PDC 2025

Pittsburgh Diffraction Conference

Hosted by Brookhaven National Laboratory

October 9 – 12, 2025

Brookhaven National Laboratory

U.S. DEPARTMENT of ENERGY

Organizers of the Pittsburgh Diffraction Conference 2025:

Vivian Stojanoff and Daniel Olds
National Synchrotron Light Source II (NSLS-II), Brookhaven National Laboratory

President and President Elect Pittsburgh Diffraction Society

Jeney Wierman (Cornell University),
Daniel Olds (Brookhaven National Laboratory)

Pittsburgh Diffraction Society Board Members in Attendance:

Daniel Olds (Brookhaven National Lab), Conference Chair 2025
Vivian Stojanoff (Brookhaven National Lab), Conference Co-Chair 2025
Jeney Wierman (CHESS), President
Simone Brixius-Anderko (University of Pittsburgh), Immediate Past-President
Matthias Zeller (Purdue University), Treasurer
Andrey Yakovenko (Argonne National Lab), Secretary
Aina Cohen (SSRL), Member at Large
Charles Lake (Indiana University of Pennsylvania), Member at Large
John P. Rose (University of Georgia), Member at Large
Leighton Coates (Oak Ridge National Lab), Member at Large

Local Organizing Committee:

Milinda Abeykoon (NSLS II)
Thomas Caswell (NSLS II)
Aina Cohen (SSRL)
Martin Fuchs (NSLS II)
Joyce Frank (MiTeGen)
Eliot Gann (NSLS II)
Boris Khaykovich (MIT)
Peter Khalifiah (SUNY)
Priyanka Ketkar (NSLS II)
Gihan Kwon (NSLS II)
Jennefer Maldonado (NSLS II)
Daniel Olds (NSLS II)
Darya Marchany-Rivera (SSRL)
Sarah Perry (UMass, Amherst)
David Sprouster (SUNY)
Alex Soares (NSLS II)
Patrick Shaw Stewart (Douglas Instruments, Ltd)
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Vivian Stojanoff (BNL)
Narayanasami Sukumar (NECAT-Cornell)
Crissy Tarver (SSRL)
James Walsh (UMass, Amherst)
Matt Whitaker (SUNY)
Jeney Wierman (CHESS)
Justyna Wodjyla (Incyte)

Special Thanks to our Incredibly Generous

Sponsors, Supporters, and On-Site Vendors

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 PDS has sponsored the annual PDC, returning to Pittsburgh, Pennsylvania area every five years. The society's 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 PhD 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 PDC.

Sidhu Award

This award honors the memory of Professor Surain S. Sidhu, who, while Professor of Physics and Director of the X-ray Laboratory at the University of Pittsburgh, was a founder of the Pittsburgh Diffraction Conference in 1943. Later, Professor Sidhu moved to Argonne National Laboratory, where he pioneered the use of the null matrix technique in neutron diffraction. This involves choosing isotopes of an element in the proportion that gives a zero net coherent scattering factor. The procedure has been widely used for studying biological materials in which the isotopic ratio of hydrogen to deuterium is appropriately adjusted. The current biennial award recognizes an outstanding contribution to crystallographic or diffraction research by a young investigator whose doctoral degree was conferred within six years before the award date. The award carries a cash prize of $2,000.

Winners of the Sidhu Award

(1967) Arthur I. Bienenstock
(1968) Robert M. Nicklow
(1969) Thomas O. Baldwin
(1970) Sung-Hou Kim
(1971) L. K. Walford
(1972) Dale E. Sayers
(1974) Bennett C. Larson & Nadrian Charles Seeman
(1975) Patrick Argos
(1978) Keith O. Hodgson & George DeTitta
(1980) Gregory A. Petsko
(1985) Douglas C. Rees
(1986) David Agard & John M. Newsam
(1988) Qun Shen
(1989) Ming Luo
(1990) Lee Brammer
(1992) Raymond Charles Stevens
(1993) Mark R. Pressprich & Todd Yates
(1994) Alice Vrielink & Jin Wang
(1995) Millie M. Georgiadis
(1996) Michael J. Regan
(1999) Changill Ban & Markus Wahl
(2000) William R. Wikoff
(2001) Laurence Sheppard
(2002) Yongjae Lee
(2003) Erica Ollmann Saphire
(2004) Yong Xiong
(2005) Chong-Yu Ruan
(2006) Peter Chupas
(2008) Michael Hanson
(2010) Hui Wu
(2013) Thomas D. Grant
(2017) Hande Öztürk
(2018) Yen-Ting Lai
(2022) Michael Martynowycz
(2024) Leora Dresselhaus-Marais

2024 Sidhu Award Winner

Dr. Leora Dresselhaus-Marais

Geballe Lab for Advanced Materials McCullough Building,
Room 241
Stanford University
476 Lomita Mall Stanford, CA 94305

leoradm@stanford.edu

Leora Dresselhaus-Marais is an Assistant Professor in the Department of Materials Science & Engineering at Stanford University, with a courtesy appointment in Mechanical Engineering, and a term appointment in Photon Science at the SLAC National Accelerator Lab. Professor Dresselhaus-Marais earned her PhD in 2018 in Physical Chemistry with Prof. Keith Nelson at MIT, where she demonstrated how shock waves initiate chemistry in RDX that couples to deformations in unique ways that enhance the sensitivity. Professor Dresselhaus-Marais did her BA (2012) and MSc in Chemistry at the University of Pennsylvania.

Before joining Stanford University, she was Lawrence Fellow at Lawrence Livermore National Lab leading a team exploring ultrafast darkfield X-ray microscopy to study mechanisms of radiation damage, shock-induced deformation, and melting in single crystals. Today, Professor Dresselhaus Marais studies how modern methods can enable new opportunities to update "old-school" materials processing and manufacturing for sustainability using X-ray Free Electron Lasers and synchrotron radiation.

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

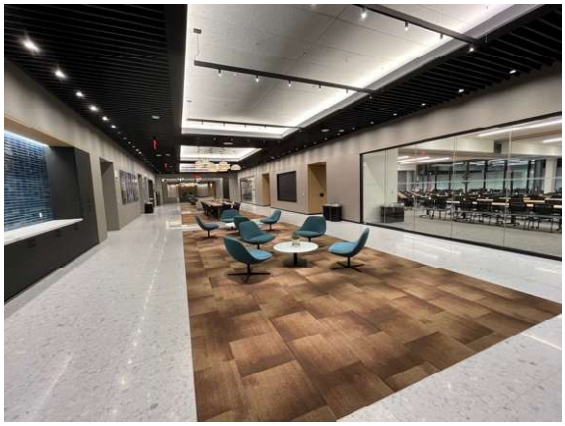
82nd Annual Pittsburgh Diffraction Conference at Brookhaven National Laboratory

Brookhaven Lab is located at the center of Long Island in an area with beautiful beaches and quaint New England style villages. Long Island's North Shore is the perfect getaway and is the gateway to the east end's scenic vineyards and wineries. For more information about the Eastern Long Island:

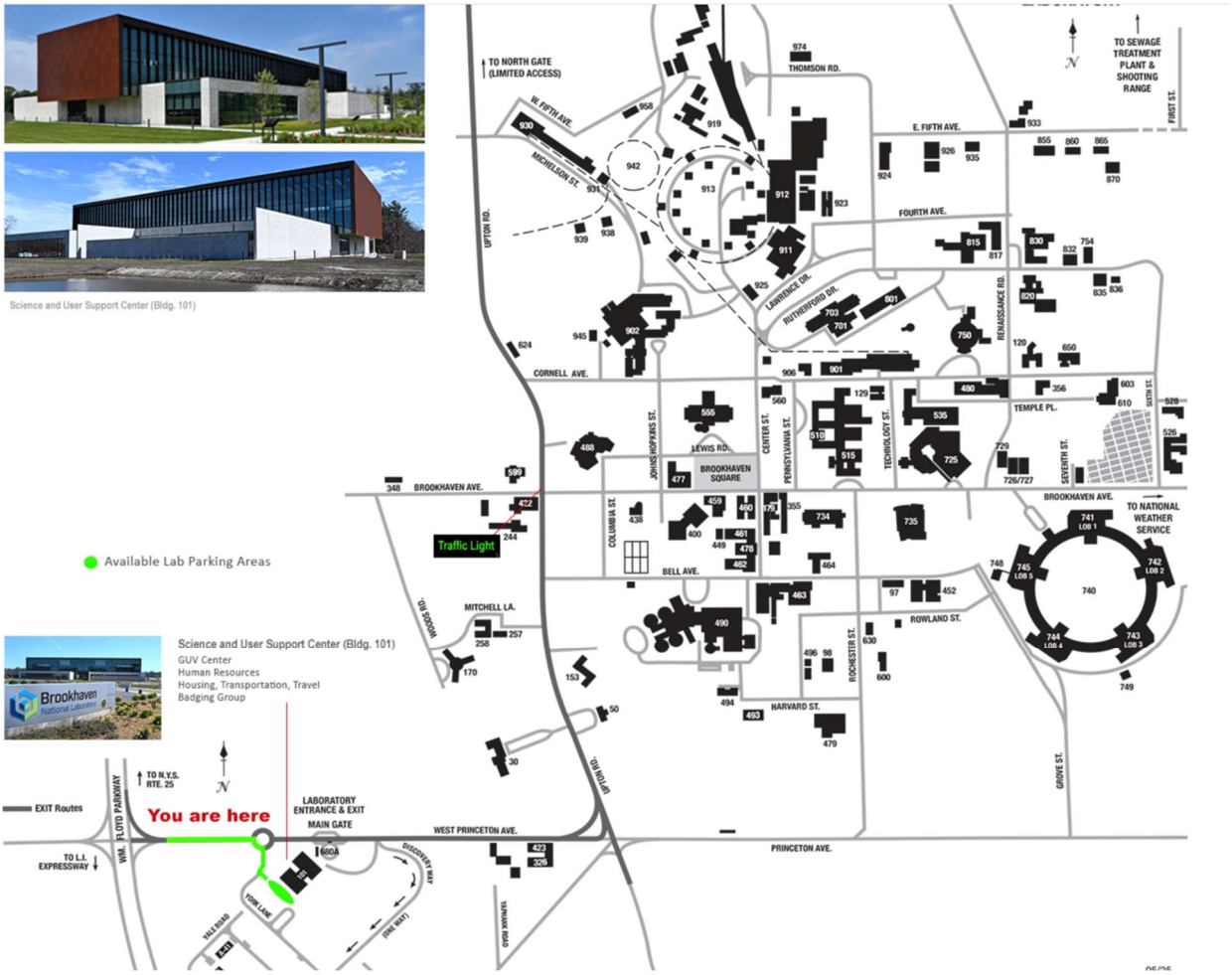
https://eastendgetaway.com/
https://www.discoverlongisland.com/

Brookhaven National Laboratory is located off of exit 68 of the Long Island Expressway (LIE/Highway 495), on William Floyd Highway (County Rt 46) north towards Wading River, on the left side.

Conference, Poster Sessions and Exhibition will take place in Science and User Support Center (Bldg 101).



Campus Map



Click HERE for larger map and directions

Parking at the Conference

Ample parking is available at SUSC, Bldg 101. Brookhaven National Lab follows the nationwide identification requirements for federal site access. Anyone 18 years of age and older must have a valid REAL ID, Enhanced ID, Passport or one of these alternate forms of ID to gain access to the Laboratory.

Transportation ----------

Arriving by Train:

Brookhaven Lab has a shuttle service to and from the closest train station on the Long Island Rail Road (LIRR) - RONKONKOMA. The shuttle service only runs on weekdays!

	Pick-ups		Drop-offs	
	Train Departs	**Train Arrives**	**Train Departs**	**Train Arrives**
Shuttle Departs BNL	**NYC/Penn Station or Grand Central**	**Ronkonkoma**	**Ronkonkoma**	**NYC/Penn Station or Grand Central**
6:25 a.m.	5:16 a.m.	6:39 a.m.	7:10 a.m.	8:25 a.m. GC
			7:28 a.m.	8:49 a.m.
8:10 a.m.	7:01 a.m. GC	8:22 a.m.	8:58 a.m.	10:16 a.m. GC
	7:24 a.m.	8:46 a.m.	9:22 a.m.	10:40 a.m.
3:40 p.m.	2:31 p.m.	3:52 p.m.	4:14 p.m.	5:36 p.m.
			4:57 p.m.	6:16 p.m.
5:45 p.m.	4:25 p.m. GC	5:44 p.m.	6:26 p.m.	7:45 p.m.
	4:34 p.m.	5:50 p.m.	6:56 p.m.	8:15 p.m. GC
	4:46 p.m. GC	6:07 p.m.	7:20 p.m.	8:38 p.m.
	4:54 p.m.	6:11 p.m.		

LIRR schedules website | LIRR Ronkonkoma Branch timetable | Getting to JFK Airport via public transit

For more information: https://www.bnl.gov/staffservices/shuttleservices.php

Hotel Shuttle Service:

A few hotels in the surrounding area offer shuttle services to the Laboratory. Check with your hotel as you make the reservation.

Home 2 Suites offers pick-up and drop-off service from the LIRR train station in Ronkonkoma. It also has a shuttle to and from the hotel and the Lab

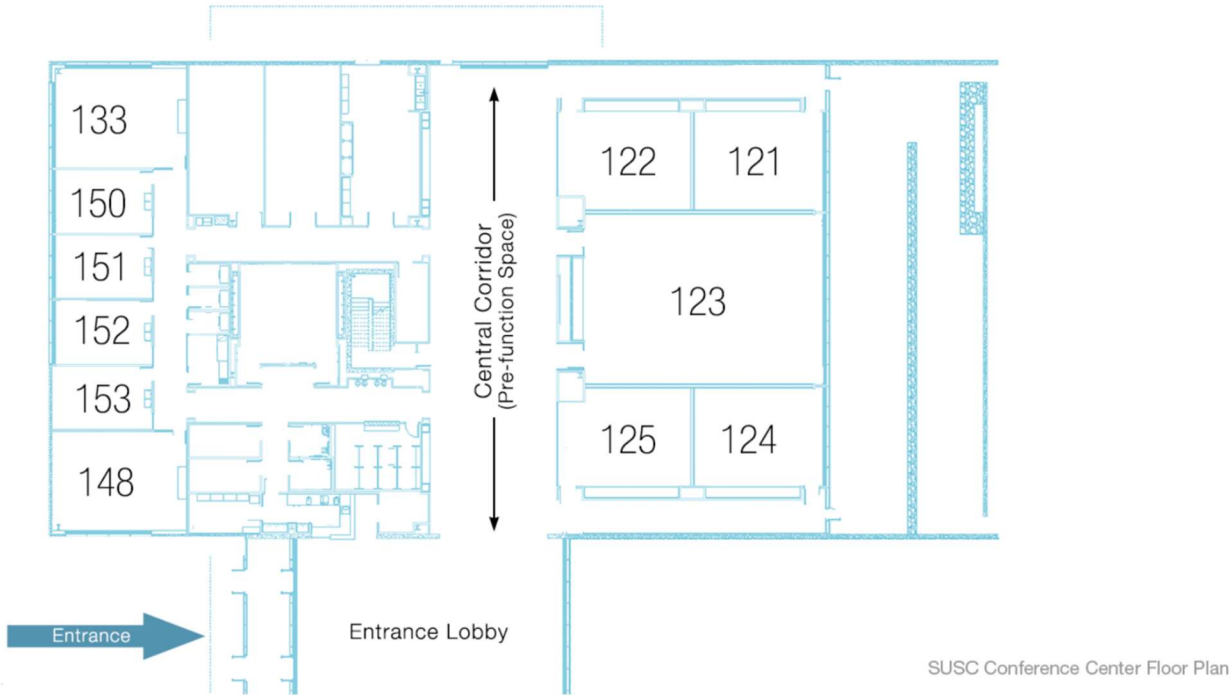
You can also arrange your own rideshare options (like Lyft or Uber) from the Hotel to the Lab and from the Lab to the Hotel.

There are no public transportation options that reach the Lab.

Registration

Registration starts at 8:00 a.m. on Friday, October 10, in SUSC (bldg. 101)

Conference Rooms



Talks and sessions will take place in room 123 of SUSC (bldg. 101)

Poster Sessions

Posters will be on display for the entire conference and can be set up in SUSC room 122. Poster boards (48" tall x 36" wide) will be provided.

Posters will be judged on Saturday, Oct. 11, from 1:30 - 2:30 p.m. for Material Sciences and from 4:30 – 6:00 p.m. for Life Sciences.

Student presenters are eligible for one of two Chung Soo Yoo Awards which carry a U$ 400.00 cash prize. To be eligible please sign-up during registration and be at your poster during the respective poster session on Saturday.

NSLS-II Tours ----------

Tours will take place Friday from 3:30 - 5:30 pm. Shuttle buses will leave SUSC (bldg. 101) promptly at 3:45 p.m. to reach NSLS-II by 4:00 p.m. Tours will be broken up by interest (life or material sciences) and visit with scientists at their beamlines. Please wear long pants (or an ankle-length skirt) and fully enclosed shoes to access the experimental floor. The tour will run until 5:00 p.m., when buses will arrive to return participants to SUSC (bldg. 101).

SIGN UP AT REGISTRATION!

Social Events ----------

Banquet Dinner
Danfords Hotel and Marina
25 E Broadway, Port Jefferson, NY 11777
Phone: +1-631-928-5200
Date: Friday, October 10, 2025, 6:30 p.m. – 9:00 p.m.
A bus will depart SUSC (bldg.101) promptly at 5:45 p.m .
Please carpool, parking is limited.
Property Map | Directions & Map

Awards Dinner
Science and User Support Center (SUSC) at Brookhaven Lab
Date: Saturday, October 11, 2025, 7 - 9 p.m.

Program

Pre-Conference Workshops

Thursday, October 9	NSLS II Bldg 745 room 183	SUSC room 148
8:30 am - 12:00 noon	**Crystal Clear Insights: Mastering Protein Crystallization** C. Tarver (SLAC), D. Marchany-Rivera (SSRL), P. Shaw Stewart (Douglas Instrument Ltd.), A. Soares (NSLS II), S. Perry (UMASS, Amherst), J. Frank (MiTeGen)	**PDF 101: A Hands on Tutorial to Total Scattering Methods** M. Abeykoon (BNL), D. Olds (BNL)
12:00 noon	Lunch Break	
1:00 - 5:00 pm	**Crystal Clear Insights: Mastering Protein Crystallization** (continued)	**PDF 101: A Hands on Tutorial to Total Scattering Methods** (continued)
5:30 pm	End of Workshops	

Main Conference Program

TIme	**Friday, October 10**	
8:00 - 9:00 am	Registration – Coffee, pastries …..Poster set-up in	
	SUSC room 123	
8:30 - 8:40 am	Opening Remarks	
8:40 - 9.10 am	Welcome **Elke Arenholz (NSLS II);** **Qun Schen (NSLS II, Former recipient Sidhu Award)**	
9:10 – 9:55 am	Keynote Lecture 1 – Material Sciences & Sidhu Award Lecture **Leora Dresselhaus-Marais (Stanford University);** *Developing X-ray Diffraction Microscopes to Visualize Mesoscale Lattice Defect Dynamics*	
10:00 – 10:20	Coffee Break/Posters/Exhibition	
10:20 - 11:05	Keynote Lecture 2 – Life Sciences **Simone Techert (DESY);** *Functions beyond static structures*	
11:05 – 11:45	Early Career Panel Discussion: Iva Milisavljevic (Alfred University), David Sprouster (SBU), Elizabeth Cote (UMass Amherst), Heather L. Donald (Pittsburgh University), Jonathan Clinger (Baylor University), Crissy Tarver (SLAC)	
11:50 – 12:20	Sponsor Talks	
12:00 - 1:30	Lunch/Posters/Exhibition	
	SUSC 123	SUSC 124
	Welcome **Sean McSweeney (NSLSII)**	
1:30 – 3:30 pm	Session 1A: *Precision by Pieces: Future Directions in Fragment-based Approaches* **Chairs: Justyna Wojdyla (Incyte) & Wuxian Shi (NSLS II)**	Session 1B: *Progress in soft materials scattering* **Chairs: Eliot Gann (BNL), Priyanka Ketkar (BNL)**
1:30 – 1:55	**Jerome Baudry**; *Leveraging protein dynamics and machine learning for fragment-based ligand discovery*	**Arithi Jayarman (UDel);** *Computational Reverse Engineering Analysis of Scattering Experiments (CREASE) for Understanding Complex Soft Material Structures*
1:55 – 2:20	**Inna Krieger;** *Crystal Fragment Screen Revealed Novel Binding Sites in Mycobacterium tuberculosis Malate Dehydrogenase*	**Dean DeLongchamp (NIST);** *Reconciling chain orientation in polymer-grafted nanoparticles between coarse-grain models and resonant soft X-ray scattering*

2:20 – 2:45	**Rachel Palte;** *WRN helicase structural flexibility showcased through fragment-based lead discovery of inhibitors*	**Siyu Wu (BNL);** *Advancing AI-Ready Infrastructure for Autonomous X-ray Scattering at the CMS Beamline*
2:45 – 3:10	**Dale Kreitler;** *The X-ray crystallographic fragment screening facility at NSLS-II*	**Greg Su (ALS);** *Membranes In Motion: Tracking Polymer Membrane Formation and Performance With In Situ X-Ray Scattering*
3:00 – 5:30 pm	NSLS II Tour – Registration Required (bus leaves SUSC at 3:30pm)	
6:00 - 10:00 pm	Banquet - Danfords Inn Port Jefferson Harbor	
10:00 pm	End of 1st Day	

OCTOBER 11 Second Day

Time	Saturday, October 11	
8:30 – 10:10 am	SUSC 123	SUSC 124
8:30 - 10:10 am	Session 2A: Metal Matters: Structure and Function of Metalloproteins **Chair: Sarah Perry**	Session 2B: Emergent Materials for the Future **Chairs: Dave Sprouster (SBU), Boris Khaykovich (MIT)**
8:30 - 8:55	**Sarah Bowman:** *The iron-y of spectroscopy coupled crystal-based metalloprotein structural methods*	**Maike Lang (UTK);** *Characterization of Radiation Effects in Ceramics with Neutron Total Scattering*
8:55 - 9:20	**Denis Rousseau***: Structural insights into the functional properties of cytochrome c oxidase*	**Joerg Neuefeind (ORNL);** *Saturated non-periodic tetrahedral networks studied with Neutron Pair Distribution functions*
9:20 - 9:45	**Rachel Narehood Austin:** *The role of metal ions in the structure and function of alkane monooxygenase (AlkB)*	**Christie Nelson (BNL)**; *Resonant X-ray Diffraction Studies of Electronic Order*
9:45 – 10:10	**Simon Vecchioni:** *DNA Metalation in Electronic Devices: A Structural Approach*	**Sean Drewery (UTK);** *Engineering Compositionally Complex Oxides for Thermal Barrier Coatings*
		Scott Misture (Alfred University); *Controlled Reduction of Oxides for Energy Applications*
10:10 - 10:30 am	Coffee Break/Posters/Exhibition	
10:30 am - 12:30 pm	Session 3A: The Shape of Change: Capturing Structural Dynamics in Action **Chair: Aina Cohen & Martin Fuchs**	Session 3B: *Energy Storage* **Chairs: Peter Khalifiah (SBU), Gihan Kwon (BNL)**
10:30 - 10:55	**Alec Follmer;** *Illuminating Allosteric Regulation in Cytochromes P450 with X-rays* ***(VIRTUAL)***	**Hailong Chen (Georgia Tech)***; X-Ray and Neutron Characterization Guided Materials Design and Development for Energy Storage Systems*
10:55 - 11:20	**Heather L. Donald;** *Time-resolved crystallography reveals a novel mechanism of Enterobacter cloacae (Ent385) AmpC b-lactamase mediated hydrolysis of ceftazidime*	**Xiaojing Huang (BNL);** *Multi-modal correlative imaging of energy storage materials*

11:20 - 11:45	**Sarah Uttormark;** *Time-resolved SWAXS monitors the formation of an RNA triple-helix*	**Jue Liu (ORNL);** *Structure and superionic transition of Li3YCl6 and Li3YBr6*
11:45 am - 12:10 pm	**Cynthia Stauffacher;** *Dissecting a Complex Enzyme Mechanism: Watching HMG-CoA Reductase in Action*	**Enyuan Hu (BNL);** *Dynamics of Materials and Interphases in Energy Storage via X-ray and Neutron Diffraction*
12:10 pm 12:30 pm	**Jonathan Clinger;** *Adventures in Cryo-trapping Time-resolved Crystallography*	

12:30 - 1:30 pm	Lunch Break/Posters/Exhibition	
1:30 – 2:30 pm	Sponsor Talk: **Pascal Hofer**	Material Science – Posters Judging
	Patrick S Stewart; *Using phase diagrams with microseeding to prepare crystal samples for routine and advanced data-collection techniques* **Robert Thorne;** *Instrumentation and methods for efficient time-resolved X-ray crystallography of biomolecular systems with sub-10 ms time resolution*	
2:30 - 4:30 pm	Session 4A: *Life at the Edge: Understanding Biology in Extreme Environments* **Chair: Jeney Wierman**	Session 4B: *In-Situ Methods at High Pressure* **Chairs: Matt Whitaker (SBU), James Walsh (UMass, Amherst)**
2:30 – 2:55	**Steven P. Cramer***; Some Like it Hot – Extremophile Protein X-Ray Diffraction at 120 °C Reveals Structural Changes*	**Pamela Burnley (University of Nevada, Las Vegas);** *The secret inner life of a deforming rock, revelations from synchrotrons x-rays*
2:55 - 3:20	**Haley Moran**; *Proteome-Wide Structural Responses to High Hydrostatic Pressure Revealed by LiP-MS*	**Rajkishna Dutta (Princeton University);** *A Novel Eight-Coordinated Phase of Mg2SiO4: Insights into the Mineralogy of Super-Earth Mantles*
3:20 – 3:45	**Peter Davies (virtual);** *Structural adaptations of ice-binding proteins to function at sub-zero temperatures*	**Alison Altman (TAMU);** *Elucidating anisotropic chemistry in lanthanide halide systems under pressure*

3:45 – 4:10	**Liliana Guerrero;** *Pushed to Extremes: Structural Responses of STEP to Temperature, Pressure, and Dehydration*	**Cathy Badding (MIT);** *In Situ Diffraction to Discover Co–Bi Binaries*
4:10 – 4:35	**Doeke Hekstra;** *Towards direct visualization of the reaction coordinates of proteins.*	**Elizabeth Cote (UMass, Amherst)**; *Martensite-type ε-VCy" high-pressure synthesis pathway revealed through in situ x-ray diffraction*
4:30 – 4:50 pm	Coffee Break/Posters/Exhibition	
4:30 - 6:00 pm	Poster Judging Session (Life Sciences)	
6:00 – 6:30 pm 6:30 – 8:00 pm	Dinner Speaker **Silvia Centeno** *Decoding the Past: Insights into Artists' Materials and Deterioration Processes* Dinner Poster Award Announcements	
8:00 pm	End of 2nd Day	

OCTOBER 12 Third Day

Sunday,October 12	SUSC 123	SUSC 124
8:30 am - 10:30 am	Session 5A: *Next-Gen Structural Biology: Expanding the Experimental Toolbox* Chairs: **Banumathi Sankaran & Narayanasami Sukumar**	Session 5B: *Advances in Computational Crystallography* **Chairs: Thomas Caswell (BNL), Jennefer Maldonado (BNL)**
8:30 - 8:55 am	**Gino Cingolani;** *Beyond Structures: The Next Frontier in Structural Biology*	**Cevdet Noyan (Columbia University)**; *Sampling Volumes in Powder Diffraction Experiments*
8:55 - 9:20 am	**Shirish Chodankar;** *Small-Angle X-ray Scattering in the Era of Integrative Structural Biology*	**James Yates (BWXT);** *Sparse sensing based data collection methods*
9:20 - 9:45 am	**Dean Myles;** *Neutron Biophysics: From Atoms to Cells*	**Saul Lapidus (ANL);** *Probing further under sharp peaks and peak shape: The beginning of pushing the capabilities of high resolution PXRD beamline 11-BM with new detectors and analysis*
9:45 - 10:10 am	**Narayanasami Sukumar;** *X-ray and Spectroscopic Insights into Metal Centers in Biomolecules*	**Adam Corrao (BNL);** *AI for powder diffraction: Bayesian optimization-driven measurements & automated analysis*
10:10 - 10:35 am	**Max Burian;** *About Data and Insight: Rethinking Collaboration and Compute in Modern Crystallography*	
10:30 - 10:50am	Coffee Break/Exhibition	
	SUSC room 123	
10:50 - 11:30am	PANEL Innovation in Motion: Challenges, Opportunities, and Strategies Lu Ma, Thomas Caswell, Eliot Gann	
11:30am - 12:00pm	Business Meeting Treasurer's Report Next Conference Closing Remarks	
12:00 pm	End of Conference	

Developing X-ray Diffraction Microscopes to Visualize Mesoscale Lattice Defect Dynamics

Leora Dresselhaus-Marais
Department of Materials Science & Engineering, Stanford University, Palo Alto, CA

Materials strengthen or shatter as they respond to their surroundings via multiscale dynamics from defects at the atomic scale. For centuries, scientists and engineers have struggled to connect a material's fundamental properties directly to its structure. Today, we understand that line defects (dislocations) pattern into 3D hierarchical networks that dictate the microstructure, setting the properties and dynamics of the system. However, our understanding of these dynamics is limited by a lack of experiments to directly resolve how dislocation pattern deep beneath a material's surface.

I developed time-resolved dark-field X-ray microscopy (DFXM) to directly image dislocations and their collective dynamics in real-time, hundreds of micrometers beneath any surface. I will describe the optical, theory, and analytical frameworks that I developed to image and interpret these subsurface dynamics from ms-fs timescales. Using this new framework, I will then present results showing a new view of how dislocation boundaries evolve in single-crystal aluminum at temperatures that have long been inaccessible to theory and experiments. Zooming in on the dislocations that comprise a tilt boundary, I will show how the stochastic motion of the boundary dislocations over 10 minutes reveal how it loses its inherent stability at 0.99 Tm. I then show my group's new developments using DFXM at X-ray free electron lasers, where we have used it to understand the microscopic origins of acoustic waves that govern thermal transport in semiconductor materials. My new approach demonstrates the groundwork for new opportunities to establish the fundamental science underlying our understanding and control of defects and strain control – across many fields of science and engineering.

Keynote Lecture 2 - Life Sciences

Functions Beyond Static Structures

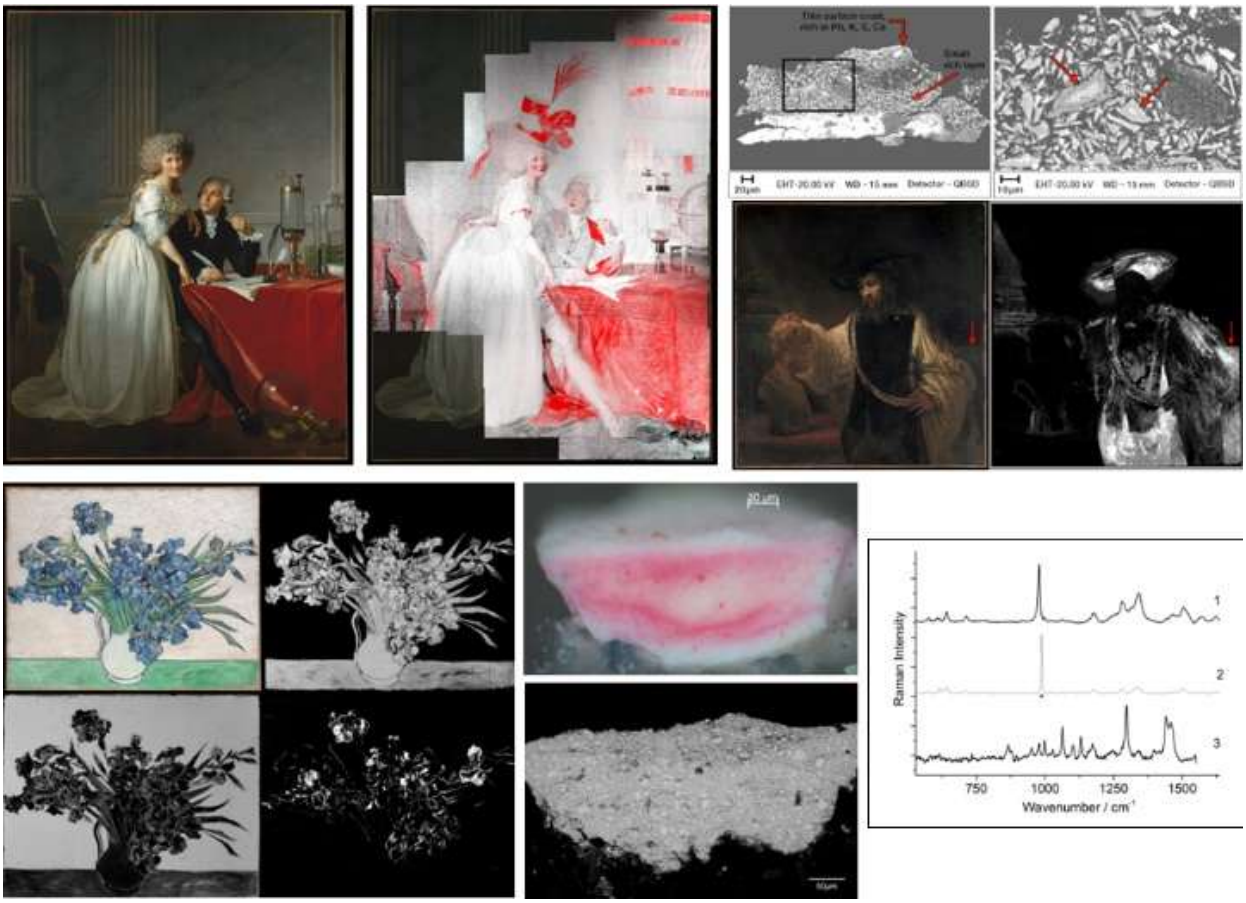
Simone Techert
Structural Dynamics of (Bio)chemical Systems
Deutsches Elektronen-Synchrotron DESY
Institute for X-ray Physics, Goettingen University

Advances in serial crystallography at X-ray free electron laser (XFEL) and synchrotron sources have opened a new window into understanding how biomacromolecules respond to a broad range of perturbations. Life is a nonequilibrium phenomenon and therefore understanding how the molecules of life function require techniques that can probe their non-equilibrium states. This lecture will provide an overview of what can be accomplished using serial crystallography to study enzyme structure and dynamics during catalysis, with selected examples of cysteine-dependent enzymes. Future goals, including higher dimensional non-equilibrium structural enzymology approaches, will also be discussed.

Awards Dinner Speaker

Decoding the Past: Insights into Artists' Materials and Deterioration Processes

Silvia A. Centeno
Department of Scientific Research, The Metropolitan Museum of Art, New York, NY, USA



This talk will present a brief overview of what we have learned about the materials and techniques in paintings by artists such as Jacques-Louis David, Vincent van Gogh, and Rembrandt van Rijn, addressing issues such as whether the works were meant by the artists to look the way they do today or if their appearances are the result of changes that have occurred over time. Also, it will highlight how conservators, scientists, art historians, and image specialists work collaboratively to get a sense of how these works may have originally looked when the examination and analyses have revealed that changes have occurred.

In these kinds of studies, the approach typically involves the use of non-invasive analytical methods, such as X-ray fluorescence mapping, multispectral imaging, and infrared reflectography followed by micro-sampling if deemed necessary and possible. For the analysis of samples, techniques such as Raman and Fourier transform infrared spectroscopies, scanning electron microscopy-energy dispersive X-ray spectrometry, X-

ray diffraction, and gas chromatography-mass spectrometry methods are generally employed.

The results of these investigations are not only crucial for increasing our appreciation of the masterpieces but also for preserving them for future generations.

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Early Career Panel

Panelists:

Iva Milisavljevic (Alfred University)

David Sprouster (Stony Brook University),

Elizabeth Cote (UMass Amherst),

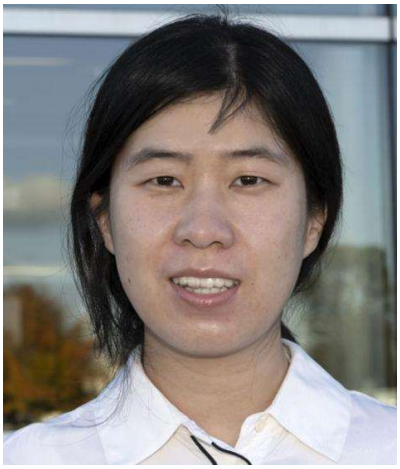
Heather L. Donald (Pittsburgh University),

Jonathan Clinger (Baylor University),

Crissy Tarver (SLAC)

Innovation Panel

Panelists:



Lu Ma (NSLS-II)

Max Burian (Dectris)

Gino Cingolani (UAB)

Eliot Gann (NSLS-II)

Thomas Caswell (NSLS-II) Sarah Perry (UMass, Amherst)

Pre-Conference Workshops

Crystal Clear Insights: Mastering Protein Crystallization

This one-day, hands-on workshop will provide participants with practical training in state-of-the-art protein crystallization techniques and their connections to emerging structural biology methods. Five interactive modules, led by experts from academia, national laboratories, and industry, will cover a broad spectrum of approaches—from classical vapor diffusion to microfluidics and acoustic droplet ejection, as well as the interface between crystallography and cryo-EM. Designed for students, postdocs, and researchers at all levels, the workshop offers unique opportunities to gain confidence in experimental methods, explore complementary technologies, and strengthen skills essential for successful structural biology research. The workshop is composed of five modules developed in parallel.

Introduction Lecture:

Gene to Structure Insights

Crissy L Tarver
High-throughput Biochemistry Laboratory Director
SLAC National Accelerator Laboratory - SSRL

The journey from a DNA sequence to a three-dimensional protein structure is one of the most powerful narratives in modern molecular biology. This lecture will explore how a simple stretch of genetic code can be transformed into purified protein, coaxed into crystals, and ultimately revealed as an atomic-level model. Along the way, we will highlight the creativity and problem solving that drive each step — from gene design and expression, to purification, crystallization, and structure determination. Beyond the technical milestones, the lecture will emphasize the excitement of seeing biology made visible at atomic resolution. Each structure is more than a scientific achievement; it is a gateway to understanding how molecular machines work and how they can be targeted in medicine. Protein structures illuminate the mechanisms of disease, reveal vulnerabilities for therapeutic intervention, and guide the design of new drugs with precision. By tracing the arc from gene synthesis to structure, this talk will inspire students and researchers to see structural biology as a means of discovery and as a foundation for translating molecular insight into medical impact.

Hands-On Tutorials:

Each Tutorial will last 2 hours. Participants will choose 3 and rotate among them.
We may not be able to satisfy all your choices. Groups will have 4-5 members; this is related to the occupation restrictions of the Labs.

Module 1. Tricks and Tips of Vapor Diffusion Methods
Learn practical approaches to vapor diffusion crystallization, from hanging to sitting drops, and practice crystal handling using lysozyme samples.

Module 2. Exploring Phase Diagrams for Crystals
Observe crystallization phase diagrams and seeding methods through a demonstration of the Oryx 8 system. Bring your own protein samples.

Module 3. Nanoliter Crystallization with Acoustic Droplet Ejection
Discover how acoustic droplet ejectors enable nano/micro-crystallization and high-throughput sample transfer. Bring your own protein samples.

Module 4. Microfluidics Crystallization and X-ray Diffraction
Gain hands-on experience with microfluidic devices for crystallization and learn how they can be used for room-temperature diffraction experiments.

Module 5. From Crystals to Cryo-EM Samples
Learn about how to handle your samples for temperature-controlled experiments and serial crystallography; devices and techniques shared between crystallography and cryo-EM.

PDF 101: A Hands on Tutorial to Total Scattering Methods

This one day workshop will introduce participants to total scattering measurements and data, the basics of modeling approaches, and series of hands-on tutorials using one of the most popular pair distribution function analysis toolset, PDFgui. In these tutorials, participants are able to learn the basics of the software, interact with beamline scientists, and gain experience fitting real world data from complex, functional materials.

Total scattering (and the associated pair distribution function technique), an extension of diffraction methods, is increasingly prevalent in modern materials studies. The unique combination of Bragg and diffuse scattering has related vacancies in high temperature ceramics to both their superionic conductivity and phase stability, nanometer-sized polar domains or nanoregions in relaxor ferroelectrics to their enhanced dielectric and piezoelectric properties, and vacancy/disorder arrays and other subtle local correlations to the mechanisms of high-Tc superconductivity. These methods have further proven critical in understanding guest-host interactions, amorphous to crystalline transitions, local spin correlations, and other disordered crystalline materials phenomena.

Modern PDF software includes small and large box approaches, and can incorporate neutron and x-ray PDF, EXAFS, and single crystal diffuse scattering data. Complex atomistic models can be used to aid scientists from diverse disciplines in understanding the inner-workings of property mechanisms, and ultimately in optimizing and controlling them through atomic structure modification. PDF in the hands of a trained expert is an immensely powerful technique to probe the local and average structure in ways few other techniques can.

Becoming an expert in all such PDF analysis methods can require extensive experience and years of practice, which makes the start for novices in this field intimidating. This workshop will prepare participants to start their own journey into the world of PDF, giving them a basis of both theoretical background, experimental guidance, experience with data analysis, and proving an opportunity to interact with experts in the method.

Speaker Abstracts

LIFE SCIENCE

Precision by Pieces: Future Directions in Fragment-based Approaches

Chairs: Justyna Wojdyla[1] & Wuxian Shi (NSLS II)[2]
[1]Incyte
[2]Center for Biomolecular Structure (CBMS), National Synchrotron Light Source II

Leveraging Protein Dynamics and Machine Learning for Fragment-Based Ligand Discovery

Jerome Baudry[1], Armin Ahmadi[1], Vineetha Menon[2], Shivangi Gupta[2], Crissy L Tarver[3]
[1] Biological Sciences, University of Alabama in Huntsville
[2]Computer Science, University of Alabama in Huntsville
[3] SLAC National Accelerator Laboratory

Fragment based discovery is very sensitive to apparently minor variations of protein structures, as these variations may have a large effect on binding sites' volumes and physicochemical properties. We show how such protein structural diversity leads to different fragment-based results, and how these different results can be integrated together.

Crystal Fragment Screen Revealed Novel Binding Sites in Mycobacterium Tuberculosis Malate Dehydrogenase

Inna Krieger
Department of Biochemistry and Biophysics, Texas A&M

Malate dehydrogenase (MDH) is an indispensable central metabolic enzyme. There are no reports of antimicrobial drug discovery on this target, likely because of its highly conserved active site. We employed crystallography-based fragment screening in combination with biochemical assays to find sites in M. tuberculosis MDH that would provide inhibitor selectivity. We identified fragment hits that engage sites distinct from the catalytic cleft. Notably, compounds bound to these alternative pockets demonstrated selective inhibition of M. tuberculosis MDH compared to human homologs. Some of the fragment-bound complexes showed conformational flexibility and novel surface features that can be used for further development of selective inhibitors. These findings underscore the power of crystal fragment-based approaches not only to identify starting points for inhibitor design but also to provide novel information on potential allosteric binding sites.

WRN Helicase Structural Flexibility Showcased Through Fragment-Based Lead Discovery of Inhibitors

Rachel Palte Kubiak
MERCK

WRN helicase has been recognized as a promising synthetic lethal target for therapeutic intervention in cancers characterized by microsatellite instability-high (MSI-H) and mismatch repair deficiency (MMRd). However, the search for effective helicase inhibitors poses significant challenges, as high-throughput biochemical screening often yields limited validated candidates, many of which show poor activity in cellular contexts. In this study we show the power of non-covalent fragment-based lead discovery in locating new druggable allosteric sites on WRN and enabling us to combat the challenging behavior of WRN during high-throughput screening hampering hit identification. Through the fragment optimization process the structural enablement of WRN with key prioritized fragments revealed multiple conformations of WRN with significant domain rotations up to 180°, including a previously uncharacterized conformation. Rooted in a combination of biochemical, biophysical, and structural approaches, we present the detailed analyses of optimized chemical matter evolved from screening hits and the unique structural abilities of WRN to accommodate diverse conformations

The X-ray crystallographic fragment screening facility at NSLS-II

Dale Kreitler
NSLS-II, Brookhaven National Laboratory

We are working to establish an x-ray crystallographic fragment screening (XCFS) user facility in the US at the Automated Macromolecular Crystallography (AMX) beamline at the National Synchrotron Light Source II (NSLS-II) in response to increasing user demand. AMX is a microfocus beamline (7x5 um; 5E12 at 13475 eV) that began user operation in 2017. AMX's fully automated collection mode which includes two orthogonal raster grid screens as the default centering protocol, currently runs at 550 samples per day and is a key component of our XCFS workflow. Fragment lab users at the NSLS-II XCFS are provided unipucks and SPINE bases. In addition, users are provided access to a wet lab next to the beamline that contains an Oryx8 (Douglas Instruments), Crystal Shifter (Oxford Labtech), and Echo 550 acoustic liquid handler for transfer of fragment compounds. Users can select from approximately 2200 compounds from commercial fragment libraries (Enamine, Life Chemicals) that are stored onsite at NSLS-II. We have developed a simple python application that can be run from the NSLS-II jupyter notebook hub to manage sample tracking and to monitor experiment progress. PanDDA analysis is performed by beamline staff (or users) on our inhouse computing cluster from an Omnissa horizon client virtual machine. Since development began in 2023, we have run 11 projects that have generated approximately 12000 samples and hundreds of hits. We are working to increase the project throughput of the facility to provide access to a larger user community.

The Metal Matters: Structure and Function of Metalloproteins

Chair: Sarah Perry
Undergraduate Program Director, Department of Chemical Engineering, University of Massachusetts Amherst

The Iron-y of Spectroscopy Coupled Crystal-Based Metalloprotein Structural Methods

Sarah Bowman [a,b], *Budziszewski, GR*[a,b] *Arnone, S,*[a] *Snell, ME,*[a,b] *Lynch, ML,*[b,c]
[a] *University at Buffalo, Jacobs School of Medicine and Biomedical Science, Department of Biochemistry, Buffalo, NY, 14203, USA*
[b] *University at Buffalo Hauptman Woodward Research Institute, Buffalo, NY,14203, USA*
[c] *University at Buffalo, Jacobs School of Medicine and Biomedical Science, Department of Structural Biology, Buffalo, NY, 14203, USA*

Diffraction-based structural biology methods are a fundamental tool for structural science, accounting for close to 85% of all macromolecular structures deposited to the Protein Data Bank. Recent advances in structural biology methods, from synchrotron, electron diffraction, and XFELs to computational advances and CryoEM, have opened the door to the investigation of more challenging target samples. This talk will highlight several research projects in the Bowman Lab on metalloprotein biology and the methods available to discover how structure contributes to their unique chemistry. Metals in proteins are ubiquitous (involving up to 50% of the proteome) and involved in many of the reactions that define life – transfer of energy, photosynthesis, metabolism, oxygen shuttling, and much more. In structural work, X-ray and electron sources used in diffraction and CryoEM studies can cause changes in oxidation state and radiation damage, obscuring the true nature of the role of the metal in the protein. We are working to make use of spectroscopy coupled to diffraction data collection to probe metalloprotein biology

Structural Insights into the Functional Properties of Cytochrome c Oxidase

Denis Rousseau, Izumi Ishigami, and Syun-Ru-Yeh
Albert Einstein College of Medicine, Bronx, NY 10461 USA
denis.rousseau@einsteinmed.edu

Cytochrome c oxidase (CcO) is the membrane-bound terminal enzyme in the electron transfer chain in all organisms that utilize oxygen to generate energy. It is responsible for >90% of the oxygen utilization in the biosphere. In mitochondria, CcO catalyzes the four-electron reduction of O2 to H2O, thereby maintaining electron flow for oxidative phosphorylation. At the same time, it harnesses the oxygen reduction energy to translocate four protons across the mitochondrial membrane, thereby augmenting the electrochemical proton gradient required for the generation of ATP by ATP-synthase. A great deal of research has been conducted to comprehend the molecular properties of CcO. However, the mechanism by which the oxygen reduction reaction is coupled to proton translocation remains poorly understood. Here, we present the chemical properties of a variety of key oxygen intermediates of bovine CcO revealed by time-resolved resonance Raman spectroscopy and the structural features of the enzyme uncovered by serial femtosecond crystallography, an innovative technique that allows structural determination at room temperature without radiation damage by using femtosecond pulses from an X-ray free electron laser for diffraction from CcO microcrystals.1,2 These data support a proton translocation mechanism in mammalian CcO which is distinct from that in bacterial oxidases.3

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The Role of Metal Ions in the Structure and Function of Alkane Monooxygenase (AlkB)

Rachel Narehood Austin
Barnard College

The transformation of alkanes to terminal alcohols using molecular oxygen as the sole oxidant and a catalyst utilizing earth-abundant metal ions exemplifies the kind of chemistry required for a sustainable future. We are focused on understanding how the structure of alkane monooxygenase (AlkB), the metalloenzyme that catalyzes the selective oxidation of most liquid alkanes in the environment, facilitates its function. Two recent cryo-EM structures of AlkB[1, 2] have answered many long-standing questions about this enzyme, but raised others. We've addressed some, but not all, of these questions with the help of EXAFS and molecular dynamics (MD) simulations.[3] Recent functional studies have also raised additional questions about the role of metal ions in this environmentally important enzyme.[4] We will point to new directions necessary to understand the catalytic power of AlkB.

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DNA Metalation in Electronic Devices: A Structural Approach

Simon Vecchioni
New York University

DNA as a vehicle for molecular programming relies on the predictability and information storage capability of the canonical Watson-Crick base pairs, A:T and G:C. With only four Watson-Crick nucleobases and the insulating behavior of natural DNA, it has become clear that there are strong limitations on DNA-based electronic systems. To address this, we present an expanded DNA alphabet based on metal-mediated DNA (mmDNA) base pairing in which we substitute hydrogen bonds between pyrimidine nucleobases with templated metal ion coordination.

Using anomalous scattering techniques, we firmly establish the ability to program metal ions (Ag^{+}, Au^{+}, Au^{3+}, Cu^{2+}, Cd^{2+}, Hg^{2+}) inside the double helix. We further capture a series of chemical reactions using time-resolved crystallography to exchange metal ions, and we use this as the basis for the rational design of a DNA-based field-effect transistor. Applications in nanoelectronics and nanophotonics are described.

The Shape of Change: Capturing Structures in Action

Chair: Aina Cohen[1] & Martin Fuchs[2]

[1]Division Director of Structural Molecular Biology (SMB), Stanford Synchrotron Radiation Lightsource (SSRL)

[2]Lead Beam line Scientists, Center for Biomolecular Structure (CBMS), NSLS II

Time-resolved Crystallography Reveals a Novel Mechanism of Enterobacter Cloacae (Ent385) AmpC β -lactamase Mediated Hydrolysis of Ceftazidime

Heather L. Donald[1], Lin, G[1]., Zinser-Peniche, P[1]., Esquillin-Torres[2], J., McElheny, C[1]., Doi, Y[1]., Sluis- Cremer, N[1]., Cohen, A[3]. and Calero, G[1].

[1]University of Pittsburgh, [2]University of Puerto Rico Humacao, [3]Stanford Synchrotron Radiation Lightsource

The continuous development of antibiotic resistance represents a global health threat. Antibiotic resistance in Gram-negative pathogens can be caused by a variety of mechanisms, including enzymatic inactivation of β-lactam antibiotics by β-lactamases. The goal of this study was to use rapid freeze-quench time-resolved (TR) crystallography to gain insight into the mechanism by which the –clinically isolated– class C β-lactamase from Enterobacter cloacae (AmpCEnt385), hydrolyses ceftazidime (CAZ); a third-generation cephalosporin. Eighteen TR data points ranging between 18 s and 45 min were acquired for the drug-resistant AmpCEnt385. Our TR data shows that CAZ shuffles between three distinct binding positions prior to the acylation step (i.e, hydrolysis of the β-lactam ring). A re-orientation of the side chain of Thr314 in AmpCEnt385 facilitates CAZ initial binding; intriguingly, Thr314 substitutions decreased the minimum inhibitory concentration for CAZ by more than 16-fold; but had minimal impact on penicillins and monobactams. Shifts in the active site were observed over the course of the acylation and deacylation reactions, including the side chain of Lys67 as it acts as a general base. We were able to observe a coherent sequence of binding steps, followed by both the acylated and deacylated states of a class C serine β-lactamase. Our results suggest that CAZ “shuffle” binding is the result of two “sticky” binding sites with affinity for the β-lactam ring, resulting in substrate trapping while large radical groups accommodate in the binding pocket. These critical insights will ultimately pave the way for developing more effective inhibitors, which is crucial in the ongoing battle against antibiotic-resistant bacteria.

Time-Resolved SWAXS Monitors the Formation of an RNA Triple-Helix

Sarah Uttormark and Pollack Lab

School of Applied and Engineering Physics Cornell University, Ithaca, NY

Single-stranded RNAs are remarkably dynamic molecules capable of folding into intricate structures that enable their multiple biological functions. Time-resolved small-angle X-ray scattering (TR-SAXS) captures global structural changes of flexible RNA ensembles; however, higher resolution structural information has been difficult to obtain. The development of wide-angle X-ray scattering (WAXS) at NSLS-II has permitted the exploration of scattering features at higher scattering angles, corresponding to sharper resolution of molecular features. Notably, static WAXS measurements revealed significant differences between unstructured and structured RNAs. The use of X-ray free-electron lasers (XFELs) now extends time-resolved X-ray scattering technology from traditional small-angles into the wide-angle regime. Together, TR-SAXS, static WAXS, and TR-WAXS are combined to reveal the folding pathway of a single-stranded RNA to a stable triple-helix.

This work used APS, NSLS-II and LCLS. *Work in the Pollack Lab is supported by NIH award R35-GM122514 and NSF BioXFEL award 1231306.*

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Illuminating Allosteric Regulation in Cytochromes P450 with X-rays

Alec Follmer
UC Davis

From metabolism and detoxification to the biosynthesis of steroids and natural products, cytochromes P450 (P450s) are heme-containing enzymes that utilize O2 for the activation of C–H bonds and serve ubiquitous roles in nearly all biological systems. Their enzymatic activity, however, requires the formation of highly reactive intermediates within their active sites that can lead to the generation of potentially harmful reactive oxygen species and/or deleterious effects to the protein itself. Accordingly, many P450s have evolved structural and dynamic mechanisms to regulate their catalytic activity that involve complex rearrangements of their protein scaffolds. Despite a general understanding and appreciation of this regulation, the specific mechanisms and the extent to which they can be generalized remains unclear. Here, I will highlight several recent insights into how the reactivity of P450s is the controlled by allosteric interactions far from the active site that were discovered using a combination of X-ray crystallographic, spectroscopic, and computational methods. Together these studies provide a new perspective on the importance that conformational dynamics play in the regulation of P450 activity.

Dissecting a Complex Enzyme Mechanism: Watching HMG-CoA Reductase in Action

Cynthia V. Stauffacher,[1] Vatsal Purohit,[1] Himani N. Patel,[2] Calvin N. Steussy,[1] Aina Cohen,[3] Timothy Schmidt,[1] Walker Hoisington,[2] Mikaela Farrugia,[2] Phillip S. Rushton,[1] Paul Helquist,[2] Olaf Wiest,[2]

[1] *Department of Biological Sciences, Purdue Institute for Cancer Research, Purdue Institute of Inflammation, Immunology and Infectious Diseases, Purdue University, West Lafayette, Indiana*

[2] *Department of Chemistry and Biochemistry, University of Notre Dame, Indiana*

[3] *Stanford Synchrotron Radiation Light Source, SLAC, National Accelerator Laboratory, Menlo Park, California*

The complex mechanisms of enzymes in their role of molecular machines in the cell employ changes across scales of structure from the assembly of large multi-macromolecular complexes to the orientation of specific amino acid residues around a substrate. Series of intermediate structures that serve as snapshots along the reaction pathway of enzymes have long been produced from both structural and computational studies. These snapshots are often far separated along timelines and sometimes can be misleading, so cutting edge techniques have been developed over the last few years to explore enzyme mechanisms directly with time-resolved structural techniques. Combining the strengths of computational dynamics and experimental time-resolved crystallography promises a more detailed understanding of the atomistic details of catalysis, in effect, a “movie” of the enzymatic reaction.

Deciphering an enzymatic mechanism is even more challenging for an enzyme that has multiple intermediates. We have chosen for our studies 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-CoA reductase, HMGR) from Pseudomonas mevalonii, which is an oxidoreductase of the isoprenoid pathway that performs three reactions to interconvert a thioester, HMG-CoA, into a primary alcohol, mevalonate, using two cofactor molecules and producing two key intermediates, mevadehyde and mevaldyl-CoA. Initial studies uncovered a set of features of PmHMGR that make it ideal for the combined application of combined computational and time-resolved experimental methods to elucidate the complete reaction pathway. The complex, multi-step reaction is reversible in bacterial HMG-CoA reductase and was discovered to proceed in the crystal. The packing of the molecule in the crystal results in a large channel that gives easy access to the active site for delivering substrates/cofactors. Both local realignment of substrates and side

chains drive the chemistry when a three helix 50 residue flap is signaled to close over the bound substrates/cofactors to produce an active enzyme.

The experimental research on PmHMGR has developed from initial snapshots, one of which surprisingly showed the creation of a thiohemiacetal intermediate in the crystal, to the development of a pH driven trigger for coordinating the reaction for time-resolved measurements, to spectroscopic studies following the NAD+/NADH transition, to time-resolved data that clearly shows the steps in formation of mevaldyl-CoA from mevalonate. Computational studies provided transition state information and through molecular dynamics examined the role of specific hydration states in the active site. The time steps of these two approaches are still far apart; current research at SSRL using developing techniques of time-resolved investigations promise to give even finer time-resolved detail on the chemical mechanism at the active site and what drives/accompanies the major rearrangement of the 50 amino acid flap which is necessary for the two oxidoreduction steps.

Adventures in Cryo-trapping Time-resolved Crystallography

Jonathan Clinger

Baylor University

Many time-resolved spectroscopies, especially those with long acquisition times, make use of cryo-trapping techniques for capturing reaction intermediates in biochemical reactions. Traditionally, these techniques have been sparingly used in crystallography due to the unique sample requirements for successful diffraction experiments. Recent advancements in crystallography, especially with the development of high flux microbeam synchrotron sources and the development of techniques to grow micro and nano protein crystals, have opened new avenues for quenching crystallography to attain biochemically relevant time resolution and become a much more attractive technique to structural biologists. However, there are both pros and cons to performing quenching experiments compared to pump-probe or mix-and-inject experiments. Cryo-trapping time-resolved crystallography therefore represents an alternative and complementary technique to serial crystallography approaches. This presentation will discuss pump-quench time-resolved data on a photoreceptor and mix-and-quench data on an enzyme and compare results from these techniques to serial crystallography experiments on similar proteins.

Life at the Edge: Understanding Biology in Extreme Environments

Chair: Jeney Wierman
MacChess Director, Cornell University

Some Like it Hot – Extremophile Protein X-Ray Diffraction at 120 °C Reveals Structural Changes

Stephen P. Cramer
SETI Institute

How does the structure of a protein change as the temperature is raised from cryogenic conditions at 100 K to 393 K? Understanding the structure and dynamics of proteins under environmental extremes is relevant for human health, biotechnological applications, and our search for life elsewhere in the universe. Here we reveal the high temperature crystal structure of a hyperthermophilic (*Pyrococcus furiosus*) rubredoxin at 393 K (120 °C), together with multiple complementary structures down to 100 K. The results are compared with molecular dynamics calculations. Significant changes in H-bonding are observed. Discussions about high-temperature protein structure and stability need to recognize that low temperature structures may not represent the high temperature case.

Proteome-Wide Structural Responses to High Hydrostatic Pressure Revealed by LiP-MS

Haley Moran
Johns Hopkins University

Understanding how protein structure and conformational flexibility respond to elevated pressure is essential for studying life in deep marine and subsurface environments. Limited proteolysis coupled with mass spectrometry (LiP-MS) enables the detection of proteome-wide structural features by monitoring protease accessibility across native and perturbed conditions. We have adapted this technique for use under high hydrostatic pressure (up to 100 MPa), enabling global structural surveys in near-native states. Applying this method to *Thermus thermophilus*, a known piezosensitive bacterium, we find that over 20% of proteins exhibit significant structural changes at 100 MPa relative to ambient pressure. These findings establish high-pressure LiP-MS (HiP-LiP) as a powerful tool for identifying pressure-sensitive protein states, generating new candidate targets for follow-up with high-pressure SAXS, crystallography, or other structural techniques at synchrotron sources.

Structural adaptations of ice-binding proteins to function at sub-zero temperatures

Peter L. Davies
Queen's University, Kingston, Ontario, Canada

Antifreeze proteins that circulate in the blood of some marine fishes and the hemolymph of overwintering insects can prevent these organisms from freezing at sub-zero temperatures. They do so binding to, and preventing the growth of, seed ice crystals. They are part of a larger group of ice-binding proteins that include ice adhesins in microorganisms, and plant proteins that prevent the recrystallization of ice in frozen tissues. What these structurally diverse proteins share is a flat surface (ice-binding site) that is relatively hydrophobic and populated by regularly arrayed short chain amino acids that are mainly alanine and threonine, but also include some valine, serine, and asparagine. These residues are thought to organize ice-like waters on the ice-binding site that then freeze the protein onto the ice crystal. The working temperature of these proteins is zero degrees Celsius and below. Their structures show less reliance on hydrophobic cores and a greater dependence on disulfide and hydrogen bonding, calcium ion coordination, and even internal water networks that help rigidify their ice-binding sites. Some of the latter proteins are extremely thermolabile and must be crystallized in a cold room. Many of these observations hold true for bacterial ice-nucleation proteins that also have subzero working temperatures.

Supported by the Canadian Institutes of Health Research.

Pushed to Extremes: Structural Responses of STEP to Temperature, Pressure, and Dehydration

Liliana Guerrero
CUNY

The striatal-enriched protein tyrosine phosphatase (STEP, PTPN5) is a neuronal phosphatase implicated in learning, memory, and neurodegeneration, and serves as a promising therapeutic target. Yet, despite decades of study, its conformational flexibility and regulatory mechanisms remain incompletely understood. Here, we probe the structural adaptability of STEP under extreme conditions of temperature, pressure, and dehydration using X-ray crystallography. By comparing structures obtained under each perturbation to a standard cryogenic reference, we uncover distinct responses of key functional motifs. Elevated temperature promotes increased sidechain heterogeneity, while high pressure stabilizes a dual conformation of the regulatory E loop, including an active-like state previously observed only in allosterically activated STEP. Dehydration, in contrast, induces long-range rearrangements, including shifts in the allosteric S-loop. Together, these results reveal how STEP samples a broader conformational landscape than previously appreciated and demonstrate that environmental extremes can unmask hidden states relevant to regulation and drug discovery. More broadly, this work highlights the utility of crystallographic perturbations as a lens to explore protein dynamics and allosteric potential.

Towards direct visualization of the reaction coordinates of proteins

Doeke Hekstra
Harvard University

We lack experimental tools to directly experimentally study the mechanical properties of proteins---their coordinated motions and the dependence thereof on applied forces, e.g. exerted by binding partners. To address this, we are developing the use of electric fields as a way to perturb protein molecules, within crystals, combined with the use of short X-ray pulses to observe the induced conformational changes. I will describe two examples: application of electric fields (and other perturbations) to an enzyme to probe its concerted motions, and application of electric fields to a K+ ion channel as a means to directly drive and observe K+ ion permeation along the physiological reaction coordinate.

The Shape of Change: Capturing Structures in Action

Chair: Aina Cohen[1] & Martin Fuchs[2]
[1]Division Director of Structural Molecular Biology (SMB), Stanford Synchrotron Radiation Lightsource (SSRL)
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Illuminating Allosteric Regulation in Cytochromes P450 with X-rays

Alec Follmer
UCDavis

From metabolism and detoxification to the biosynthesis of steroids and natural products, cytochromes P450 (P450s) are heme-containing enzymes that utilize O2 for the activation of C–H bonds and serve ubiquitous roles in nearly all biological systems. Their enzymatic activity, however, requires the formation of highly reactive intermediates within their active sites that can lead to the generation of potentially harmful reactive oxygen species and/or deleterious effects to the protein itself. Accordingly, many P450s have evolved structural and dynamic mechanisms to regulate their catalytic activity that involve complex rearrangements of their protein scaffolds. Despite a general understanding and appreciation of this regulation, the specific mechanisms and the extent to which they can be generalized remains unclear. Here, I will highlight several recent insights into how the reactivity of P450s is the controlled by allosteric interactions far from the active site that were discovered using a combination of X-ray crystallographic, spectroscopic, and computational methods. Together these studies provide a new perspective on the importance that conformational dynamics play in the regulation of P450 activity.

Time-resolved crystallography reveals a novel mechanism of Enterobacter cloacae (Ent385) AmpC b-lactamase mediated hydrolysis of ceftazidime

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The continuous development of antibiotic resistance represents a global health threat. Antibiotic resistance in Gram-negative pathogens can be caused by a variety of mechanism, including enzymatic inactivation of β-lactam antibiotics by β-lactamases. The goal of this study was to use rapid freeze-quench time-resolved (TR) crystallography to gain insight into the mechanism by which the –clinically isolated– class C β-lactamase from *Enterobacter cloacae* (AmpC $^{Ent385)}$), hydrolyses ceftazidime (CAZ); a third-generation cephalosporin.

Eighteen TR data points ranging between 18 s and 45 min were acquired for the drug-resistant AmpC ^{Ent385} .Our TR data shows that CAZ shuffles between three distinct binding positions prior to the acylation step (i.e, hydrolysis of the β-lactam ring). A re-orientation of the side chain of Thr314 in AmpC ^{Ent385} facilitates CAZ initial binding; intriguingly, Thr314 substitutions decreased the minimum inhibitory concentration for CAZ by more than 16-fold; but had minimal impact on penicillins and monobactams. Shifts in the active site were observed over the course of the acylation and deacylation reactions, including the side chain of Lys67, moving away from the catalytic serine and towards Tyr150 as it acts as a general base.

We were able to observe a coherent sequence of binding steps, followed by both the acylated and deacylated states of a class C serine β-lactamase. Our results suggest that CAZ “shuffle” binding is the result of two “sticky” binding sites with affinity for the b-lactam ring, resulting in substrate trapping while large radical groups accommodate in the binding pocket. These critical insights will ultimately pave the way for developing more effective inhibitors, which is crucial in the ongoing battle against antibiotic-resistant bacteria.

Time-resolved SWAXS monitors the formation of an RNA triple-helix

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Single-stranded RNAs are remarkably dynamic molecules capable of folding into intricate structures that enable their multiple biological functions. Time-resolved small-angle X-ray scattering (TR-SAXS) captures global structural changes of flexible RNA ensembles; however, higher resolution structural information has been difficult to obtain. The development of wide-angle X-ray scattering (WAXS) at NSLS-II has permitted the exploration of scattering features at higher scattering angles, corresponding to sharper resolution of molecular features. Notably, static WAXS measurements revealed significant differences between unstructured and structured RNAs. The use of X-ray free-electron lasers (XFELs) now extends time-resolved X-ray scattering technology from traditional small-angles into the wide-angle regime. Together, TR-SAXS, static WAXS, and TR-WAXS are combined to reveal the folding pathway of a single-stranded RNA to a stable triple-helix.

This work used APS, NSLS-II and LCLS. Work in the Pollack Lab is supported by NIH award R35-GM122514 and NSF BioXFEL award 1231306.

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Dissecting a Complex Enzyme Mechanism: Watching HMG-CoA Reductase in Action

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The complex mechanisms of enzymes in their role of molecular machines in the cell employ changes across scales of structure from the assembly of large multi-macromolecular complexes to the orientation of specific amino acid residues around a substrate. Series of intermediate structures that serve as snapshots along the reaction pathway of enzymes have long been produced from both structural and computational studies. These snapshots are often far separated along timelines and sometimes can be misleading, so cutting edge techniques have been developed over the last few years to explore enzyme mechanisms directly with time-resolved structural techniques. Combining the strengths of computational dynamics and experimental time-resolved crystallography promises a more detailed understanding of the atomistic details of catalysis, in effect, a “movie” of the enzymatic reaction.

Deciphering an enzymatic mechanism is even more challenging for an enzyme that has multiple intermediates. We have chosen for our studies 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-CoA reductase, HMGR) from Pseudomonas mevalonii, which is an oxidoreductase of the isoprenoid pathway that performs three reactions to interconvert a thioester, HMG-CoA, into a primary alcohol, mevalonate, using two cofactor molecules and producing two key intermediates, mevadehyde and mevaldyl-CoA. Initial studies uncovered a set of features of PmHMGR that make it ideal for the combined application of combined computational and time-resolved experimental methods to elucidate the complete reaction pathway. The complex, multi-step reaction is reversible in bacterial HMG-CoA reductase and was discovered to proceed in the crystal. The packing of the molecule in the crystal results in a large channel that gives easy access to the active site for delivering substrates/cofactors. Both local realignment of substrates and side chains drive the chemistry when a three helix 50 residue flap is signaled to close over the bound substrates/cofactors to produce an active enzyme.

The experimental research on PmHMGR has developed from initial snapshots, one of which surprisingly showed the creation of a thiohemiacetal intermediate in the crystal, to the development of a pH driven trigger for coordinating the reaction for time-resolved measurements, to spectroscopic studies following the NAD+/NADH transition, to time-resolved data that clearly shows the steps in formation of mevaldyl-CoA from mevalonate. Computational studies provided transition state information and through molecular dynamics examined the role of specific hydration states in the active site. The time steps of these two approaches are still far apart; current research at SSRL using developing techniques of time-resolved investigations promise to give even finer time-resolved detail on the chemical mechanism at the active site and what drives/accompanies the major rearrangement of the 50 amino acid flap which is necessary for the two oxidoreduction steps.

Adventures in Cryo-trapping Time-resolved Crystallography

Jonathan Clinger
Baylor University

Many time-resolved spectroscopies, especially those with long acquisition times, make use of cryo-trapping techniques for capturing reaction intermediates in biochemical reactions. Traditionally, these techniques have been sparingly used in crystallography due to the unique sample requirements for successful diffraction experiments. Recent advancements in crystallography, especially with the development of high flux microbeam synchrotron sources and the development of techniques to grow micro and nano protein crystals, have opened new avenues for quenching crystallography to attain biochemically relevant time resolution and become a much more attractive technique to structural biologists. However, there are both pros and cons to performing quenching experiments compared to pump-probe or mix-and-inject experiments. Cryo-trapping time-resolved crystallography therefore represents an alternative and complementary technique to serial crystallography approaches. This presentation will discuss pump-quench time-resolved data on a photoreceptor and mix-and-quench data on an enzyme and compare results from these techniques to serial crystallography experiments on similar proteins.

Next-Gen Structural Biology: Expanding the Experimental Toolbox

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Beyond Structures: The Next Frontier in Structural Biology

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Over the past three decades, structural biology has undergone a profound transformation, driven by breakthroughs in both experimental and computational approaches. In this presentation, I will highlight landmark developments that have reshaped the field and discuss how cryogenic electron microscopy (cryo-EM) and AlphaFold have opened unprecedented opportunities to investigate large, asymmetric, and dynamic molecular assemblies. I will then turn to the future, offering a (subjective) perspective on emerging directions, persistent challenges, and key knowledge gaps that must be addressed to advance our understanding of biological structure and function.

Small-Angle X-ray Scattering in the Era of Integrative Structural Biology

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The advancement of structural biology increasingly relies on integrative, multimodal approaches, with small-angle X-ray scattering (SAXS) serving as a critical component. When combined with molecular dynamics simulations, atomistic models, and predictive frameworks such as AlphaFold, SAXS enables the resolution of complex structural questions in the life sciences. The Life Sciences X-ray Scattering (LiX) beamline at NSLS-II is a highly automated platform optimized for high-throughput measurements. It incorporates offline sample mixing, in-line size-exclusion chromatography, and integration with multi-angle light scattering, thereby allowing direct separation and characterization of heterogeneous mixtures at the beamline. These capabilities extend the applicability of SAXS to weakly scattering and transient systems while supporting concentration-dependent studies and systematic analyses of macromolecular assembly pathways. Data interpretation is further enhanced through GROMACS-based molecular dynamics simulations, which integrate atomistic structures from crystallography or AlphaFold with SAXS-derived constraints to refine structural models with improved accuracy.

Neutron Biophysics: From Atoms to Cells

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Neutron scattering applications range from atomic-resolution studies of individual hydrogen atoms in enzymes, through to multi-scale analyses of hierarchical structures and assemblies in biological complexes, membranes and in living cells. We will briefly describe the capabilities that are available for structural biology at ORNL neutron facilities, with special focus on current instrumentation and software for neutron protein crystallographic analysis. We will highlight development of new transformative Dynamical Nuclear Polarization (DNP) capabilities for neutron diffraction, which enable the scattering lengths of hydrogen atoms to be amplified and tuned in situ - and in beam - during data collection, maximizing the scattering from hydrogen atoms and enhancing visibility and fidelity in biological structures. We provide perspective on the development and the use of these tools to provide novel information on the dynamics, structure, function and interfacial relationships in complex biological systems.

Integrating X-ray Crystallography and XANES Spectroscopy to Probe Redox and Structural Features of a Type I Copper Protein and a Cobalamin Transport Protein

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Understanding the oxidation states of transition metals in metalloproteins is critical for elucidating their biochemical functions. Among the available techniques, X-ray Absorption Near Edge Structure (XANES) spectroscopy provides an element-specific probe of the electronic environment and redox state of metal centers, complementing structural information from X-ray crystallography. We applied single-crystal XANES spectroscopy to characterize the copper site in amicyanin, a Type I electron transfer protein. Amicyanin contains a single copper ion in a distorted tetrahedral geometry and mediates electron transfer between methylamine dehydrogenase and cytochrome c_{551}i. Comparison of XANES spectra from oxidized and reduced states with high-resolution crystallographic data reveals correlations between electronic structure and subtle geometric changes at the copper center. This approach was further extended to the human Intrinsic Factor (IF), a glycoprotein essential for cobalamin (vitamin B_{12}) transport and absorption in the ileum. Deficiency of IF leads to pernicious anemia and neurological disorders. Preliminary XANES analysis of the cobalamin cofactor, suggests that this method may help resolve structural ambiguities in the binding environment. Together, these studies demonstrate the power of combining X-ray crystallography and XANES spectroscopy to achieve a more complete understanding of structure–function relationships in metalloproteins, from copper-based electron transfer to cobalamin transport.

About Data and Insight: Rethinking Collaboration and Compute in Modern Crystallography

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Crystallography and electron microscopy now produce insight at the speed of data. Detectors, compression, and pipelines have compounded throughput, but our ability to turn data into decisions still hits friction: environment setup, reproducibility, compute bottlenecks, and collaboration drag. This talk outlines a prag-matic model for distributed, elastic, and reproducible analysis—moving from “data handling” to “insight distribution”- and shows how DECTRIS CLOUD operationalizes it across MX and CryoEM.
At MX beamlines, streaming diffraction images into DECTRIS CLOUD triggers indexing, integration, and early model-building on data arrival; preliminary maps and refinement updates are available within minutes, supporting in-beam strategy decisions while preserving full provenance. For cryoEM, movie stacks become immediately addressable, launching motion correction, CTF estimation, particle picking, and 2D/3D classification in containerized, versioned environments; distributed teams review identical re-sults concurrently and can rerun analyses reproducibly without environment drift. Together, these cases reduce time-to-insight during data taking, eliminate “works-on-my-machine” failures, and ensure consistent results across sites.
Across both cases, three principles proved decisive: (1) Reproducible environments (versioned containers owned by the community, not by a single workstation), (2) Elastic compute (scale up for the heavy steps, scale down when idle), and (3) Frictionless collaboration (share data, code, parameters, and results as first-class, auditable objects). We argue that the next 10× in structural science won’t come from a single faster step, but from faster agreement across teams: shorter loops from data to shared, trusted insight.

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Speaker Abstracts

MATERIAL SCIENCE

Progress in Soft Materials Scattering

Chairs: Eliot Gann[1] & Priyanka Ketkar[1]
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Machine Learning Based Computational Methods For Analyzing Small Angle Scattering Data from Soft Materials

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In this talk, I will share the recent developments we have made with Computational Reverse Engineering Analysis of Scattering Experiments (CREASE) and its applications in interpreting data from small-angle scattering experiments. CREASE takes as input experimentally measured 1D or 2D scattering profiles and uses a genetic algorithm to output the distributions of key structural features as well as representative 3D real-space structures whose computed scattering profiles match the experimental scattering input. I will demonstrate in my talk a few examples of how CREASE and CREASE-2D have been used to understand SAXS and SANS patterns from our experimental collaborators, which were otherwise difficult to interpret with existing analytical models.

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Reconciling chain orientation in polymer-grafted nanoparticles between coarse-grain models and resonant soft X-ray scattering

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Orientation and conformation in nanoscale amorphous regions often dominate the properties of soft materials. Robust correlations between structure in these amorphous regions and important properties are not well developed due to a lack of measurements with high spatial resolution and a sensitivity to molecular orientation. For example, radial polymer chain orientation with significant spatial variation is typically predicted in computational models of polymer-grafted nanoparticles (PGNs), but it has not been validated due to this measurement gap. I will describe our approach to solving this issue using polarized resonant soft X-ray scattering (P-RSoXS), which combines principles of soft X-ray spectroscopy, small-angle scattering, and data fusion with real-space imaging to produce a molecular scale structure measurement for soft materials.

I will focus on the P-RSoXS of polymer-grafted nanoparticles (PGNs). The most unique structural motif of PGNs is the high-density region in the corona where polymer chains are “stretched” under significant confinement. We apply our approach to measure the orientation of polystyrene (PS) chains grafted to gold nanoparticles. Using a GPU-accelerated virtual RSoXS instrument, we measure the thickness of the anisotropic region of the corona and the extent of chain orientation within it. Radial chain orientation is observed that decays in magnitude away from the particle, and differences in this nanoscale orientation landscape are observed between particles of different graft density. The shape of this nanoscale orientation landscape can be reconciled with computational predictions, and quantitative agreement is possible with data fusion that incorporates experimental data from other measurements, coarse-grain simulation results, and atomistic conformation information. These results demonstrate the power of P-RSoXS to quantify and discover orientational aspects of structure in PGN systems and illustrate a framework that can be applied broadly to semicrystalline or amorphous polymers with a range of chemistries and chemical heterogeneity.

Advancing AI-Ready Infrastructure for Autonomous X-ray Scattering at the CMS Beamline

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With the growing demand for high-throughput beamtime utilization and data-driven materials research, the Complex Materials Scattering (CMS) beamline at the National Synchrotron Light Source II (NSLS-II) has made significant progress toward enabling autonomous, self-driving X-ray scattering experiments. These efforts aim to enhance AI readiness across several key domains, including experimental and data infrastructure standardization, workflow modularization, experiment control automation, feedback loop compatibility, and human-in-the-loop interfaces. These developments mark a key step toward our long-term goal of transforming CMS into a smart lab-in-beamline platform—supporting high-throughput and autonomous in situ X-ray scattering experiments.

Membranes In Motion: Tracking Polymer Membrane Formation and Performance with *In Situ* X-Ray Scattering

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Polymer membranes have the potential to address water and energy security challenges. They can selectively permit solutes over a range of sizes for applications ranging from filtration, desalination, fuel cells, and critical minerals recovery. However, their widespread use is hindered by a limited understanding of the complex processes that govern membrane self-assembly under realistic environmental conditions and translating fundamental studies to manufacturing-relevant fabrication. X-ray scattering can probe characteristic length scales from angstroms to hundreds of nanometers and is amenable to relatively fast measurements, making it well suited for revealing important dynamic processes associated with membrane formation, performance, and degradation. In situ and operando characterization is required to properly understand the structure and self-assembly of polymers in environments relevant to specific applications. This presentation will show how in situ x-ray scattering can reveal information on polymers in solution, morphology evolution during membrane formation, and membrane fouling during operation.

Block copolymers can be fabricated into isoporous membranes via an industrially relevant self-assembly plus nonsolvent-induced phase separation (SNIPS) process. In SNIPS, block copolymer micelles provide a template for an ordered array of pores at the membrane surface, and these pores impart selectivity. We will show how in situ resonant soft x-ray scattering can probe the core-shell morphology of these micelles in relevant solvent blends used for membrane fabrication. Moreover, in situ grazing incidence x-ray scattering reveals the nanoscale morphology evolution during solvent evaporation after casting, a critical step in SNIPS membrane formation. This provides key structural signatures that enable a better understanding of what leads to an isoporous structure and how other factors, like the incorporation of additives, impacts membrane morphology.

Membrane fouling, or the unwanted deposition of contaminants on membrane surfaces or within pores, increases mass transfer resistance and leads to flux decline. Fouling remains a challenge in membrane technologies. We will show the development of a membrane flow cell adapted for operando transmission x-ray diffraction and scattering that enables watching fouling under realistic pressures and flows. This provides opportunities for new fundamental insight into the mechanisms of foulant precipitation, including in complex waters, and foulant removal.

Emergent Materials for the Future

Chairs: David Sprouster[1] & Boris Khaykovich[2]
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Characterization of Radiation Effects in Ceramics with Neutron Total Scattering

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The development of durable nuclear ceramics has been central to efforts for advanced fission and fusion energy systems. There still exist, however, large gaps in the understanding of fundamental modes of structural degradation under harsh environments, including self-irradiation. We have shown that neutron total scattering measurements with pair distribution function (PDF) analysis can be utilized to uniquely characterize defects and disorder in a wide range of nuclear ceramics (e.g., pyrochlore, spinel, and actinide oxides). Key to this approach are swift heavy ions with a penetration depth of ~100 µm, which is high enough to produce sufficient quantities of irradiated material for characterization using bulk techniques [1]. Irradiation experiments are performed at the UNILAC accelerator at the GSI Helmholtz Center (Darmstadt, Germany) using typically Au ions of about 2 GeV kinetic energy. Irradiated samples were investigated at the Nanoscale Ordered Materials Diffractometer (NOMAD) beamline at the Spallation Neutron Source (Oak Ridge National Laboratory, USA). These measurements enable detailed analysis of both cation and anion defect behavior, and short-range order, which is particularly important for the investigation of amorphous materials. Recent results for several ceramics demonstrate that structural changes are more complex than previously thought with distinct processes occurring over different length scales [2,3]. For example, disordered pyrochlore and spinel oxides [4] are composed of local structural units that maintain atomic order and exist in configurations that are different than the expected average structure determined using traditional techniques (e.g., X-ray diffraction). Here, we will highlight the importance of short- and medium-range analysis for a comprehensive description of nuclear ceramics [5].

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Saturated non-periodic tetrahedral networks studied with Neutron Pair Distribution functions

Joerg Neuefeind

Oak Ridge National Laboratory

The Nanoscale Ordered Materials Diffractometer is the only instrument at the Spallation Neutron Source specifically build to measure (neutron weighted) Pair Distribution Functions. The Pair Distribution Function is sensitive to short range order independent of long range periodicity and is therefore indispensable when the local order is key to understanding the system in question. Saturated non-periodic tetrahedral systems such as amorphous SiO2 have long resisted efforts to build a three dimensional model that is a) saturated, b) non-periodic and c) consistent with diffraction data. It is shown how accurate neutron diffraction data can help build such a model and how that model, once it exists, can effortlessly explain the structure of other similar systems such as GeO2, ZnCl2 and BeF2.

Resonant X-ray Diffraction Studies of Electronic Order

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Resonant x-ray diffraction, which adds spectroscopic sensitivity to the well-known strengths of x-ray diffraction, can be used to characterize electronic order— magnetic, charge/orbital, multipolar— in emergent materials. In the first part of this talk, the resonant x-ray diffraction capabilities at NSLS-II beamline 4-ID are described. Key capabilities include the broad energy range of 2.4 to 23 keV that provides access to transition metal, lanthanide, and actinide absorption edges; polarization control and analysis; and sample environment chambers for temperature control between 2 K and 1100°C.

In the second part of the talk, specific examples of resonant x-ray diffraction studies that exploit its polarization dependence are described. In a bulk single-crystal platelet of $Cr_2Ge_2Te_6$— a semiconductor that remains ferromagnetic in the 2D limit[1]— an MgO analyzer crystal provides sufficient suppression of the structural scattering to reveal magnetic scattering in the rotated polarization channel. Temperature-dependent studies complement magnetic measurements,[2,3] and reciprocal space scans indicate 3D ferromagnetism below T_c. In the quantum spin liquid candidate a-$RuCl_3$, polarization-resolved measurements of the azimuthal dependence of the scattering shed light on the magnetic structure, which is found to be robust to structural disorder including stacking faults and twinning.[4] Finally, recent studies combining variable incident polarization with full polarization analysis are described.

Work performed in collaboration with E. Horsley, S. Kim, Y.-J. Kim, Y. Liu, C. Petrovic, and J. Sears. This research used beamline 4-ID of the National Synchrotron Light Source II, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Brookhaven National Laboratory under Contract No. DE-SC0012704.

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Engineering Compositionally Complex Oxides for Thermal Barrier Coatings

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To meet the demands of next-generation technologies, strategic material design is required to leverage diverse properties while mitigating performance degradation in extreme environments. We explore the compositional design of low thermal conductivity rare earth titanate pyrochlore (RE2Ti2O7) ceramics, taking advantage of the large range of ionic radii available in the Lanthanide series (RE = La to Lu, large to small). Corresponding average and local atomic structures are examined using a combination of neutron, X-ray, and electron scattering probes, deployed across length scales to untangle complex structure-property relationships. An emphasis is placed on determining whether samples are true solid solutions or multi-phase systems containing nanoscale inhomogeneities or locally disordered/distorted regions. We demonstrate, through compositional-complexity, the ability to independently tune the average and local atomic structures, achieving low thermal conductivity without compromising the inherent bulk mechanical stiffness of these materials. Structural stability and select properties are also examined under extreme conditions such as ultra-high temperatures (up to 2800 °C) using aerodynamic levitation and laser heating with in-situ scattering, evaluating suitability for thermal protection system applications. By combining multi-scale characterization with intentional compositional design, we establish a framework for developing and assessing the performance of next-generation materials.

Controlled Reduction of Oxides for Energy Applications

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In-situ techniques offer insights into reaction mechanisms and microstructure development that may be missed entirely when studying complex materials systems. Application of in-situ XRD, neutron powder diffraction and scanning electron microscopy in studies of oxides under reducing conditions have been useful in tailoring materials properties. Examples include synthesis of reforming catalysts, photocatalysts and battery materials. The talk will review recent work on selective oxide reduction to form surface metal colloids on oxide surfaces which in turn have controlled crystallographic faceting – along with extensions of this work to make defect-rich oxide battery materials and photocatalysts with improved properties.

A key topic of interest is introduction of large numbers of oxygen vacancies in high-performance battery materials as a means of tuning properties. An example of controlled reduction of $Nb_{12}WO_{33}$ will be reviewed, where a 'volcano plot' of extent of reduction vs. battery capacity is evident and the reduced samples outperform the fully oxidized, well-crystalline versions by as much as 50 mAh/g across charge rates from 0.1 to 10C. X-ray absorption spectroscopy (including in-situ cycling) reveals the extent of reduction of the Nb and W ions and their behavior before, during and after lithiation, while electrochemical testing yields new knowledge of Li diffusion rates and charge transfer with oxygen defects.

Nickel/cobalt aluminate spinel is another interesting case study, where reducing atmospheres extract reducible cations from the MAl_2O_4 spinel starting phase. The spinel materials are chemically highly tailorable, and selectively reduced materials are useful as catalysts for fuel reforming, for example. Here we present a study employing *in-situ* scanning electron microscopy as the primary tool for tracking the reduction processes dynamically. In-situ neutron powder diffraction, Raman spectroscopy, and S/TEM complement the FEGSEM data. The talk will show how we track first the evolution of the faceting of the base oxide particles, then sintering of particles, and finally Ni/Co exsolution and resorption – as functions of temperature and oxygen partial pressure. Reduction of the metal nanoparticles from the oxide yields surface sockets and the time/temperature profiles determine the self-organization of the metal particles on the oxide surfaces, as well as the particle sintering. We find that rapid mass transport occurs on the surface, enabling the metal nanoparticles to diffuse along the surface and coalesce. Using catalysis measurements, we show that the oxide surfaces are in fact oxygen deficient, allowing rapid near-surface oxygen ion transport and that simple control over the reduction reactions yields optimized catalysis behavior. We consider possible mechanisms that enable rapid diffusion of particles on these unique oxide surfaces.

Energy Storage

Chairs: Peter Khalifiah[1] & Gihan Kwon[2]
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X-Ray and Neutron Characterization Guided Materials Design and Development for Energy Storage Systems

Hailong Chen

The Woodruff School of Mechanical Engineering

Georgia Institute of Technology

In situ and operando XRD has been widely used in battery research in the past two to three decades, yet designing setups that truly capture real-time conditions in electrochemical devices remains challenging. In the past decade, our group has developed a diverse suite of in situ XRD and PDF tools for various synthesis methods (solid state, hydrothermal, molten salt and electrodeposition), as well as operando XRD and PDF tools for functioning batteries and other electrochemical systems, leveraging synchrotron, lab X-ray, and neutron sources.

In this talk, I will report our recent work using variable-temperature in situ synchrotron and neutron diffraction to reveal the detailed crystal structure of halide solid electrolytes, and how these insights have guided the design of novel materials with record-high room-temperature ionic conductivity. Our studies revealed that the superionic transition (SIC) in the Li_3YCl_6 solid electrolyte originates from collective anion motion triggered by increasing temperature. Building on this understanding, we designed and synthesized new halide electrolytes, achieving room-temperature ionic conductivities up to 12 mS/cm[1]. I will also briefly highlight our recent progress in developing novel low-cost and sustainable cathode materials, where operando XRD and energy dispersive XRD provided useful insights[2, 3].

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Multi-modal correlative imaging of energy storage materials

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Scanning probe microscopy offers a unique opportunity to detect structural and chemical heterogeneities over a large sample volume with high spatial resolution and sensitivity. In this presentation, we will show our recent works on applying the multi-modal imaging capability to study energy storage materials, including mapping the doping [1,2] or phase-separation [3] induced lattice distortion inside cathode particles, and enhancing electrochemical performance by inducing various core-shell gradients [4,5,6]. We will also describe the design and capabilities of two new nanoprobe beamlines at NSLS-II, Advanced Nanoscale Imaging (ANI) and Tender X-ray Nanoprobe (TXN) [7].

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Structure and superionic transition of Li3YCl6 and Li3YBr6

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Halide-based Li^+ solid-state electrolytes have garnered renewed interest in recent years, primarily due to their significantly enhanced Li^+ ionic conductivity and excellent stability with high-voltage cathodes. It is well established that synthetic conditions and protocols can drastically influence cation defect chemistry, thereby impacting Li^+ mobility in these materials. Despite extensive research, considerable debate remains regarding the fundamental mechanisms governing Li^+ transport, particularly about the Li+ superionic transitions in these compounds, which continue to impede performance optimization. In this talk, I will present a comprehensive structural picture of Li_3YCl_6 and Li_3YBr_6 synthesized under varying conditions. Additionally, I will discuss the different structural origins of the superionic transitions observed in these two compounds.

Dynamics of Materials and Interphases in Energy Storage via X-ray and Neutron Diffraction

Enyuan Hu

Chemistry Division, Brookhaven National Laboratory

Energy storage plays a critical role in modern society, with increasing demands for low cost, high energy density, safety, and supply chain resilience. These evolving requirements introduce greater structural complexity to energy storage materials. Compounding this complexity, systems like batteries operate under cyclic electrochemical stimuli—charging and discharging—that drive dynamic structural changes often beyond the scope of conventional phase diagrams.

Another key challenge lies in the formation of interphases—reaction products at the electrode–electrolyte interface, often catalyzed by electrochemical processes. These interphases are essential for kinetically stabilizing battery systems that are inherently thermodynamically unstable, enabling long-term cycling. Understanding their composition and evolution is crucial for rational materials design and improved system stability.

In this talk, I will present our group's efforts to address these challenges using synchrotron and neutron scattering techniques. We employ time-resolved XRD to capture phase transitions during fast charging, discharging, and material synthesis. We extend these studies to operando PDF to probe the real-time dynamics of amorphous components. To investigate systems involving oxygen redox, we utilize neutron diffraction and PDF to reveal subtle structural changes. Most recently, we have applied synchrotron XRD and PDF to uncover elusive interphase components in batteries, identifying their formation pathways and tracking their dynamic evolution. These studies provide mechanistic insights into fast transformations, oxygen redox, and interphase evolution, guiding rational design for improved performance.

In-Situ Methods at High Pressure

Chairs: Matt Whitaker[1], James Walsh[2]
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The Secret Inner Life of a Deforming Rock, Revelations from Synchrotron X-rays

Pamela Burnley, University of Nevada, Las Vegas

Probing deforming crystalline aggregates (rocks) using white beam synchrotron X-ray diffraction provides information about the behavior of various grain populations within the aggregate; revealing that not all grains are 'doing the same thing'. Elastic plastic self-consistent (EPSC) models provide a means of interpreting these complex x-ray diffraction data sets. EPSC is a forward modeling technique that takes into account the elastic and plastic properties of the material at the single crystal level and then calculates how an aggregate of crystals will behave by treating each grain as an elliptical inclusion in a homogeneous matrix constructed of an average of the rest of the grains. As part of its output, the model calculates the d-spacing for user specified diffraction lines, allowing the individual behavior of various diffraction lines in the context of the overall aggregate behavior to be understood. EPSC models can be used to interpret which deformation mechanisms are operating in a material and to identify situations where unanticipated deformation mechanisms are at work. We have found that EPSC models work well for understanding the deformation of olivine, clinopyroxene, and quartz aggregates, as well as synthetic materials such as alumina and Mg alloy. Bulk strength measurements as well as critical resolved shear stresses for dislocation slip derived from the models agree well with literature values. We have also used EPSC-produced bulk strength measurements combined with ultrasonic velocity measurements to measure acoustoelastic constants for a variety of materials at high pressure.

A Novel Eight-Coordinated Phase of Mg_2SiO_4: Insights into the Mineralogy of Super-Earth Mantles

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The discovery of thousands of extrasolar planets has sparked strong interest in understanding the behavior of candidate exoplanetary materials at pressure and temperature conditions beyond those found within the Earth. Based on the measured densities of exoplanets, many are likely constituted primarily of rock and metal like Earth. For an Earth-like composition, the core-mantle boundary conditions of these exoplanets and can reach ~1300 GPa, well beyond the limits of conventional static high-pressure experiments. Shock wave method can reach ultra-high pressures but can only access very high temperature liquid states. High-powered laser facilities such as the National Ignition Facility (NIF) provide a novel approach to ramp compress and probe materials to ultrahigh pressures in the solid state. Historically, shock compression experiments have been limited in their ability to directly resolve crystal structures, as in situ X-ray diffraction capabilities were not available. Consequently, structural determinations relied on shock-recovered samples rather than in situ measurements. The TARDIS (target diffraction in situ) diagnostic at the NIF provides a unique opportunity for timeresolved X-ray diffraction during dynamic compression, offering insights into the structural transitions at extreme pressures and enabling the direct identification of novel high-pressure phases. In this work, we have compressed forsterite, Mg_2SiO_4, a primary constituent of planetary mantles, to 1.05 terapascals (1.05 million atmospheres). Using nanosecond X-ray diffraction to probe the atomic-level structure, we observe the metastable Forsterite-III phase at pressures below 565 GPa. At ultra-high pressures, Mg_2SiO_4 adopts a thorium phosphide-type structure in which, unlike all other known silicate minerals, silicon is in eight-fold coordination with oxygen. Our results are consistent with computations based on density functional theory. The thermophysical and chemical properties of this newly discovered mineral phase will influence the structure, dynamics, and evolution of large, rocky exoplanets.

Elucidating Anisotropic Chemistry in Lanthanide Halide Systems Under Pressure

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Lanthanide dihalides have long served as model systems for understanding the impact of electron counting and configurational changes in f-element materials for decades. Crucially, these systems exhibit a dichotomy of behaviors defined by their electronic structures and governed by electron count. They are either well considered as salt-like with charge separated ions or they engage in delocalized bonding that promotes metallicity. This dichotomy is also structure directing, introducing significant structural diversity into this class of materials. However, this dichotomy is not just defined by lanthanide identity and can be tuned based on extrinsic factors such as temperature and pressure. Counterintuitively, the application of pressure can be used to reduce the dimensionality of these materials, driven by electronic structure changes.

We expect that the application of anisotropic force to anisotropic materials will yield a cooperative effect to promote further anisotropy. However, the details of such interactions remain not well elucidated, in particular how anisotropic force influences a material across length scales from atomic level chemistry to nanostructure and finally bulk properties. Herein, we report on a direct comparison of anisotropic lanthanide halide materials synthesized under isotropic compression conditions as compared with uniaxial deformation. Using a large volume press at Brookhaven National Laboratory, we synthesized different polymorphs of lanthanide halides. Through both in situ diffraction studies of structure formation and ex-situ analysis of recovered samples, we elucidated differences in structure and properties that are further supported by calculations. Together, these efforts provide greater chemical context for the use of an expanded mechanochemical toolkit to control solid-state chemistry.

In Situ Diffraction to Discover Co–Bi Binaries

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Permanent magnets are crucial components of generators and motors, which in turn enable many technologies, such as wind turbines and electric cars. Designing new permanent magnets requires a fundamental understanding of how orbital angular momentum impacts coercivity. To target large magnetic anisotropy in a ferromagnet, we considered systems where a transition metal provides the spin moment, and a heavy main group element bolsters strong spin-orbit coupling. Within this framework, the Co–Bi systems emerge as an appealing platform for discovery, leading us to investigate the Co–Bi system for permanent magnetism using high-pressure ab initio structure predictions and high-pressure synthesis. Performing *in situ* synchrotron powder X-ray diffraction and ambient pressure single crystal diffraction revealed four novel compounds, with theory predicting ferromagnetism and large magnetocrystalline anisotropy energy in β-CoBi and β-$CoBi_2$, rivaling or exceeding that of established magnets such as CoPt and Nd–Fe–B [1]. The Co-Bi system has been previously shown a phase which exhibits superconductivity [2,3], indicating that Co–Bi system could be a platform for understanding the competition and coexistence of emergent correlated properties in a chemically simple binary system.

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Martensite-type ε-$VC_{y''}$ high-pressure synthesis pathway revealed through *in situ* x-ray diffraction

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High-pressure synthesis is a powerful tool in materials discovery and can often uncover new metastable phases, even in well studied systems such as transition metal carbides. The vanadium–carbon system has been thoroughly studied due to its importance in the steel industry as well as its many high resilience materials used across a range of industries [1,2]. Although this system is prized for strength and resiliency properties, there are surprisingly few studies exploring phases stabilized under extreme pressure [3]. Here we employ high pressure-high temperature (HP-HT) synthesis with the multi-anvil press to investigate the crystal chemistry of vanadium carbides under extreme pressure.

During HP-HT synthesis, *in situ* synchrotron XRD data reveals significant volume expansion in the bcc-V precursor phase, consistent with carbon incorporating into the metal lattice interstices. This provides a time-resolved glimpse into the growth dynamics of the competing phases. Here we present *in situ* synchrotron XRD data collected during the synthesis and recovery of the novel martensite type phase, ε-$VC_{y''}$. We also present single crystal dataset recovered from synthesis experiments carried out between 0.5 and 5.1 GPa. This discovery of a novel vanadium analogue of the well-known martensite structure demonstrates that high pressure may be a rich source for other non-equilibrium carbide materials, and that *in situ* XRD can reveal the path towards novel materials.

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Advances in Computational Crystallography

Chairs: Thomas Caswell[1] & Jennefer Maldonado[1]
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Sampling Volumes in Powder Diffraction Experiments

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We present a simple analytical formalism based on the Lorentz-Scherrer equation and Bernoulli statistics for estimating the fraction of crystallites (and the associated uncertainty parameters) contributing to all finite Bragg peaks of a typical powder pattern obtained from a static polycrystalline sample[1]. We test and validate this formalism using numerical simulations, and show that they can be applied to experiments using monochromatic or polychromatic (pink-beam) radiation. Our results show that enhancing the sampling efficiency of a given powder diffraction experiment for such samples requires optimizing the sum of the multiplicities of reflections included in the pattern along with the wavelength used in acquiring the pattern. Utilizing these equations in planning powder diffraction experiments with optimized sampling efficiency is also discussed.

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Compressed and Sparse Sensing Techniques

James Yates

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For extremely measurement hungry techniques, such as XRD-CT or 3D-PDF very large numbers of diffraction measurements, often on the order of thousands, must be taken to reconstruct the signal of interest. More efficient sampling of these signals using compressed sensing and AI techniques allows for a dramatic reduction in necessary measurements, often by more than an order of magnitude. A scientifically interesting measurement is generally structured in some way, making it low in information content from an information theory perspective. For any signal that is not constructed purely of random, uncorrelated noise, conventional measurement of a signal produces a large amount of redundant information.

Compressed sensing methods often require an order of magnitude fewer samples to provide a provably identical signal to one which is conventionally sampled. With prior knowledge about the signal being measured, even sparser sampling of a signal can produce a useful measurement. In certain contexts, the problem of compressed and sparse sensing is equivalent to a denoising problem. Here we show an AI diffusion model sparse sensing method applied to XRD-CT capable of providing a useful measurement using an order of magnitude fewer measured diffraction patterns. The development of such techniques will enable experiments, such as in situ and time resolved experiments, that are simply impossible using conventional sampling techniques.

Probing further under sharp peaks and peak shape: The beginning of pushing the capabilities of high resolution PXRD beamline 11-BM with new detectors and analysis

Saul Lapidus

Argonne National Laboratory

Powder diffraction is a key bulk probe to the understanding of the crystallographic nature of materials, especially when single crystal studies are impractical. This talk will lay out the power of high-resolution powder diffraction and specifically the capabilities of beamline 11-BM, that has been a mainstay of material science and powder diffraction studies in the USA.

With the APS upgrade now complete, new detectors have been implemented at beamline 11-BM, small GaAs area detectors, replacing the classic scintillation detectors and the capabilities and challenges, both computation and experiment, that lay ahead (with both current accomplishments and future possibilities discussed). These developments will allow for more detailed crystallographic understanding and deeper material science studies and experiments from high-resolution powder diffraction at 11-BM.

AI for powder diffraction: Bayesian optimization-driven measurements & automated analysis

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Powder diffraction is ubiquitous in materials discovery, serving as a fundamental materials characterization technique that has evolved alongside both technological developments and material challenges. Modern diffraction instruments, particularly those at large-scale user facilities such as the National Synchrotron Light Source II (NSLS-II), enable data collection rates that significantly outpace traditional analysis methods, making data analysis and interpretation the bottleneck to discovery and innovation. This mismatch of measurement and analysis times is exacerbated by the increasing need for additional characterization techniques to fully understand the structure and function of complex materials. Typically, these techniques are available only on specific synchrotron beamlines, and complementary measurements are conducted days to months apart, blocking real-time insights and limiting their value to a combined post-measurement analysis. Recently, we demonstrated artificial intelligence (AI) driven multi-beamline synchrotron experiments in which combined powder diffraction and X-ray absorption fine structure (XAFS) measurements were orchestrated in real time. This world-first achievement opens many opportunities for human-AI collaborative operating modes in which AI works alongside facility users to enhance scientific output.

Using the *Bluesky* data acquisition platform at NSLS-II we conducted multi-modal, multi-beamline, and AI-driven experiments in real time across spatially distant beamlines, with asynchronous measurements occurring at vastly different rates. An ensemble of AI agents, each with a defined task scope, enables autonomous operating modes in which both raw data and products of scientific analyses (*e.g.,* Rietveld refinement, XAFS modelling) are processed in real time to guide subsequent measurements. This highly extensible and modular framework features a plug-and-play architecture that allows researchers to easily substitute agents for data reduction, analysis, or decision-making, depending on experimental needs. We also developed a digital twin for diffraction measurements to enable offline agent development and performance evaluation. We will discuss the design and implementation of our experimental framework that integrates AI with variable autonomy and demonstrate enhanced efficiency in exploring mappable samples. Finally, efforts underway to automate powder diffraction analysis will be discussed, including data analysis pipelines and parallelized workflows that utilize high performance computing resources.

Poster Abstracts

Life Sciences Posters

Elucidating a mechanistic role of colibactin induced DNA damage

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The human gut microbiota constitutes a complex, diverse community of microorganisms that influence human physiology, clinical responses to drugs, and disease progression[1]. The natural product colibactin, along with its associated biosynthetic gene cluster, is an example system for the role microbially derived small molecules play in the human microbiome. This is particularly relevant in the human gut, where host microbiota is involved in various disorders, including colorectal cancer pathogenesis. Colibactins are hybrid polyketide-nonribosomal peptides that are bona fide virulence factors and are suspected of promoting colorectal carcinogenesis[2]. Owing to difficulty in isolating *E. coli* natural active colibactins we will utilize synthetic colibactin analogs[3] to investigate and elucidate mechanisms by which colibactins damage and bring about genotoxic effects in human gut microbiome. In this current work we establish that our synthetic colibactin analogs show similar levels of activities as the naturally virulent colibactins. Our results indicate that colibactin interacts with DNA by forming DNA-alkylated adducts at adenine rich motifs. We verified DNA alkylation alkylation by gel electrophoresis, enzymatic reaction and mass spectrometry. ClbS, a gene product of the *pks* island, confers resistance to genotoxicity of colibactin in host bacteria by functioning as a cyclopropane hydrolase[1]. In this work, we confirmed the enzyme activity of ClbS against synthetic colibactin analogs. Our results aim to delineate the mechanistic role of colibactin in DNA-damage associated tumorigenesis. We hope that these results show the finer details of the complex chemistry of host-microbe and microbe-microbe interactions.

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How evolution shaped the structure of cholesterol-metabolizing cytochrome P450 11A1, a key player in steroid hormone biosynthesis

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The ancestral cytochrome P450 11A1 (CYP11A1) enzyme plays a critical role in the synthesis of steroid hormones. It catalyzes the first and rate-limiting step in the conversion of cholesterol to pregnenolone via side-chain cleavage. Pregnenolone serves as the precursor for steroid hormones controlling immune and stress response, salt homeostasis, and sex differentiation. Thus, the evolution of CYP11A1 is tightly connected to the rise of steroidogenesis as a hallmark feature of higher vertebrates. Recently, a vertebrate ancestor of CYP11A1 has been resurrected via ancestral sequence reconstruction (ASR). Notably, the ancestral CYP11A1 has a drastically decreased catalytic activity towards cholesterol compared to the extant bovine isoform. Instead, the cholesterol precursor desmosterol is the preferred substrate. We hypothesize that differences in substrate preference occur due to structural rearrangements which were shaped by evolution.

To understand the structural basis for changes in substrate preference, we aimed to determine the structure of the vertebrate ancestral CYP11A1 enzyme in complex with cholesterol and desmosterol, respectively, using X-ray crystallography. We generated recombinant CYP11A1 ancestor in complex with cholesterol and desmosterol, respectively, and with obtained crystals could solve both structures to 2.4 Å and 2.9 Å, respectively. Both structures exhibit the highly conserved P450 protein fold with the heme prosthetic group in the active site. Our data reveals significant modifications of the substrate access channel and active site architecture when compared to the extant bovine CYP11A1 isoform. In the ancestral CYP11A1 enzyme, desmosterol adopts a classical sterol binding pose that closely resembles that of cholesterol bound to the bovine structure. Our most exciting finding is that cholesterol is bound in a flipped orientation in the ancestral CYP11A1 active site, an arrangement that likely renders it less favorable for catalysis compared to desmosterol. Complementary molecular dynamics simulation further confirm a preference for desmosterol as substrate.

The structure of the vertebrate CYP11A1 ancestor sheds light on the evolution of human steroidogenesis, highlighting how this enzyme became increasingly specialized over time. These findings also contribute to our understanding of how CYP11A1 enzyme evolution shaped the transition of life from water to land and enabled the rise of higher vertebrates.

The Reciprocal Space Station Forum for Structural Biology

Kevin M Dalton[1] *and the Astronauts*[-1]

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Structural Insights into the Mechanism of Selective Inhibition of Mitochondrial Complex III by Antifungal Agents

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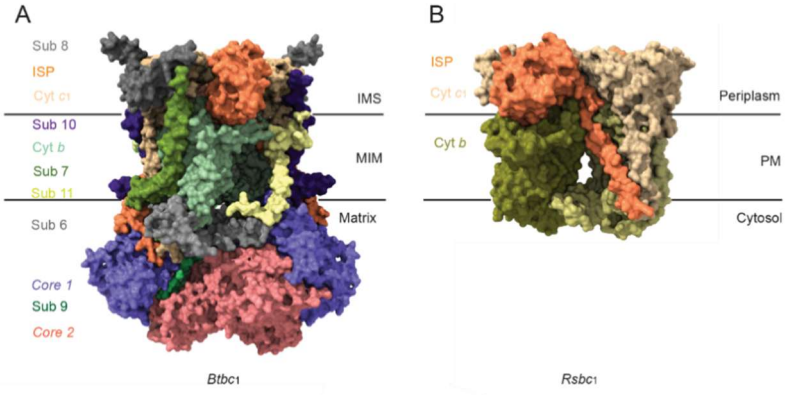
Cytochrome bc_1 as well as other complexes of the electron transport chain, are validated drug targets especially for pesticides and antibiotics. However, drugs that inhibit infections (for example *Plasmodium* spp.) are often toxic to mammalian hosts undermining or restricting their use. Here, we explore differences in sequence and structure of cyt bc_1 that, in conjunction with differential cellular response to drugs, are sufficiently large to offer vulnerabilities to pharmacological exploitation. This study employs purified cyt bc_1 from the photosynthetic bacterium *Cereibacter* (formerly *Rhodobacter*) *sphaeroides* ($Rsbc_1$) and from bovine mitochondria ($Btbc_1$) as representatives for bacterial/fungal and mammalian enzymes. We provide experimental evidence that this system has predictive power based on activity studies, X-ray crystallography and cryo EM structure determinations. We elucidated the mechanism of selective inhibition of cyt bc_1 by atovaquone and identified pyramoxadone as a veritable fungicide with much greater selectivity for $Rsbc_1$ over $Btbc_1$. Furthermore, pyramoxadone has low cytotoxicity in cultured human cells and outperforms currently available fungicides. This study provides an example that subtle differences in sequence and structure of some of the most highly conserved proteins can be exploited successfully.



56,000 fps Hybrid Pixel Array X-ray DetectorAiming for Time Resolved and Imaging Experiments

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It has been more than ten years since HPAD (Hybrid Pixel Array Detectors) have been widely utilized as X-ray diffraction and imaging detectors. Thanks to their single photon counting capability, HPADs provide images with minimal background noise and a wide dynamic range. Due to limitations of the fabrication process, most HPADs are made with a monolithic sensor and tiled readout ICs. In a conventional HPAD, there were so-called “inter-chip pixels” on the edges of the readout ICs. These inter-chip pixels are 1.5 times wider and/or taller than non-inter-chip pixels. This means we are losing position information for photons impinging on those pixels.

We have successfully addressed this inter-chip pixel problem by utilizing a redistribution layer on the Silicon sensor. So, in this detector, non-uniformity in a single sensor module is eliminated.

This new detector is designed based on the UFXC32k IC [1] and its high-energy resolution variant, the LNPX32k IC, for a soft X-ray application detector. Both ICs were designed by the AGH University of Science and Technology and are part of the XSPA [2] detector series. XSPA detectors employ a modular design with each monolithic sensor module consisting of 16 UHXC chips providing 1024 x 512, 76 µm x 76 µm pixels. The absence of inter-chip pixels between ROICs leads to improved image quality.

The XSPA detector series is applicable for X-ray imaging and for time-resolved X-ray measurements. Dealing with “inter-chip pixels” is a key feature for imaging, and for time-resolved measurements, we understand that the frame rate is as important as the size of the pixels and the area of the detector. Thanks to UFXC32k IC’s high count-rate and fast operation capability, combined with our high data throughput backend circuits, the XSPA-500k is capable of up to 56,000 fps with full-frame readout and 100,000 fps with 100 lines ROI in the center of the modules with continuous exposure (zero-deadtime mode operation with 2-bit counter/pixel). If non-continuous exposure (burst-mode operation [3]) is allowed, it can achieve over 970,000 fps with an approximately 2% duty ratio. By using the LNPX32k IC, we can operate our detector with as low as 400 eV X-rays.

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Shoot First, Ask Later: High Throughput Crystallography Screen Towards the Identification of New Cyclophilin D Inhibitor Fragments

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Ischemic strokes account for about 87% of all strokes in the United States and for 62% worldwide [1]. One of the main complications of ischemic stroke is ischemia-reperfusion injury, in which cells begin to rapidly die during the restoration of blood flow to the ischemic tissue, which is exacerbated by dysregulation of the mitochondrial transition pore (mPTP) [2]. Cyclophilin D (CypD) is a peptidylprolyl isomerase that isomerizes proline residues in polypeptides and is key modulator of the mPTP. Currently, there is only one FDA-approved cyclophilin inhibitor, Cyclosporine A (CsA), which is an immunosuppressant. Though CsA is very potent, it binds to several cyclophilin isoforms with nanomolar affinity [3]. Previously, our group developed a series of peptidic macrocycle inhibitors that were able to selectively inhibit CypD *in vitro* through specific chemical modifications of the macrocycles which took advantage of structural differences in the diverse S2 pocket of the CypD's active site. Presently, we sought to design CypD inhibitors with a more favorable small molecule chemistry by employing high throughput structural fragment screening. We soaked over 1,000 small molecule fragments into the CypD crystals, which generated over 400 co-crystal structures and informed parallel computational pipelines to identify lead compounds. Rigorous biochemical characterization of these compounds has resulted in several potential leads that can inform subsequent generations of inhibitors.

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The Future of Macromolecular Crystallography Experiments: Autonomous Experiments as Extension to Your Laboratory

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The NIH-NIGMS and DOE-BER MX beamlines at the NSLS-II have implemented the hybrid access to enable significantly higher sample and user throughput without compromising data quality. In this access mode, all samples undergo automated data collection which includes multiple automated data reduction pipelines, or raster screening the night before scheduled time. During normal operation, up to three groups are scheduled with each 3 hours of continuous time for manual data collection. Many users forfeit the scheduled manual collection and about two thirds (2/3) of the groups rely on this dedicated beamtime to collect additional data sets as required. Users are provided an experimental report with tables and figures prior to beamtime helping them making best educated choices as to which samples should undergo manual collection.

The hybrid data collection mode allows for both very high throughput and best data quality; it provides best access to MX resources by making best uses of automated workflow and manual collection from expertly trained MX users. We encourage groups relying on MX data collection at Synchrotron facilities to consider such resource as a natural extension of their own lab. By relying on NSLS-II standBy access, users rely on the automated workflow to evaluate crystal samples as frequently as needed, so that users can rapidly optimize crystal quality to achieve structure ready diffraction data with minimum interaction with beamline instrumentation and data reduction / analysis.

Looking ahead, one can imagine that synchrotron facilities are best suited to deliver publication ready structures for MX users with insufficient computing resources and or expertise. The NSLS-II MX beamlines have implemented remote post-processing infrastructures, toward automated data analysis and structure refinement. Planned tools and workflows will be presented.

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Structural Insights into the Human Elongin-A Activation Domain Highlight a Conserved Pol II Interaction Site

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Elongation factors are well known for their role in enhancing transcription efficiency through RNA polymerase II (Pol II). Their functions are essential for maintaining the elongation rate required for efficient mRNA splicing and proper organismal development. Elongin-A is a key transcription factor, acting as the transcriptionally active subunit of the heterotrimeric complex. Here, we present the crystal structure of human apo Elongin-A597–682. The activation domain includes both the Pol II binding region and the region necessary for interaction with the Cul5 enzyme in ubiquitination. Our analysis demonstrates that Elongin-A homologs are conserved at both the sequence and structural levels, particularly within the Pol II interacting region. Furthermore, in-silico modeling combined with existing literature reveals that Elongin-A structurally resembles an F-box protein, functioning with adaptor proteins Elongin B and C to assemble the Cul5-mediated ubiquitin ligase complex, thereby regulating Pol II degradation. Together, these findings provide new insights into the functional role of human Elongin-A.

Polymer-based microfluidic chips for time-resolved serial crystallography

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Improvements in the brilliance of X-ray beams and the sample handling technology needed for these new advanced X-ray sources has inspired a new generation of experiments looking to obtain time- resolved information of the structural changes of proteins. While serial crystallography techniques for delivering thousands of crystals to the X-ray beam were originally developed to circumvent the severe radiation damage caused by these brilliant sources, this technology is also uniquely enabling for performing time-resolved experiments. Fixed-target platforms with easy mounting of crystals serve as a beneficial strategy for crystal delivery in serial crystallography experiments at both XFELs and synchrotrons. Fixed-target platforms offer the advantage of fixing the position of crystals in a well-defined array thereby improving the sample utilization rate and producing relatively high target 'hit' rates. However, the device material surrounding the crystal can result in significant background scattering and adversely affect signal-noise ratios.

We have developed a fixed-target device that uses the hydrodynamic trapping approach described by Lyubimov *et al*., *Acta Cryst D* (2015) to create an array of crystals that are connected to a fluidic network. These devices are fabricated using a thiol-ene-epoxy based resin known as OSTEMER. OSTEMER enables a 2-step curing process – an initial rapid UV based curing process followed by thermal curing which helps it to stiffen and bond to various substrates leading to the formation of a sealed device. OSTEMER has good X-ray transparency properties, and the optical transparency of OSTEMER allows for light-based triggering for time-resolved studies, while the microfluidic aspect facilitates the use of chemical triggering. We evaluate the performance of these chips through the Design of Experiments approach by flowing crystals/polymer bead suspensions with varying number densities and flow rates. Insights gained from these experiments will help to optimize the loading conditions to ensure maximum occupancy of traps with single crystal per trap. We anticipate that this novel polymer-based microchip will enable time-resolved structural studies of a range of proteins at both XFEL and synchrotron sources.

Structural elucidation of rationally designed CRYGS protein variants for enhanced therapeutic potential in treating retinal neuroinflammation

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Background The structural and functional differences in proteins play a major role in disease etiology, particularly in neurodegenerative conditions such as glaucoma and age-related macular degeneration (AMD). These retinal disorders involve progressive neuroinflammation, yet most current treatments are palliative and fail to address this underlying cause of vision loss1. Developing therapeutics that directly target neuroinflammation is thus of growing clinical interest.

Aims This study seeks to provide a structural understanding of Crystallin-γS (CrygS), a protein that is naturally upregulated in the retina following injury or disease. CrygS has shown anti-inflammatory and regenerative effects in injured retinal cells, likely through direct interaction with Interleukin-1β (IL1β), a key pro-inflammatory cytokine2. We aim to improve the therapeutic potential of CrygS through structure-guided protein engineering and elucidate the structures of the CrygS variants through X-ray crystallography.

Methods We computationally designed and analyzed several thermodynamically stable, single-amino-acid variants of CrygS to enhance its binding affinity to IL1β. Molecular docking and binding energy calculations identified one variant, mutA, with significantly improved anti-inflammatory activity in a retinal cell culture system ($p = 0.0001$). To enhance clinical viability, we introduced a second point mutation (C25X) to eliminate a cysteine residue involved in disulfide bond formation and aggregation. Proteins were expressed in *E. coli*, purified using nickel affinity and size exclusion chromatography, and crystallized via sitting-drop vapor diffusion.

Findings We successfully solved the X-ray crystal structures of wild-type CrygS, mutA, and the double mutant mutA+C25S to resolutions of 1.83 Å, 1.45 Å, and 1.51 Å, respectively. All structures crystallized in the P3121 space group. Structural comparisons have revealed minimal differences thus far.

Conclusion This study provides the first structural analysis of CrygS variants with demonstrated therapeutic relevance. Our findings support the potential of CrygS-based protein therapeutics in treating retinal degenerative diseases and offer a framework for structure-guided optimization of neuroprotective proteins.

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Photonic Insights from a MOF-like Methionine Structure

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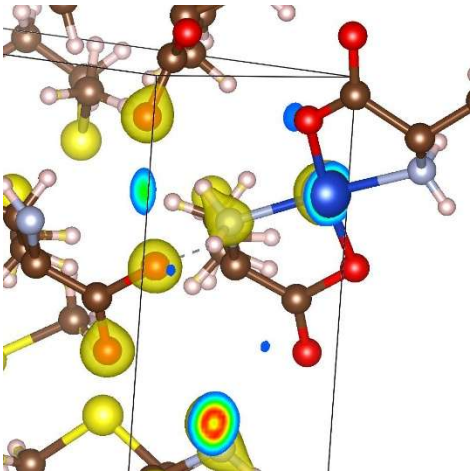

We report the structure of a MOF-like construct of methionine bound to Cu(II) as determined by crystal electron diffraction (MicroED). While the overall framework geometry and connectivity are similar to previously characterized structures, in our system, a denser lattice packing stabilizes a novel crystalline form. Optical reflectance measurements combined with modeling of the complex dielectric function reveal changes in spectral intensity and band-edge features relative to known polymorphs. To rationalize these observations, we performed electronic structure and optical response calculations using PBE density functional theory (DFT), directly comparing the newly observed structure with *apo* structures. This comparative analysis demonstrates that the incorporation of copper, when coupled to subtle variations in molecular assembly, produces pronounced differences in the optical behavior of the resulting material. Together, these results highlight the value of MicroED for resolving new polymorphs and underscore the importance of detailed structure-property relationships in guiding the design of bio-inspired photonic materials.

Fixed-Target Microfluidic Device for Anaerobic Crystallography

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Understanding a protein's structure, its interactions with other molecules, and regulatory mechanisms, is a key to figure out its role in biology. Structural analysis is important to identify this functional background, and also to advance drug discovery, disease modeling, and personalized medicine. Among the numerous potential targets, redox-sensitive metalloproteins, which are involved in key processes of respiration and oxygen transport are inherently unstable in aerobic environments because oxygen can alter the metal site and/or surrounding protein structure. These challenges have resulted in considerable difficulties in elucidating details of the structure function relationship. Apart from being unstable at ambient conditions, this vulnerability becomes even more pronounced during X-ray diffraction experiments, where intense radiation from synchrotron sources causes structural alterations that can deviate significantly from the native state. Cryogenic cooling has traditionally been employed to reduce such damage as it slows down radiation-induced reactions and minimizes oxygen diffusion. However, the same mechanisms that slow the effects of radiation damage also prevent direct observation of functional motions within the proteins. To overcome these limitations, we are developing an anaerobic fixed-target device that will be used for data collection at ambient temperatures.

To create a stable anaerobic environment inside the device, we are exploring different types of novel, X-ray compatible materials such as graphene and Ostemer. Graphene, due to its atomic thinness and impermeability to gases, serves as a key oxygen barrier while Ostemer is a UV-curable polymer that takes advantage of thiol-ene click chemistry. By using an excess of thiol groups we can enable oxygen scavenging function, meaning that the thiol groups in the material react with and consume oxygen molecules, thus removing the oxidizing species that would otherwise damage the protein crystals during data collection. We are validating our approach via colorimetric studies using the redox sensitive dye methyl viologen, as well as Hemoglobin and (Methyl-Coenzyme M Reductase) MCR as more challenging protein targets. We look forward to demonstrating the efficacy of our devices for both static and time-resolved structure determination.

Cracking the carbon code: the X-Ray structure of Carbon-based Quantum Dots

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Carbon-based quantum dots are particles made of single or multiple atomic layers with sizes being less than 10 nm.[1] They exhibit remarkable properties such as tunable photoluminescence and bandgap,[2] good water solubility, low toxicity, and bio-compatibility.[3] Carbon-based quantum dots have been explored for applications in the fields of drug delivery,[4] catalysis,[5] photovoltaic devices,[6] and supercapacitors,[7] among others. Crystallography has been pivotal in the development of next-generation materials by enabling the understanding of the atomic structure of materials and its relationship with their properties.[8] Until this study, no X-ray crystal structure of carbon-based quantum dots nor of their doped-derivatives has been published, as many of the preparations in literature have not yielded single crystal quality products. In this article, we demonstrate for the first time the crystal structure of carbon-based quantum dots and their nitrogen doped and sulfur doped derivatives. Beyond establishing these X-ray structures, our work highlights a critical need for advancing crystallographic methods at the interface of small-molecule and macromolecular research, which we call mesomolecular crystallography, and motivates the development of more sophisticated computational tools specifically tailored to non-protein macromolecules.

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Protein Dynamics and Kinetic Studies - Room Temperature and Time-Resolved Serial Crystallography

Wuxian Shi, Alexei Soares, Kevin Rollet, Babak Andi, Edwin O. Lazo, Dale Kreitler, Stuart F. Myers, Jun Aishima, Venkateswaran Shekar, Robert Schaffer, Sean McSweeney, Martin R. Fuchs
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Room-temperature (RT) macromolecular X-ray crystallography (MX) enables the observation of alternative protein conformations under physiologically relevant conditions. By varying the temperature during RT crystallography, functionally important states can be revealed, as demonstrated in multi-temperature structures of the SARS-CoV-2 main protease [Ebrahim 2022]. RT crystallography is also well-suited for ligand-binding studies, where transient allosteric sites are often eliminated by cryocooling of the crystals and can be captured only at RT. Accessing structural snapshots of intermediates in catalytic transitions is essential for fully understanding enzyme function. To expand this capability, we are implementing chemical reaction triggering to probe the widest possible range of systems using droplet delivery [Mehrabi 2019]. The Frontier Macromolecular Crystallography (FMX) beamline (17-ID-2) at NSLS-II [Schneider 2021], with its ultrabright and tunable microfocus beam, is ideally suited for RT and time-resolved measurements using multi-crystal and serial crystallography approaches. We're now accepting first commissioning user proposals for time-resolved serial crystallography.

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Using phase diagrams with microseeding to prepare crystal samples for routine and advanced data-collection techniques

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Serial data collection and microED techniques typically require “slurries” of tiny, well-ordered crystals [1]. Neutron diffraction requires very large single crystals. Making samples for these techniques is often a complex process requiring many rounds of optimization. To guide them in this task, protein crystallizers often keep a notional phase diagram in mind, which has four zones: an undersaturated zone where protein always remains in solution, a metastable zone where crystals will grow when seeds are added, a crystal nucleation zone where crystals appear spontaneously, and a protein precipitation zone. However, the shape of real-life phase diagrams can vary, making the interpretation of experimental results difficult. It is therefore very helpful to determine the phase diagrams of individual target proteins experimentally. Douglas Instruments, in collaboration with the University of Southampton, has introduced a simple and rapid method of generating custom phase diagrams using just 15 – 60 µL of protein [Fig. 1]. A simple approach uses the microbatch-under-oil method to avoid concentrating the sample drop (as would occur in a vapor diffusion setup), and by carrying out the same procedure with and without a seedstock, the metastable zone can be identified [2]. Moreover, advanced methods often require relatively large sample volumes, and microbatch can easily be scaled up to 50 µL or larger “batches” using robotics. A new variation of the method eliminates the need for oil by using a sitting drop setup, where solutions are dispensed to the reservoirs that exactly balance the concentrations of the drops. We present case studies where phase diagrams were used to increase control and crystal quality for routine and advanced data collection.

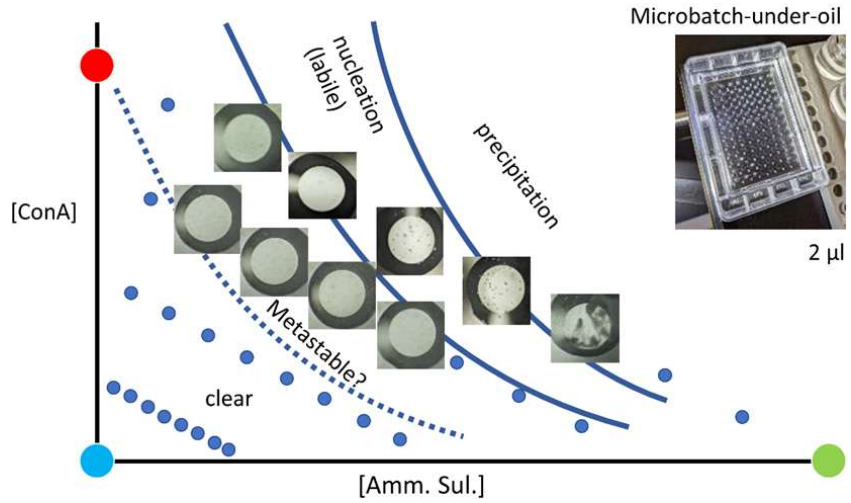

Figure 1. The rapid determination of a protein's phase diagram using a microbatch-under-oil format. Blue circles indicate the conditions that were tested. Images of the wells are shown in conditions of interest. All points on the accessible phase diagram can be reached by mixing the three ingredients shown: protein stock (red circle), precipitant or cocktail stock (green circle) and a diluent, normally water (cyan circle). To find the border of the metastable zone (dotted line) the experiment was repeated with the addition of a seed-stock (results not shown).

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Protein Dynamics and Kinetic Studies - Room Temperature and Time-Resolved Serial Crystallography

Wuxian Shi, Alexei Soares, Kevin Rollet, Babak Andi, Edwin O. Lazo, Dale Kreitler, Stuart F. Myers, Jun Aishima, Venkateswaran Shekar, Robert Schaffer, Sean McSweeney, Martin R. Fuchs
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Room-temperature (RT) macromolecular X-ray crystallography (MX) enables the observation of alternative protein conformations under physiologically relevant conditions. By varying the temperature during RT crystallography, functionally important states can be revealed, as demonstrated in multi-temperature structures of the SARS-CoV-2 main protease [Ebrahim 2022]. RT crystallography is also well-suited for ligand-binding studies, where transient allosteric sites are often eliminated by cryocooling of the crystals and can be captured only at RT. Accessing structural snapshots of intermediates in catalytic transitions is essential for fully understanding enzyme function. To expand this capability, we are implementing chemical reaction triggering to probe the widest possible range of systems using droplet delivery [Mehrabi 2019]. The Frontier Macromolecular Crystallography (FMX) beamline (17-ID-2) at NSLS-II [Schneider 2021], with its ultrabright and tunable microfocus beam, is ideally suited for RT and time-resolved measurements using multi-crystal and serial crystallography approaches. We're now accepting first commissioning user proposals for time-resolved serial crystallography.

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Validating Metal Identities in 70 Metalloprotein Models with Particle-Induced X-ray Emission and X-ray Fluorescence Spectroscopy

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Metals play essential structural and catalytic roles in biological mechanisms, yet many are modeled incorrectly. The scientific community utilizes these models often without knowledge of the potential errors. Using Particle Induced X-ray Emission (PIXE) and X-ray Fluorescence X-ray Spectroscopy (XRFS) to identify the metals present unambiguously, we examined the original protein samples used to submit 70 models to the Protein Data Bank (PDB), 28 from the North-East Structural Genomics consortium, and 42 from the Seattle Structural Genomics Center for Infectious Diseases. The results were arranged into distinct classes based on the PIXE and XRFS measurements. The original model (OM) and new data were inconsistent in 39 (56%); extra metals not in the OM were detected in 30 (43%); and the OM metals and data agreed in one. In many of the 70 samples, problematic metal assignments can be identified from the difference electron density in the original structural model. The PIXE and XRFS results were in good agreement, confirming the potential for using high-throughput XRFS more routinely to disambiguate metalloprotein identities. Our overall findings are a cause for concern, since PDB models are widely used by researchers unaware of the potential misidentification of metals. They are particularly worrying where machine learning is used to create artificial intelligence models to predict metal position, but more critically metal identity. On the positive side, the results demonstrate that metal identity can be rapidly determined, even from samples that have been prepared years before the measurement.

The Exploration of Macromolecular Electron Transport Mechanisms using Signatures from X-ray Induced Radiation Chemistry

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X-ray-induced radiation chemistry perturbs protein structures during crystallographic data collection, despite cryocooling to 100 K. However, at this temperature, free radicals produced through radiolysis are mostly immobilized, but mobile electrons and holes remain active. Low individual X-ray doses come close to mimicking the electron environment in vivo. We use this to our advantage, exploring these signals and introducing a quantitative tool for mapping putative electron flow in macromolecules. Examples are given from a range of proteins and complexes with known electron transport pathways, and from structural proteins as controls. The technique shows considerable promise in visualizing electron pathways and other mechanistic information at the atomic scale, introducing a new tool to the field.

Instrumentation and Methods for Efficient Time-Resolved X-Ray Crystallography of Biomolecular Systems with Sub-10 Ms Time Resolution

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Time-resolved X-ray crystallography has great promise to illuminate structure-function relations and key steps of enzymatic reactions with atomic resolution. The dominant methods for chemically initiated reactions require complex instrumentation at the X-ray beamline, significant effort to operate and maintain this instrumentation, and enormous numbers (~10^5-10^9) of crystals per time point.

We describe instrumentation and methods that enable high-throughput time-resolved study of biomolecular systems using standard crystallography sample supports and mail-in X-ray data collection at standard high-throughput cryocrystallography synchrotron beamlines.

We have designed and constructed an automated instrument that allows rapid reaction initiation by mixing of crystals and substrate/ligand solution, rapid capture of structural states via thermal quenching with no pre-cooling perturbations, and that yields time resolutions in the single millisecond range, comparable to the best achieved by any non-photo-initiated method in both crystallography and cryo-electron microscopy. Our approach to reaction initiation has advantages of simplicity, robustness, low cost, adaptability to diverse ligand solutions and small minimum volume requirements, making it well suited to routine laboratory use and to high-throughput screening.

We have also designed a gravity-based instrument that can be constructed by undergraduates using off-the-shelf and 3-D printed parts costing ~$600 and that yields time resolutions of ~10 ms. These two instruments promise to democratize access to time-resolved study of biomolecular systems.

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Quantitative Cellular Tomography, the Future Bio-Imaging Beamline at NSLS-II – Enable Whole-Cell Analysis

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Cryo- soft X-ray tomography (cryo-SXT) is a high resolution three-dimensional (3D) imaging technique that harnesses the power of soft X-ray energies to visualize structures in whole, native-state cells without sectioning or labelling before imaging. The technique is applicable to various cell types, delivering high-throughput, quantitative, and high (tens of nanometers) spatial resolution anatomy of cells. At NSLS-II, BNL we will design and build the Quantitative Cellular Tomography beamline (QCT) for the cryo-SXT technique, as part of a suite of NSLS-II instruments dedicated to biological imaging. The QCT instrument will provide automated sample exchange and data collection, including preliminary data analysis to ensure the collection of statistically significant numbers of cells.

Poster Abstracts

Material Science Posters

Temperature-Dependent Antiferromagnetic Fluctuations in Sr_2IrO_4 Probed by X-ray Photon Correlation Spectroscopy

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Abstract

The layered iridate Sr_2IrO_4 has emerged as a model system to explore the interplay of spin-orbit interaction (SOI), electron correlations, and structural distortions, with its antiferromagnetic (AFM) order below $T_N \approx 240$ K arising from a $J_{eff} = 1/2$ state that produces strong magnetic anisotropy and cuprate-like physics [1, 2]. Previous resonant x-ray and neutron diffraction studies have identified second-order AFM transitions accompanied by diffuse magnetic scattering, highlighting the presence of critical fluctuations near T_N and underscoring the need to understand their temperature-dependent evolution for deeper insights into spin correlations in 5d oxides [3, 4]. X-ray Photon Correlation Spectroscopy (XPCS) offers a powerful approach to probe slow dynamics and critical fluctuations, as it directly captures temporal correlations of speckle patterns to reveal microscopic relaxation processes, enabling the understanding of dynamic heterogeneity, critical slowing down, and the nature of ordering across structural, magnetic, and electronic phase transitions [5, 6].

In the presentation, I will show our recent experiments where we employ resonant XPCS at the Ir L_3-edge to probe both static and dynamic behavior of the structurally forbidden but magnetically allowed Bragg peak positions, which provide direct sensitivity to the AFM order parameter. Speckle patterns recorded at the AFM Bragg peak positions provide direct access to temporal correlation functions and relaxation dynamics near the AFM transition. By analyzing the temperature- and Q-dependence of the speckle dynamics, we aim to uncover the dynamics of the AFM domain fluctuations and signatures of critical slowing down as T_N is approached. Furthermore, comparison of static magnetic Bragg intensities and correlation lengths with the spatio-temporal fluctuations enables us to correlate magnetic morphology with temporal evolution near T_N. These results will advance our understanding of SOI-driven magnetism in Sr_2IrO_4 and shed light on broader questions of quantum spin correlations and complex dynamical behavior in strongly correlated 5d systems [2, 7].

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Development of a Next-Generation In-Situ Annealing Platform for Synchrotron-Based Thin-Films Materials Characterization

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In collaboration with NSLS-II, IBM is developing a fully-integrated next-generation in-situ annealing system to accelerate materials discovery and process optimization. This automated, remote-access platform will enable high-throughput structural characterization during annealing, reducing experimental timelines from several months to just a few days. The system will support simultaneous measurements of 2D X-ray diffraction (XRD), sheet resistance, and surface roughness, allowing real-time monitoring of texture evolution during annealing cycles reaching up to 1000°C.

IBM has been a pioneer in synchrotron-based accelerated analysis for over 30 years, beginning with a manually loaded in-situ chamber at the original NSLS and later transitioning to an automated, remote-access end station at the Brockhouse sector of the Canadian Light Source. That system has been available to general users and in continuous operation for more than eight years. Across these platforms, IBM has supported the annealing of about 1000 samples annually, achieving throughput rates of 2–4 anneals per hour.

Key innovations in the new system include an enlarged output window for area detection that enables the measurement of texture evolution, and the replacement of toxic, costly beryllium X-ray windows with a clear thermoplastic. The selected plastic offers sufficient X-ray transparency, vacuum resilience, and optical clarity, enabling simultaneous optical and X-ray access through chamber ports at a fraction of the cost, eliminating the safety concerns linked to the presence of beryllium. Precise temperature calibration using eutectic films (e.g., Indium, Tin, Aluminum, Gold, Silver) ensures accurate thermal control across a broad range. Automated sample loading and unloading have also been successfully demonstrated, using a compact 4-axis laboratory robot.

We have successfully demonstrated the new system at the ISR (4-ID) beamline using standalone signal I/O and software. The next phase involves integrating the system into NSLS-II's infrastructure to enable AI-driven, machine-controlled experimentation. This collaboration between IBM and Brookhaven National Laboratory will establish a robust platform for rapid materials development, device prototyping, and automated processing and analysis, as well as workforce development in microelectronics and advanced materials research.

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Resolving Solvation Structure of Aqueous $ZnCl_2$ Electrolytes via Molecular Dynamics and X-ray Total Scattering

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Aqueous $ZnCl_2$ solutions are promising electrolytes for zinc-ion batteries, particularly in the water-in-salt (WIS) regime where electrochemical stability is enhanced. In this study, we investigate the structural and transport properties of $ZnCl_2$ solutions across a wide concentration range using machine learning (ML)-assisted molecular dynamics (MD) and X-ray total scattering.

By developing a neural network potential (NNP) model, we perform MD simulations with *ab initio* accuracy but at much larger lengths and longer timescales. We obtain a complete solvation structure of Zn^{2+} and quantify the population of different motifs of the first solvation shell. The structural predictions are validated by X-ray total scattering experiments. Agreement between simulated and experimental S(q), as shown in Figure (b), confirms accurate capture of solvation-shell evolution from hydrated Zn^{2+} to chlorinated clusters.

Transport properties, including conductivity and Zn^{2+} transference number, are derived from MD trajectories (Figure (c) and (d)). We propose a three-stage transport mechanism, transitioning from independent ion diffusion to correlated ion cluster migration, and finally to Zn^{2+} conduction through polymeric Zn-Cl networks at high concentration.

Our study provides atomic scale insights into the structure and transport properties of the $ZnCl_2$ electrolyte and can aid the optimization and development of WIS electrolytes.

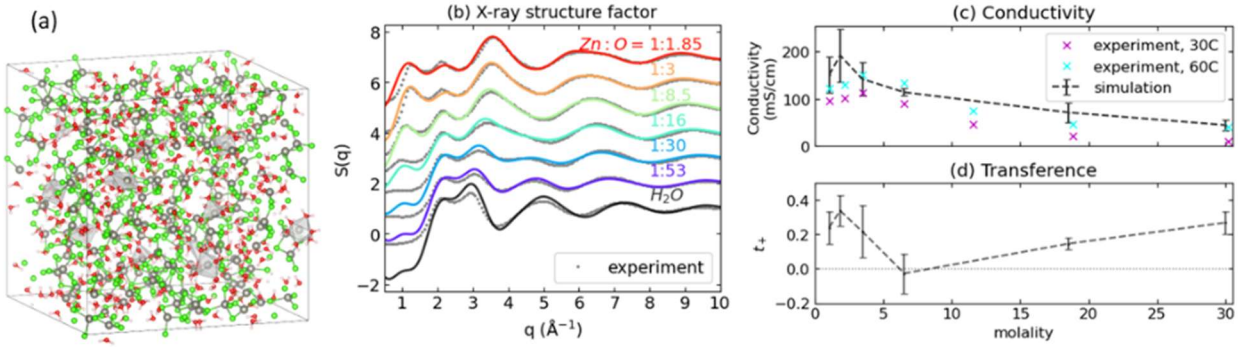

Figure: (a) An MD snapshot. (b) Simulated and experimentally measured structure factors. (c,d) Transport properties, including (c) conductivity and (d) transference number.

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CAPS: A modular crystal analyzer system for x-ray powder diffraction

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Abstract We describe a novel arrangement of a perfect crystal and linear position-sensitive detector designed to maximize the intensity and resolution in a powder diffraction experiment. The basic concept has been described in [2], using an area detector for readout. They used multiple crystal analyzers to increase the throughput of the system, with the output of the 13 analyzer crystals detected by the pixelated detector. We chose to use one-dimensional detectors, with the position sensitivity transverse to the beam, and discrete crystals. Together with a miniature goniometer, the combination forms a compact module, several of which could be placed at will on a goniometer 2-Theta circle. This arrangement allows the minimization of the angular scan range necessary to cover a large 2-Theta range, thus also minimizing data collection time.

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In-Situ Synchrotron X-ray Diffraction Study of Thermochemical Materials for Long-Term Electrothermal Energy Storage Application

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Thermochemical materials (TCMs) which store/release energy by utilizing endothermic/exothermic thermochemical reactions are promising candidates for long-term electrothermal energy storage applications because of their high energy density and minimal energy losses. Thermochemical reactions and the associated structural changes like phase transition, crystallite size evolution, etc. occurring at elevated temperatures under specific environments cannot readily be accessed by post-mortem characterization techniques. However, knowledge of such changes in materials under operating conditions is crucial for the development and deployment of robust, safe and efficient energy storage technologies that rely on thermochemical reactions. X-ray diffraction (XRD) is a versatile non-destructive technique to probe the structure of materials for various applications. Specifically, *in-situ* XRD can be used to monitor material interaction and structural changes under operating conditions like high temperature, gas flow, pressure, etc. to study dynamic processes in real-time and optimize materials properties for specific applications.

$CaO/CaCO_3$ TCM system is a cost effective and sustainable material system with high energy density that has great potential as an electrothermal energy storage material, especially for grid-scale applications. However, the degradation in conversion efficiency with increasing thermal cycles (charge/discharge) presents a major challenge in the practical application of this system. In this study, we use high energy *in-situ* synchrotron XRD at 28-ID-2 XPD beamline at National Synchrotron Light Source II (NSLS-II) to study the phase conversion and structural changes in $CaO/CaCO_3$ system during thermochemical reactions at elevated temperatures and under specific gaseous environments. The use of high-flux synchrotron source provides high quality data with superior signal-to-noise ratio along with faster acquisition times enabling rapid measurement to capture dynamic processes and/or transient phases in real-time.

By analyzing the evolving XRD patterns during thermochemical reactions at high temperatures and special environments – carbonation at 650 °C with CO_2 gas flow and calcination at 850 °C with N_2 gas flow – we compare the conversion rates between micro and nano particles as a function of thermal cycling revealing the effect of particle size on the conversion efficiency. Further, we study the effects of dopant on the conversion efficiency and associated structural changes as a function of number of thermal cycles. We calculate crystallite size and discuss the crystalline quality and structural evolution during thermochemical conversion. Finally, we compare our results with those from thermogravimetric analysis (TGA), which is extensively used in such research areas, and discuss the implications of our findings. Our study provides direct mechanistic insights into the structural evolution and degradation of conversion efficiency with increasing number of thermal cycles in the $CaO/CaCO_3$ TCM system. Such knowledge adds to the comprehensive understanding of structure-property-performance relationship in the TCM system which is necessary for its successful implementation in electrothermal energy storage technologies.

Submicron strain mapping of crystals grown in glass at 5-ID, NSLS-II

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Glass-ceramics are engineered composites fabricated by heating and partially crystallizing glasses for properties tailored between the two or more phases [1]. Laser-heating confines this crystallization to microscale single crystals to fabricate optically active architectures in otherwise passive glass media. By either heating method, property mismatches between the glass and crystal lead to residual strains which can alter local properties, lead to lattice curvature [2], or induce material cracking. Most measurements and models focus on the bulk and do not account for the local heterogeneities of microstrains within individual single crystals [3]. Here, we develop methods for mapping these microstrains at the 5-ID beamline at the National Synchrotron Light Source II via scanning X-ray diffraction measurements with a submicron focused monochromatic beam. We show microstrain heterogeneities across spherulites grown in congruently crystallized bulk $LaBGeO_5$ glasses, and by mapping through a series of incident X-ray energies to construct three-dimensional reciprocal space maps, we demonstrate comparable microstrains in laser-fabricated single crystals in the same system. This behavior is not unique to one system and significant microstrain heterogeneities develop in other optically active laser-fabricated single crystals like $LiNbO_3$ incongruently crystallized in lithium niobosilicate glasses. Measuring these local microstrains helps to inform the local properties of single crystals grown in glass, while the technique can be expanded to map microstrains in other heterogenous material systems.

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4.

Structural Design of Wadsley-Roth Share Phases: A Pathway to Enhanced Lithiation

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Wadsley-Roth (WR) shear phases are promising candidates for next-generation, fast-charging, and durable Li-ion batteries. WR phases are complex crystallographic frameworks that are comprised of edge- and corner-shared MO_6 octahedra (M = Nb, Ta, etc.) organized in blocks of various sizes, connected either directly through edge-sharing or edge-sharing and metal tetrahedra MO_4 (M = W, Mo, V, etc.) at the block corners. What makes them unique is that the adjacent blocks are offset, creating crystallographic shear planes. This results in a three-dimensional framework that enables fast ionic conduction within the blocks and electronic transport along the shear planes.

This study explores performance-enhancing strategies undertaken for various WR phases, including $TiNb_2O_7$, $Nb_{12}WO_{33}$, and $Nb_{12}MoO_{33}$ through detailed structural and electrochemical analysis. Advanced characterization techniques, including powder XRD with Rietveld refinement, XPS, XANES, EXAFS, TEM, SEM, and others, were used to investigate structure-property relationships in these materials, including post-cycling. The results indicated a significant increase in the 10C capacity (233 mAh/g) of a poorly-crystalline $TiNb_2O_7$ annealed at 800°C compared to a highly crystalline material. This improvement was attributed to the complex local atomic and architectural changes that occur during crystallization. In the $Nb_{12}WO_{33}$ phase, the intentional introduction of oxygen defects led to a 1.4X increase in Li-ion diffusivity and a 27% reduction in cell resistance, significantly improving rate performance. In another study, the same WR material was subjected to the synthesis approach in which the formed $Nb_{12}WO_{33}$ phase was reduced in-situ under $5\%H_2/N_2$ flowing gas, and then re-oxidized in a controlled fashion to control defect content. The reduction-reoxidation synthesis provided the means to restructure the material and change the defect type and concentration, creating the pathways to improved Li-ion diffusivity and enhanced electron conductivity. In the final study, the kinetically limited crystallization of $Nb_{12}MoO_{33}$ enabled the synthesis of non-equilibrium extended defect-rich material, helping to facilitate both electron and ionic transport. Electrochemical cycling of such defect-rich material resulted in remarkable lithiation capacities of 200 and 151 mAh/g at 10C and 20C cycling rates, respectively. These findings highlight the critical role of atomic-scale features, point defects, and microstructure in enhancing electrochemical behavior, offering insights for the rational design of high-performance battery materials.

Kinetically Tuned Topotaxial Intergrowth Stabilizes Cobalt-free $LiNiO_2$ Cathode: Insights from in situ/operando SXRD

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In situ synchrotron X-ray diffraction (SXRD) provides direct, time-resolved insight into reaction pathways during solid-state synthesis, capturing metastable intermediates that are essential for "synthesis-by-design" yet often elude ab initio prediction. Focusing on cobalt-free $LiNiO_2$ (LNO), we combine time/temperature-resolved in situ SXRD with ex situ electron microscopy to map the structural evolution from hydroxide precursors to the product phase.

The in situ SXRD data reveal a quasi-binary pathway that proceeds through a dual-phase intermediate. High-angle annular dark-field STEM confirms a topotaxial intergrowth in which stoichiometric layered $LiNiO_2$ is intrinsically interlocked with a minor rocksalt $Li_xNi_{2-x}O_2$ phase within a shared close-packed oxygen framework. Within a finite temperature–dwell window, we observe a miscibility gap characterized by distinct Li occupancies in the two component phases. This window provides kinetic knobs—sintering temperature and dwell time—that deterministically tune the layered/rocksalt fraction and domain scale, enabling targeted control over electrochemical behavior.

Linking structure to function, operando SXRD during cycling shows that the interlocked architecture suppresses the abrupt c-axis collapse associated with the H2→H3 transition, smoothing high-voltage structural evolution. Complementary transmission X-ray nanotomography on aged electrodes reveals mitigated intergranular cracking relative to single-phase LNO, consistent with strain accommodation at the layered/rocksalt interfaces. Electrochemically, the optimized composite sustains operation to 4.8 V vs. $Li|Li^+$ with ~88% capacity retention after 1,000 cycles at 2C, and exhibits robust performance in 2 Ah pouch cells, underscoring scalability beyond coin-cell testing.

Together, these results establish a synthesis-by-design route that monitors and intercepts metastable pathways to engineer strain-accommodating topotaxial interfaces—stabilizing Ni-rich layered oxides without foreign-element doping.

Our synthesis-by-design workflow and the resulting multimodal, time-stamped datasets (in situ SXRD, HAADF-STEM, X-ray nanotomography) enable physics-informed training for AI to learn pathway kinetics and recommend optimal synthesis conditions, accelerating the discovery of novel materials for energy storage.

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Application of Modulation Enhanced Diffraction to Determine Cation Movement in Zeolites

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Caliandro et al.[11] demonstrated that the locations of gas molecules within zeolites can be identified using diffraction data acquired during pulsed dosing experiments. This technique has been used to measure changes in Palladium cation occupancies within zeolite Chabazite during cyclic exposure to oxidizing and reducing gas pulses.[2] The poster will present step-by-step details of the application of this technique.

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Advancing Materials Research through Combined X-ray Absorption and Scattering Capabilities at QAS

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The Quick X-ray Absorption and Scattering beamline (QAS, 7-BM) at the National Synchrotron Light Source II (NSLS-II) provides a versatile platform for in situ and operando characterization of functional materials under realistic working conditions. QAS uniquely integrates high-throughput X-ray absorption spectroscopy (XAS) with complementary X-ray diffraction (XRD) and diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). These combined approaches yield numerous advantages including comprehensive analysis, real-time monitoring, and the maximization of data yield during valuable beamtime. Notably, their integration often reveals unexpected material properties and bridges gaps across varied research areas.

The combination of XRD and XAS provides an all-encompassing perspective on material structure and properties. While XRD probes phase identification, crystal structure, and microstructural features, XAS reveals electronic configurations, oxidation states, and local coordination environments. Together, these insights enable a more complete understanding of structural–electronic correlations.

Similarly, pairing XAS with DRIFTS offers unique advantages for catalysis research. XAS elucidates details of active site structure, while DRIFTS captures surface interactions and molecular dynamics under reaction conditions. The complementary information obtained allows for a comprehensive view of catalytic transformations.

By leveraging these integrated techniques at QAS (7-BM), we advance the understanding of functional material properties and establish a foundation for future innovations across energy, environmental, and chemical sciences.

On-the-fly SAXS/WAXS analysis for Soft Materials Processing

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In-situ characterization of polymers under processing conditions is critical for developing materials that can withstand extreme conditions; however, it poses significant challenges due to the dynamic and often unpredictable nature of these environments. The characterization of these processes, along with the corresponding on-the-fly analysis and visualization, are crucial for understanding material dynamics and optimizing the efficiency of photon usage in synchrotrons. This talk will present the transformative capabilities of in-situ SAXS/WAXS experiments at the CMS beamline of NSLS-II in studying soft materials, such as polymers, under various processing conditions, including heating, stretching, pressing, poling, and exposure to humidity, even extreme conditions like high pressure. We will present case studies of in-situ SAXS/WAXS experiments conducted on polymers, which reveal important insights into the materials' structural dynamics and stability. Additionally, the talk will cover advanced data analysis methods enabled by artificial intelligence and machine learning, which enhance the interpretation of complex data sets and contribute to a more accurate and efficient analysis of structural changes. These studies are expected to contribute significantly to the development of next-generation soft, reconfigurable materials designed for applications in challenging conditions.