LHDAC cannot access. Laser heated samples in the DAC often experience re-crystallization processes and may also be accompanied by phase transformations and/or chemical reactions. A SCXRD data collection and data analysis approach on such a complex sample is possible and allows the deconvolution of the reaction products as well as explicit structure solution and *in-situ* chemical characterization of new compounds.
Here, we will review the sample preparation and DAC loading details that are necessary prior to such an experiment, describe the data collection procedures and discuss the data processing steps. Examples on carbonates, metal oxides and weak scattering compounds will be presented in order to demonstrate the merits of the method, but also how cutting-edge software and hardware developments can overcome the remaining challenges. We will finally discuss the prospects of large-scale technological advances in synchrotron facilities, such as the APS-U upgrade,

and describe how changes in high pressure diffraction beamlines can work in favor of such composite experiments.

Session 5B-4: Materials under High Pressure – Using Diffraction Techniques to understand Synthesis Pathways, Phase Transitions and Phase Behaviors

How Phase Changes Impact Nanothread Polymerization

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High-pressure, solid-state organic synthesis can provide access to novel reaction pathways and has been shown to produce materials with bonding environments and structural motifs that are difficult or impossible to obtain through solution-phase reactions.[1] However, unlike these solution-phase reactions, where molecules are extremely mobile, high-pressure, solid-state reactions are highly dependent on crystal packing with reactions typically proceeding via a closest-contact pathway. Accordingly, our understanding of the high-pressure reactivity of these systems is intrinsically tied to our ability to determine the structure and phase behavior of organic molecules at extreme pressures.

Diamond nanothreads are an emerging class of one-dimensional, crystalline carbon nanomaterials synthesized from small unsaturated ring systems (e.g., benzene[1]) that provide a unique platform to systematically study the reactivity of small molecules at pressure. Nanothreads are typically synthesized through a series of high-pressure, solid-state [4 + 2] Diels-Alder cycloaddition reactions along the molecular stacking axis to produce polymers containing an extended sp^3 carbon core. These diamond-like materials are predicted to combine the flexibility of conventional polymers with the superlative properties of diamond.[2-4] However, high-pressure phase changes and the number of competing reaction pathways that are viable at the pressures required for nanothread synthesis often results in the formation of chemically inhomogeneous products with inconsistent chemical bonding. This issue is compounded for precursors which contain pendant functional groups that can also partake in additional side-reactions.

Here we highlight our work on the formation of chemically homogeneous, functionalized nanothreads for which the structures, properties, and reactivity can be accurately determined.[5, 6] The impact of high-pressure phase changes on nanothread forming reactions will be discussed, and how forces (e.g., H-bonding) can be used to reduce the likelihood of high-pressure phase changes and allow us to accurately predict reaction pathways based on ambient pressure structures. A particular focus will be placed on our efforts to synthesize nanothreads which retain functionality at pressure, and our successes in the formation of carboxylate (-COOH) rich nanothread polymers. Additionally, this talk will highlight how high-pressure phase changes provide answers to longstanding questions in fields that are unrelated to high-pressure polymerization.

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Session 5B-5: Materials under High Pressure – Using Diffraction Techniques to understand Synthesis Pathways, Phase Transitions and Phase Behaviors

High Pressure Studies of Quantum Materials with Neutron Scattering

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I shall describe the scientific interest in high pressure neutron scattering experiments as a probe of quantum materials. I will then discuss the technical challenges and opportunities for progress in the quality of scattering data and pressure range accessible. Finally, I shall provide examples of state of high-pressure neutron scattering experiments on quantum materials and an outlook on future areas of scientific impact.

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The 80th Annual Pittsburgh Diffraction Conference

at the Wyndham University Center, Pittsburgh
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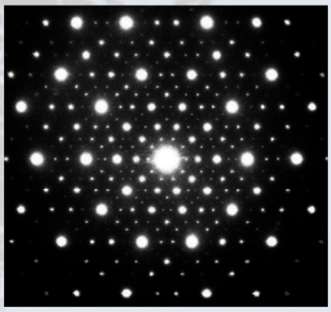

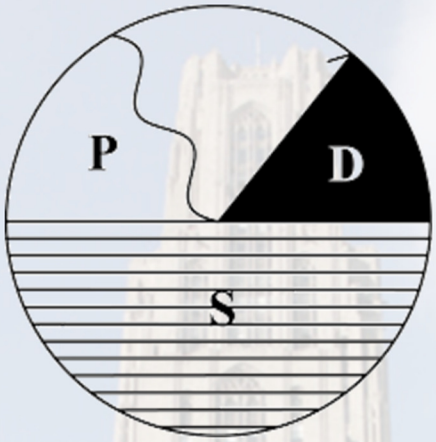

Poster Abstracts

Posters are sorted alphabetically by last name of the presenter.

Student presenters, please sign up for the **Chung Soo Yoo Award**, which carries a $400 cash prize, related to materials and biological research, respectively.

Unique Conformations of the Acute Myeloid Leukemia-Associated Src-family kinase, Fgr, Induced by ATP-Site Inhibitors

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The Src-family kinase Fgr is expressed primarily in myeloid hematopoietic cells where it normally contributes to control of innate immune responses. Overexpression and constitutive activation of Fgr is a common finding in acute myeloid leukemia (AML), and ATP-competitive inhibitors of Fgr show promise as antileukemic agents. These inhibitors include the pyrrolopyrimidine, A-419259, as well as the N-phenylbenzamide, TL02-59, both of which are orally active and suppress bone marrow engraftment of AML cells in mouse models. Like other members of the Src family, Fgr is comprised of an N-terminal unique region followed by regulatory SH3 and SH2 domains, the catalytic kinase domain, and a negative regulatory C-terminal tail. Previous crystal structures of Src and other family members demonstrate that intramolecular interactions between these domains regulate kinase activity. In the 'closed' conformation, the SH3 domain binds to the linker connecting the SH2 and kinase domains, while the SH2 domain engages the tyrosine phosphorylated C-terminal tail. In the present study, we solved X-ray crystal structures of near-full-length Fgr (SH3-SH2-kinase-tail) bound to A-419259 and TL02-59 and observed unique inhibitor-induced conformations. A-419259 induced a 'closed' Fgr conformation, with the SH3 and SH2 domains bound to the SH2-kinase linker and C-terminal tail, respectively, as observed previously for Src. In the crystal, the Fgr:A-419259 complex packed as an asymmetrical homodimer such that the activation loop of one monomer inserted into the active site of the other, providing a snapshot of the trans-autophosphorylation event required for kinase activation. By contrast, TL02-59 binding induced SH2 domain displacement from the C-terminal tail and SH3 domain release from the linker. Solution studies using

hydrogen-deuterium exchange mass spectrometry were consistent with the crystal structures, with A-419259 reducing and TL02-59 enhancing solvent exposure of the SH3 domain. These structures demonstrate that allosteric connections between the kinase and regulatory domains of Src-family kinases are influenced by the ligand bound to the active site, raising the possibility of allosteric inhibitor discovery.

Investigating SARS-CoV-2 ORF3a binding to human TRAF2 and TRAF3

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Severe COVID-19, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), is characterized by widespread inflammation and cytokine storm. A number of viral proteins are capable of inducing inflammatory responses, one of which is open reading frame 3a (ORF3a). ORF3a from SARS-CoV shares 73% homology with SARS-CoV-2 ORF3a and was shown to stimulate NF-κB signaling and inflammasome activation in a manner dependent on TNF receptor-associated factor 3 (TRAF3). TRAF family proteins regulate NF-κB activation and thereby influence inflammatory responses. Both ORF3a homologs contain a TRAF-binding consensus sequence, and SARS-CoV ORF3a has been shown to bind to human TRAFs 2, 3, and 6. We therefore investigated the binding of SARS-CoV-2 ORF3a to TRAF proteins as a potential mechanism through which SARS-CoV-2 may activate inflammatory signaling.

TRAF-C domains from TRAFs 2 and 3 were purified and co-crystallized with a peptide from ORF3a containing the TRAF-binding sequence (PIQAS). Fluorescence polarization (FP) was used to assess binding affinity between TRAF-C domains and ORF3a peptide, and dual-luciferase assays were used to measure NF-κB activation in cells transfected with wildtype ORF3a or ORF3a in which the TRAF-binding sequence was mutated to alanines. X-ray crystallography revealed that the ORF3a peptide is able to bind to the TRAF-C domain of TRAFs 2 and 3, and FP indicated that the binding affinity for these interactions is low. When we compared NF-κB activation in HEK293T cells expressing WT vs mutant ORF3a, there was no significant difference. Therefore, while ORF3a has the potential to bind to TRAFs, this interaction does not significantly contribute to inflammatory signaling through NF-κB in our assay.

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Structural Mechanisms for VMAT2 Inhibition by Tetrabenazine and Reserpine.

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The Vesicular Monoamine Transporter 2 (VMAT2) is the primary transporter responsible for loading neurotransmitters such as dopamine, serotonin and norepinephrine into synaptic vesicles. This critical role in neurotransmission has implicated it in a variety of diseases including Parkinson's and Huntington's disease, drug addiction, and depression. Despite this importance, there is limited information on the mechanisms underpinning VMAT2 function. To date, there are currently no solved structures for VMAT2; most likely due to its small size and difficulty. Here we use cryoEM to solve the structure of VMAT2 in complex with two known inhibitors: tetrabenazine (TBZ), and reserpine (RES). To counter its small size and limited extracellular features, we devise a novel method of creating a fiducial intrinsic to the native protein sequence to drive particle alignment. Using this method, we are able to identify potential TBZ and RES binding sites, and find that these inhibitors stabilize an occluded and outward-open conformation respectively. The extracellular loops (EL) in particular appear to play a significant role in transporter conformational sampling, either working together to cinch the extracellular gate close in an occluded conformation or lay completely apart in the outward-open state. Of particular importance is a tryptophan in EL 6/7, which works to plug the extracellular gate just below the ligand. We hope that these new insights will help inform future drug development, and provide a stepping stone for future studies elucidating the structural mechanisms underpinning VMAT2 substrate transport.

Using Serial Crystallography to Determine DJ-1 Mechanism

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Determining the molecular basis of the remarkable catalytic power of enzymes is a central challenge in biochemistry. The recent advent of time-resolved serial X-ray crystallography using X-ray free electron (XFELs) and third-generation synchrotron sources allows enzymes to be observed in real-time as they catalyze their reaction in the crystal. We used a new approach to mix-and-inject serial crystallography (MISC) at the Advanced Photon Source (BioCARS 14-ID) to follow the conversion of methylglyoxal to lactate by human DJ-1 through Laue diffraction. The high flux of the pink X-ray beam permitted exposure times as low as 3.6 microseconds, in contrast to millisecond exposure times typical at monochromatic beamlines. DJ-1's glyoxalase activity has been controversial, with proposals that the enzyme acts only on glycated macromolecular substrates. Using custom-built injectors, we collected a time series of datasets and observed the formation of covalent catalytic intermediates in DJ-1 microcrystals after mixing with methylglyoxal at time delays ranging from 3-30 s. Our results establish that DJ-1 acts directly on methylglyoxal and demonstrate the feasibility of performing MISC experiments at synchrotron beamlines.

Exploration of Reaction Pathways during Rapid Joule Synthesis Through Time–Resolved In–Situ High–Energy Powder X–ray Diffraction

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High-temperature (>1000 K) techniques with sub-second reaction rates, like rapid Joule synthesis, offer a unique approach for fast and energy-efficient manufacturing of materials with outstanding mechanical, electrical, thermal, and magnetic properties to advance battery, thermoelectric device, catalyst, and ultra-high-temperature thermal protection technologies. However, harnessing the full potential of these methods requires a comprehensive fundamental understanding of the thermodynamic and kinetic pathways. The study of atomic level ordering can provide these insights, but the reaction rates challenge the observation of the phase progression through direct methods like in-situ x-ray diffraction. The characterization of these reactions has the following requirements:

1. High-energy X-rays to penetrate into the bulk of materials.
2. A single photon counting detector for high-energy x-rays with a high frame rate and low signal-to-noise ratio to capture all the phase transformations during the fast-kinetical processes.
3. The high flux of a synchrotron dedicated to high-energy x-rays.
4. Strategies to efficiently analyze the large (>15,000 frames) data sets obtained.

An apparatus for time-resolved in-situ rapid Joule synthesis and a data collection strategy were developed. Leveraging the high-energy x-rays and other capabilities at APS beamline 11-ID-C and PETRA P21.1, the phase transformations and reaction kinetics resulting from heating metal nitrate salts (Zn, Cu, Co) were investigated.

New antimalarial agent CK-2-68 at unintended target, still discriminating Plasmodium from Host Cytochrome bc1.

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Malaria is a devasting disease in humans and other vertebrates. With 247 million cases in the population, the annual number of fatalities is over 600,000 in 2021. Medicines, developed to disrupt the live cycle of the malaria-causing organism Plasmodium spp., are constantly challenged by the emergence of resistance mutations. Enzymes of the respiratory chain are indispensable in metabolic processes and differ sufficiently by evolutionary adaptation that they are considered valuable targets. In fact, complex III or cytochrome bc1 is currently the sole validated target. The quinolone derivative CK-2-68 that was reported to target NDH2 (complex I) of Plasmodium was however found to cause mutations in cytochrome bc1 (complex III) in selection experiments. Here, we report on the cryo-EM structure of CK-2-68 bound to mammalian cytochrome bc1. Bos Taurus bc1 with the inhibitor bound at the Q(positive) site reveals the principles of the resistance mutations in Plasmodium as well as the lack of inhibitory action in the host enzyme. Our findings are supported by AlphaFold 2 models of Pf cyt b/c1/isp and activity assays where possible.

Conformation-dependent Inhibition of the AML-associated Src-family kinase Fgr by ATP-site inhibitors

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Development of acute myeloid leukemia (AML) is often associated with overexpression of non-receptor protein-tyrosine kinases, including three members of the Src family: Hck, Lyn and Fgr. Our group is actively investigating small molecule Src-family kinase inhibitors with significant anti-AML efficacy. These inhibitors include the pyrrolopyrimidine A-419259 and the *N*-phenylbenzamide TL02-59; the latter compound potently inhibits Fgr and Lyn *in vitro* and reverses bone marrow engraftment of the human AML cell line MV4-11 in a mouse model of AML. We recently solved X-ray crystal structures of near-full-length Fgr, consisting of the SH3, SH2, and kinase domains plus the tyrosine-phosphorylated tail, in complex with each inhibitor. A-419259 bound to the Fgr ATP-site with the regulatory SH3 and SH2 domains packed against the back of the kinase domain, resulting in a closed conformation observed in previous structures of Hck with this inhibitor. However, while TL02-59 also bound to the Fgr ATP-site, it induced allosteric displacement of the SH3 and SH2 domains from their regulatory positions, resulting in an open conformation. To explore the effect of allosteric domain displacement on the TL02-59 inhibitory mechanism, Fgr mutants were generated with enhanced SH3 domain interaction with the SH2-kinase linker (high affinity linker or 'HAL' mutants). Fluorescence polarization assays confirmed enhanced intramolecular SH3:linker interaction, thus favoring the closed conformation. X-ray crystallography of an Fgr SH3-SH2-linker protein with the HAL substitution showed that the orientation of the SH3 and SH2 domains is virtually identical to that observed in the structure of the near-full-length kinase, suggesting that the high affinity linker does not impact the overall closed kinase conformation. To test the effect of HAL substitutions on Fgr sensitivity to TL02-59, we created an

active form of Fgr by fusing it to the coiled-coil (CC) domain of the breakpoint cluster region protein (Bcr). Expression of CC-Fgr (wild-type and HAL mutants) transformed TF-1 cells into a cytokine independent phenotype and rendered them sensitive to Fgr inhibition by TL02-59. Stabilizing the closed conformation by introduction of the HAL mutations enhanced TL02-59 potency in these cells, suggesting that TL02-59 prefers a single Fgr kinase conformation. Ongoing work is directed toward identification of small molecules that mimic the effect of HAL substitutions on Fgr. Combining allosteric modulators that lock a single Fgr conformation are predicted to synergize with TL02-59 and suppress the evolution of resistance mutations, a common limitation of many clinical ATP-site kinase inhibitors currently in use for AML and other cancers.

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Elucidating the effect of polymorphism and cation disorder on the nonlinear optical properties of the Cu_2ZnGeS_4 diamond-like semiconductor

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Diamond-like Cu_2ZnGeS_4 polymorphs have been studied over the years for applications in solar cells and photochemical catalysis. Although much work has been published, the single crystal structures and the nonlinear optical (NLO) properties of the polymorphs have not been determined. Our work includes the comprehensive characterization of the structures, preparation of phase-pure α- and β-polymorphs, and assessment of NLO properties. X-ray single crystal diffraction, X-ray powder diffraction (XRPD), neutron diffraction, electron diffraction, and high-angle annular dark field scanning transmission electron microscopy (HAADF-STEM) were utilized to definitely ascertain the structures. The structures obtained from single crystal X-ray diffraction data agreed with the previously published structure types, wurtz-stannite ($Pmn2_1$) for the high-temperature α-phase and stannite ($I\text{-}42m$) for the low-temperature β-phase. Neutron diffraction was needed to verify these structures, due to the isoelectronic nature of the metal cations. Furthermore, electron diffraction and HAADF-STEM imaging support the existence of a third polymorph, γ, prepared at high temperature with a wurtz-stannite-like structure but having metal cation disorder. The disorder was exacerbated when the reactants were subjected to an ice-water quench, as opposed to a controlled slow cooling. For the NLO results, the high-temperature α-phase had the largest second harmonic generation response of the three polymorphs with a $\chi^{(2)}$ value of 17.3 pm/V, while the quenched γ-phase and the low-temperature β-phase had values of 5.04 and 0.5 pm/V, respectively. Finally, the quenched γ-phase possessed the largest laser-induced damage threshold of 1.0 GW/cm^2 with the high-temperature α-phase and the β-phase coming in lower at 0.5 and 0.3 GW/cm^2, on par or better than some commercial standards. These results indicate polymorphs can possess drastically different NLO properties, and that different heat treatment, including cooling procedures, should be investigated when preparing new NLO materials.

Cryo-EM structure of an orphan membrane transporter bound to an antiepileptic drug

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The synaptic vesicle protein 2 (SV2) family is comprised of three transmembrane glycoproteins – A, B, and C, expressed throughout the brain. Based on sequence homology, these proteins are members of the major facilitator superfamily of membrane transport proteins, but no functions have been conclusively identified in the literature for any SV2 family members to date. SV2A/B double knockout mice appear normal at birth but suffer from severe seizures, neurological abnormalities, and early death. SV2A has been identified as the binding site for the antiepileptic drug levetiracetam (Keppra), which is broadly used to treat epilepsy patients. These observations suggest that the SV2 family plays a crucial role in normal neurotransmission. SV2 family members contain twelve transmembrane helices with a large pentapeptide-repeat luminal domain (LD) and an intracellular domain with a long N-terminal disordered region. Here we present a cryo-EM structure of SV2B in an outward-open conformation complexed with padsevonil, a levetiracetam-related molecule that has been investigated clinically as an antiepileptic drug. Taking advantage of the rigid LD on the protein as well as the Selectris energy filter on the University of Pittsburgh's Titan Krios microscope allowed us to resolve the structure of SV2B in detergent without an antibody-based fiducial, which is typically needed to solve cryo-EM structures for membrane proteins of this size. Density for padsevonil is located close to the intracellular half of SV2B, with readily observed side chain densities for many of the residues involved in binding. The ordered portion of the intracellular domain forms a bundle containing two alpha-helices splayed out perpendicular to the detergent micelle, a feature that has not been seen in other MFS transporters.

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Structural and Mechanistic Characterization of Protective Broad Spectrum Non-Neutralizing Antibodies Targeting Crimean-Congo Hemorrhagic Fever

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Crimean-Congo hemorrhagic fever virus (CCHFV) causes a debilitating hemorrhagic fever with a mortality rate as high as 40%. With recent spread to new countries and no approved vaccine or therapeutics available, CCHFV is viewed as a priority public health threat by the WHO. Recently, a set of human and mouse non-neutralizing monoclonal antibodies (mAb) targeting CCHFV glycoprotein GP38 showed to confer post-exposure protection. Here, we reveal the broad cross protection properties of these mAbs as well as initial insights into how they confer this protection. Starting with a collection of anti-GP38 mAbs gathered from Turkey survivors (CC5) and mice (13G8), mAbs were screened against strains across the six clades of CCHFV as well as Aigai Virus using BioLayer Interferometry (BLI) as well as a novel CCHFV cell spread assay. X-ray crystallography was utilized to perform fine epitope mapping of two antigenic sites on clinically relevant Hoti GP38 with mutagenesis used to probe mAb resiliency and broad-spectrum potential. *In vivo* efficacy studies were

also performed. Through the collection of mAbs, five antigenic sites were identified. The epitope of Site 1 and Site 4 mAbs were mapped using 13G8/CC5-17 and CC5-20 respectively. The conserved nature of these sites, along with biochemical data revealed that these mAbs could productively engage 80%-100% of known CCHFV species and be resilient to epitope drift. Through *in vivo* and cell spread assays revealed that affinity was not the sole determinate for protection and highlights other factors involved in protection. This study highlights that non-neutralizing mAbs targeting GP38 can serve as a broad-spectrum option for pre-and-post treatment for CCHF. Also, it reveals key considerations in developing mAbs that target nairovirus GP38s as well as those seeking to leverage the non-neutralizing pathways responsible for protection.

Unveiling the Fusogenic and Membrane-Modulating Potential of Trp- and Arg-Rich Antimicrobial Peptides

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This research tackles the escalating threat of antibiotic resistance by investigating antimicrobial peptides (AMPs) as viable alternatives to traditional antibiotics. We focus on two linear AMPs, LE-53 (12-mer) and LE-55 (16-mer), which are designed to be non-helical. LE-53 is shorter, and yet it displays more potent antibacterial activity against Gram-negative (G(-)) and Gram-positive (G(+)) bacteria compared to LE-55. Both are non-toxic to eukaryotic cells. LE-53's higher efficacy is attributed to higher hydrophobicity (H) and hydrophobic moment (μH) in comparison to LE-55. Circular dichroism (CD) reveals that LE-53 and LE-55 both adopt a random coil structure in lipid model membranes (LMMs) mimicking G(-) and G(+) bacteria, so secondary structure is not the cause of the potency difference. Intriguing insights from X-ray diffuse scattering (XDS) reveal that increased lipid chain order in LE-53 could be an important difference between the two AMPs. Moreover, our XDS study uncovers a significant link between LE-53's headgroup-hydrocarbon interfacial position in G(-) and G(+) LMMs, compared to a deeper, hydrocarbon location for LE-55. Neutron reflectometry (NR) confirms the AMP locations determined using XDS. Solution small angle X-ray scattering (SAXS) reveals LE-53's remarkable capacity to induce vesicle fusion in bacterial LMMs, but not in eukaryotic LMMs. This unique
characteristic likely contributes to its heightened antibacterial activity, presenting promising opportunities to address antibiotic-resistant strains without compromising human cell integrity.

Application of quantum crystallography to study relativistic effects

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Keywords: quantum crystallography, relativistic effects, Hirshfeld atom refinement

During the last 50 years, relativistic quantum chemistry has undergone significant development and methodological progress. Nowadays, it is well-known that a relativistic quantum formalism is necessary for the study of compounds with heavy elements [1].
However, studies of the chemical properties of compounds and crystals containing heavy elements are challenging for both theoreticians and experimentalists. The Schrödinger equation is no longer applicable and experimental studies require special facilities and conditions to be considered. A perspective method is quantum crystallography which relies on the XRD data to describe crystal structure in unprecedented detail. Hirshfeld atom refinement (HAR) [2] is a method that uses tailor-made aspherical atomic scattering factors, obtained from the quantum mechanical calculations which can be done at different levels of theory, to refine atomic positions and their ADPs in the standard least-square refinement. It has been shown that HAR overcomes all the shortcomings of the Independent Atom Model (IAM), yielding more accurate hydrogen atom positions and enabling the refinement of hydrogen atom ADPs [3]. Furthermore, HAR was successfully applied to small and big, light and heavy molecules [3]. During the last few years, new software for HAR was extensively developed and nowadays modelling of disorder, even for structures with heavy elements, is possible. On the other hand, intensities

of the diffracted beam are affected not only by relativistic effects but also by absorption, anharmonic motion, anomalous dispersion, and many other effects which highly influence electron density distribution in the crystal and, in consequence, derived properties. Thus, studies of the relativistic effects with HAR require the collection of outstanding quality X-ray diffraction data sets in terms of resolution, absorption corrections, and error model.
Here, we present the results of relativistic Hirshfeld atom refinements [4] carried out as implemented in Tonto [5] or NoSpherA2 [6] for high-resolution X-ray diffraction data sets. The outcome of DFT-based refinements with the nonrelativistic and quasi-relativistic approaches will be compared, including an analysis of the nature of the Me–X bonds in Au crystal [7], the role of modelling disorder [8], and a description of aurophilic interactions [9].

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Do Protein Surface Amino Acids Correlate to Crystallization Conditions?

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Virtually every stage in the genome to structure process has undergone a revolution over the past 30 years; recombinant methods coupled with affinity purification for preparing proteins, synchrotrons as X-ray sources, cryo-cooling of crystals, ever faster detectors, and improved computers and software for data processing and structure determination. The only stage in the process that has not been made faster is crystallization screening, how the requisite crystals are actually obtained. Screening is the first stage in determining the significant factors to a process. For macromolecule crystallization, this typically involves subjecting the target protein to a random campaign of chemical assaults, in hopes that it eventually surrenders and forms an ordered array, a crystal. More often than not the protein either does nothing, randomly aggregates, or denatures and dies. On occasion one will hit upon conditions that lead to some form of self-associated outcome, which may range from nicely faceted crystals (that may or may not diffract) to ugly lumps of something that is crystalline in name only. After screening comes the second stage, the optimization of the hits obtained. Screening is a random search using known fixed chemical conditions in search of unknown but fixed crystallization conditions. We postulate that if one can, through analysis, estimate likely crystallization conditions for a given protein then a more targeted screening process would be faster, use less materials, and have a higher probability of success. Proteins interact with their environment through the amino acids on their surface, and we hypothesized that the surface solvent accessible population of a given protein could be correlated with its crystallization conditions. Our initial efforts are to test the correlation of protein surface amino acid compositions with their crystallization conditions, in the anticipation that the reverse process can be employed where the surface amino acid distribution of an unknown protein can be used to estimate its likely crystallization conditions.

Advances in *operando* and *in situ* high-energy x-ray scattering with APS-U

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The Structural Science group at the APS operates a suite of four high-energy x-ray (HEX) beamlines dedicated to in situ and operando experiments ranging from synthesis to studies of interaction of atomic order with macroscopic properties in functional materials to operando studies of complex real devices. In the last years, the program was highly demanded and contributed to more than 350 publications each year, which reflects the impact of HEX diffraction on fundamental and applied science.

After the upgrade of the APS, beamline 11-ID-D will complement this suite of beamlines, which will enable a combination of total scattering with small angle scattering and focusing in the sub micrometer range. With this, the length-scale gap between the resolution in reciprocal and real space to provide a complete picture of the structure of materials will be closed. Multimodal setups and photon energies between 26 keV and 120 keV with highest flux will enable complex in situ and operando diffraction experiments. The priorities will be on the discovery of new materials by automated panoramic syntheses and the mapping of local atomic ordering during operation with high-spatial resolution to understand the role of inhomogeneities and interfaces on macroscopic performance, especially in energy storage and conversion systems.

Upcoming opportunities for research in materials science at the upgraded APS will be discussed.

Structural basis of NINJ1-mediated plasma membrane rupture in lytic cell death

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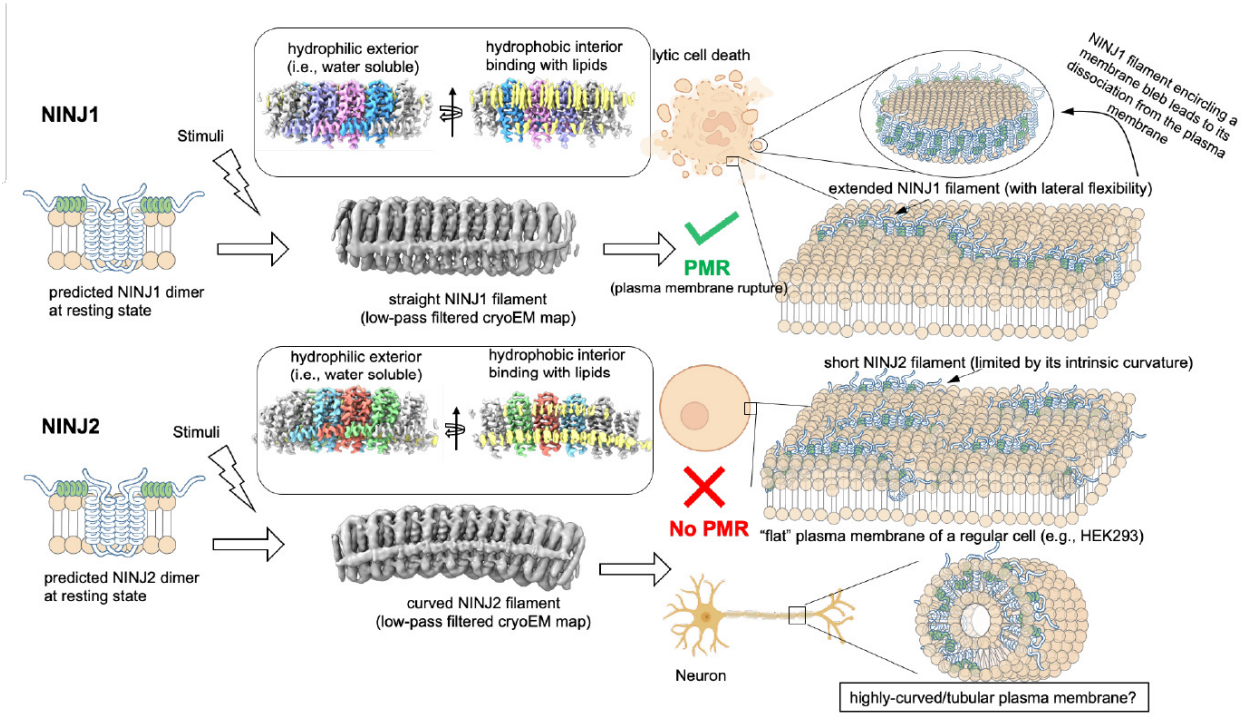
Cell death is crucial for tissue homeostasis, immune responses, removal of defective cells, and warding off diseases like cancer. There are various cell death mechanisms, including non-lytic ones like apoptosis, which prevents inflammation by not releasing cellular components. In contrast, lytic cell death, such as pyroptosis and necroptosis, results in plasma membrane rupture (PMR) and the release of cellular contents. This type of death is essential in defending against pathogens, with the release of damage associated molecular patterns (DAMPs) alerting immune system and triggering inflammatory responses. However, dysregulation of such processes can lead to chronic inflammatory diseases, underscoring the importance of regulated PMR, the final cataclysmic step in lytic cell death.

Historically, PMR was seen as a passive event from cellular swelling after membrane leakage, thought to be caused by Gasdermin-D (GSDMD) pores in pyroptosis or by mixed-lineage kinase domainlike protein (MLKL) in necroptosis. Recent studies highlight ninjurin-1 (NINJ1), a 16 kDa membrane protein, as an active PMR executioner. Initially linked to axonal regeneration post nerve injury, NINJ1 induces PMR through oligomerization activated by death signals. Surprisingly, its analogous protein, NINJ2, despite sharing sequence and structure similarities, doesn’t induce PMR.

We employed cryogenic electron microscopy (cryoEM) to understand NINJ1's role and NINJ2's limitations in facilitation of PMR. Our cryoEM analyses revealed both NINJ1 and NINJ2 proteins form linear filaments, with one side binding to lipids (i.e., hydrophobic interior) and the other being water-soluble (i.e., hydrophilic exterior). Notably, while the straight NINJ1 filament could encircle and dissolve a membrane bleb, leading to PMR, the inherently curved NINJ2 filament couldn't. Our observations indicate that this difference in ability to induce PMR could be attributed to differential lipid binding patterns between NINJ1 and NINJ2 filaments. While strong lipid binding in the extracellular side leaflet of the membrane bilayer empowers NINJ1’s PMR-inducing capacity, unique and different interaction pattern

of NINJ2 with cholesterol and lipids in the cytoplasmic side leaflet of the membrane bilayer contributes to its curvature and incapacity in inducing PMR. Intriguingly, NINJ2 might function like NINJ1 in high curvature membranes, such as neuron axons.



Small Molecule Allosteric Modulators of the AML-associated Src-family Kinase, Hck

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Hck and Fgr are members of the Src family of non-receptor tyrosine kinases expressed in myeloid hem-atopoietic cells where high-level expression can drive the formation of acute myeloid leukemia (AML). While ATP competitive inhibitors of Hck and Fgr show promise for AML therapy, their ultimate clinical efficacy is likely to be limited by the emergence of resistance mutations. Combination therapy targeting a single protein with allosteric and ATP-site inhibitors dramatically reduced resistance potential in various cancers and may have potential for AML as well. Our group has identified two small molecules with a shared pyrimidine diamine core (PDA1 & PDA2) that have potential as allosteric Hck inhibitors. Surface plasmon resonance (SPR) and NMR spectroscopy indicate that these compounds recognize a shared binding site involving the PPII-helix binding surface of the regulatory SH3 domain. *In vitro* kinase activity assays and hydrogen-deuterium exchange mass spectrometry (HDX-MS) reveal that despite the shared binding site, the compounds have opposite effects on overall kinase activity and dynamics. PDA1 stabi-lized overall Hck dynamics and did not affect kinase activity, while PDA2 disrupted the closed confor-mation of the kinase and stimulated kinase activity. To test PDA1's efficacy in cells, we transformed human TF-1 myeloid leukemia cells with a kinase-active chimeric protein that fuses Hck to the coiled-coil (cc) domain of Bcr. Treatment of TF-1/cc-Hck cells with PDA1 resulted in growth suppression which was significantly reduced with the introduction of PDA1-resistant mutants,

providing evidence for on-target activity. To determine the structural basis of PDA1 interaction with Hck, X-ray crystallography was used to map the binding site. While this effort yielded a high-resolution crystal structure of near-full-length Hck, electron density for PDA1 was not observed which may reflect the relatively low affinity of this compound and competition with the SH2-kinase linker at the SH3 domain. However, the crystal structure of the kinase domain, which was bound to the ATP-site inhibitor A-419259, revealed an extended structure of the activation loop for the first time. *In silico* docking and molecular dynamics simulations suggest that PDA1, but not PDA2, stabilizes the closed, inactive, conformation of Hck by bridging the SH3 and kinase domains. Ongoing studies are exploring the structural basis of pyrimidine diamine interaction with Hck and the potential for the combination of PDA1 with ATP-site inhibitors to reduce the potential for acquired resistance.

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Mutagenesis of the Homodimerization Interface Suppresses Multiple HIV-1 Nef Functions without Altering the Core Fold

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The HIV-1 Nef protein is essential for viral infectivity, high-titer replication *in vivo*, and immune evasion of infected cells. Nef performs these functions through diverse interactions with host cell proteins involved in signal transduction and endolysosomal trafficking. Previous crystallography studies have demonstrated that Nef forms homodimers when complexed to the regulatory SH3 and SH2 domains of Src family kinases. Nef mutants based on the crystallographic dimer interface are defective for most if not all Nef functions, including kinase activation. Here we combined neutron reflectometry of recombinant, full-length myristoylated Nef bound to model lipid bilayers with molecular simulations to demonstrate that Nef alone forms homodimers when associated with the membrane. A dimerization-defective mutant, Nef-L112D, destabilized the membrane-bound dimer, validating the central role of this residue in previous homodimer crystal structures. Neutron reflectometry data also show that the homodimeric Nef core region is displaced from the plasma membrane for host cell partner protein engagement, including Src-family kinases. The X-ray crystal structure of the Nef-L112D mutant in complex with the SH3 domain of the Src-family kinase Hck revealed a monomeric 1:1 complex, in contrast to the 2:2 dimeric complex previously observed with wild-type Nef. The overall fold of the Nef-112D core in the complex was nearly identical to that of wild-type Nef observed in previous structures, including the interaction interface with the SH3 domain. This structure demonstrates that the L112D mutation does not alter the Nef core fold, supporting the conclusion that the diverse phenotypic effects associated with this mutation are due solely to the dimerization defect. Our integrated approach provides direct evidence supporting the intrinsic capacity of Nef to homodimerize

at lipid bilayers, providing fresh insight into the pleiotropic actions of homodimer interface mutations on HIV-1 Nef function. These results have important implications for ongoing studies of inhibitors targeting this viral protein, which may act in a similar fashion as these mutations to disrupt Nef quaternary structure.

The Online Tool for Associative Experimental Design of Protein Crystallization

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The online associative experimental design (OAED) is a new tool that can be used to design experiments for protein crystallization. The OAED is a web-based application that allows users to input information about their protein and experimental conditions, and then generates a ranked list of experiments that are likely to be successful. The OAED has several advantages over the offline AED version [1]. First, it is more accessible, as it can be accessed from anywhere with an internet connection. Second, it is more up-to-date, as the computation is constantly being updated based on experimental data. Third, it is more efficient, as it can generate a list of experiments in a matter of seconds. The OAED continues to provide all advantage features of the AED analysis [1]. (1) Improving condition: The analysis approach can be used to improve existing crystallization conditions. For example, if the existing conditions are not readily repeatable, or if they do not give crystals diffracting to a sufficient resolution, the analysis approach can reveal an expanded range of conditions, some or many of which may resolve these problems. (2) Robust conditions: Crystallization is a stochastic process so that the same conditions can produce different outcomes. The analysis approach can help to find more robust crystallization conditions, which are less sensitive to the concentration of one or more of the components present. (3) New space group search: By finding new conditions the tool can potentially extend the space groups obtained in the initial screening experiments for a new packing arrangement. (4) Other features: In addition, the tool is able to generate screening conditions that can result in crystals of proteins with improved diffraction resolution and improved crystal size. The OAED is a valuable tool for the crystallography community. It can help researchers to design experiments that are more likely to be successful, and it can save them time and resources. The OAED is still under development, with a number of features

being considered to extend its utility. It has the potential to revolutionize the way that protein crystallization experiments are designed and screened.

1. Dinc I, Pusey ML, Aygun RS. Optimizing Associative Experimental Design for Protein Crystallization Screening. IEEE Trans Nanobioscience. 2016 Mar;15(2):101-12. doi: 10.1109/TNB.2016.2536030. Epub 2016 Feb 29. PMID: 26955046; PMCID: PMC4898777.

Intermediate states of nucleotide addition by HIV-1 Reverse Transcriptase

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Reverse transcription of the HIV-1 retrovirus single-stranded RNA genome into double stranded DNA is an essential step in HIV-1 replication, and reverse transcriptase (RT), the enzyme that catalyzes this reaction, is a target for antiretroviral therapy. HIV-1 RT structures have been available for a number of years. However, these structures represent thermodynamically stable species and do not inform on kinetic intermediates. Moreover, structures for the RT-T/P-dNTP complexes utilized substrate analogs (i.e., a dideoxy-terminated primer or a non-hydrolyzable dNTP) which prevent phosphodiester (PDE) bond formation. Therefore, there are no available structures of a "live" dNTP in RT's active site and existing structures may not reflect catalytically active complexes. To address this issue, we took advantage of the single particle cryo-EM framework devising "diffusion/blot experiments" where enzyme and substrates are applied on opposite sides of a grid allowing mixing of both reaction components by diffusion on the grid through the matrix of holes. By freezing grids after short diffusion times (4-12 s), we have for the first time elucidated four intermediate kinetic states during HIV-1 Reverse Transcriptase (RT) mediated nucleotide (dATP) incorporation. Furthermore, the presence of a catalytically active 3'-OH primer and a "live" dATP in our experiments allowed observation of a Mg^{2+} ion in a new alternative position that could play a role in PDE bond formation. Collectively, this study provides insights into a fundamental chemical reaction that impacts polymerase fidelity, nucleoside inhibitor drug design, and mechanisms of drug resistance.

Ear Muffs for Elephants: Rational Engineering of Conformational Dynamics with Backbone Modification

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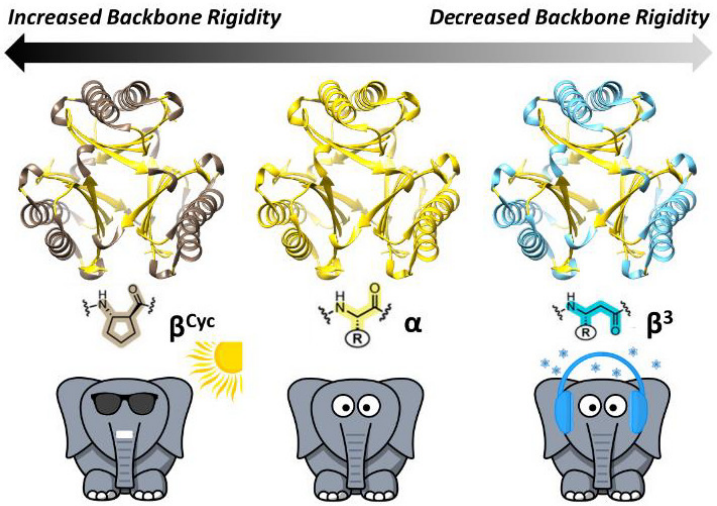

Rational design of flexible protein regions has been used to modulate enzymatic activity and engineer protein catalysts with improved cold tolerance, substrate specificity, and promiscuous activity. One approach that has been developed to engineer protein properties is the incorporation of backbone modified amino acids into native protein sequences through total chemical synthesis: so-called "heterogeneous backbone substitution." These backbone modifications have been shown to maintain local secondary structures and recreate diverse tertiary folds. Less explored than structural mimicry is the use of chemical backbone alteration to modulate protein function. One property that can be readily tuned through backbone changes that is less amenable to traditional side-chain mutagenesis is conformational dynamics. The ability to mimic or maintain sidechain properties, while modulating local rigidity in native L-α-protein folds has the potential to enable the rational design of conformational dynamics in enzymes. To this end, we are exploring the application of sequence-specific backbone modification in the enzyme 4-oxalocrotonate tautomerase to locally increase or decrease chain rigidity in a targeted manner. Here, we describe progress toward the synthesis, purification, structural characterization, and functional analysis of the prototype enzyme and variants.

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In Situ High-Energy X-ray Scattering at 11-ID-C After the Advanced Photon Source Upgrade

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Beamline 11-ID-C, situated at the Advanced Photon Source within Argonne National Laboratory, represents a cutting-edge facility designed for high-energy scattering experiments. This beamline harnesses the power of a monochromatic 106 keV X-ray beam, affording it "neutron"-like penetration capabilities. Remarkably, this energy level enables X-rays to potentially penetrate steel containers with 10mm-thick walls, facilitating data collection from samples enclosed within.

11-ID-C is equipped with X-ray lenses capable of vertically focusing the beam to a remarkable size of 2.5 microns. This breakthrough capability paves the way for in-depth investigations of layered materials using Grazing Incident X-ray scattering. Additionally, it enables the study of ultrathin layers within electrochemical systems, such as those found in coin-cell batteries.

The beamline hosts a versatile array of 2-D detectors, including a VAREX 4343 for general data acquisition and a precision Pilatus 300kW CdTe strip detector. Additionally, researchers have access to a high-performance, large-area Pilatus 2M CdTe detector. These Pilatus detectors employ single-photon counting technology, enabling the capture of faint X-ray scattering signals. Notably, they can acquire data at a remarkable rate of up to 500Hz, making them ideal for time-resolved studies.

Complementing its impressive instrumentation, 11-ID-C is equipped with a diverse range of sample environments, including high-temperature Linkam furnaces, cryogenic Cryostream system, controlled-atmosphere flow cells, electrochemical systems, a stress-strain load frame, and high-throughput sample changers. This comprehensive suite of tools serves the powder diffraction community, facilitating rapid and dependable data collection in various *in situ* processes. Researchers can explore energy storage, catalysis, gas sorption and separations, ion exchange, solid-state synthesis, and much more, thanks to the capabilities of beamline 11-ID-C.

After the APS Upgrade project is completed, x-rays will be generated by a new superconducting undulator (SCU), which will increase the flux by 10 times. This

poster will present the cutting-edge capabilities of beamline 11-ID-C and the exciting possibilities it offers for high-energy scattering experiments, material characterization, and *in situ* process studies.

Characterization of an Alternate Conformation of the HIV-1 Capsid Protein CTD Dimer using ^{19}F NMR and Weighted Ensemble MD

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The HIV-1 capsid protein assembles into a conical shell during viral maturation, encasing and protecting the viral RNA genome. The C-terminal domain (CTD) of the two-domain capsid protein dimerizes, and this dimer connects individual chains in the mature capsid lattice. Previous NMR studies have shown that different dimer arrangements can be formed; however, the structure and function of any alternate dimers are unknown. To explore the conformational landscape of the CTD dimer, we carried out atomistic molecular dynamics simulations using the weighted ensemble rare-events sampling strategy, generating an ensemble of conformations for the alternate dimer orientation. To assess whether the conformations detected experimentally match those in our simulations, we measured interconversion rates between the two alternate dimers using high sensitivity ^{19}F NMR. Overall, the measured experimental rates agree with the rate constants calculated directly from our simulations, and the alternate CTD dimer interface may mimic the orientation of the CTD dimer that connects pentameric and hexameric subunits in the mature, fully assembled capsid. Our results demonstrate the advantages of pairing atomistic rare-event sampling with ^{19}F NMR and may help elucidate the HIV-1 capsid assembly process resulting in the intrinsic structural polymorphism of capsid cores.