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Princeton-Nature Conference

3rd Frontiers in Electron Microscopy for Physical and Life Sciences

October 16-18, 2024

Princeton University, New Jersey

Program and Abstracts

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3rd Frontiers in Electron Microscopy for Physical and Life Sciences

Princeton University

Maeder Hall
86 Olden Street
Princeton, NJ 08544

October 16-18, 2024

SCIENTIFIC COMMITTEE

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Table of Contents

Welcome	**3**
General Information	**4-9**
Conference Program	**10-12**
Speaker Bios	**13-22**
Speaker Abstracts	**23-43**
Poster Abstracts	**44-82**
Speaker Abstract List, Alphabetical by Last Name	**83**
Poster Presenter List, Board Number + Flash Talk Assignments	**84-85**
Delegate List, Alphabetical by Last Name	**86-88**

Welcome

Dear Colleagues and Friends,

We welcome you to the Nature Conference, "Frontiers in Electron Microscopy for Physical and Life Sciences." This marks the third event in our series, hosted by Princeton University and Nature Research since 2018. This conference presents a wonderful opportunity to foster multidisciplinary collaboration and bring together leading scholars and emerging researchers from the physical and life sciences. We aim to stimulate discussions around innovative technologies and methodologies in electron-based imaging and analysis, illustrating the powerful concept that collaboration can lead to outcomes greater than the sum of their parts - “two plus two equals five.” This event holds immense potential to drive scientific advances and inspire new research avenues.

In partnership with Princeton University and Nature Research, this conference brings together world-leading experts and early-career researchers to explore and advance cutting-edge technologies and methods in electron microscopy (EM). Since its inception in the 1930s, EM has transformed our understanding of the world, enabling us to visualize and manipulate increasingly smaller objects at resolutions down to the single-atom level.

Over the years, the communities in physical and life sciences have significantly advanced EM instrumentation and methodologies. However, these developments have often occurred in isolation, constrained by traditional disciplines and niche applications. The rapid advances and deep impact of EM research in recent years has prompted a reevaluation of our approaches to research and education. We aim to share knowledge and drive innovation by uniting key EM researchers from both physical and life sciences. For instance, materials scientists have utilized cryo-EM to investigate amorphous and crystalline energy materials, while life scientists have benefited from tailored advances in graphene sample preparation. This cross-pollination offers incredible potential for advancing fundamental science and technology.

This year’s conference will delve into recent breakthroughs in electron microscopy instrumentation and methods that have propelled progress in the physical and life sciences. Over the next three days, we look forward to growing our research community through engaging invited talks, flash presentations, featured poster sessions, social events, and panel discussions with Nature editors. Our sessions will cover topics including:

- 2D Materials
- Machine learning and computational approaches
- Methods of development and sample preparation
- In-situ methods – catalysis

We hope your time during this conference at Princeton is fruitful and inspiring, filled with stimulating discussions, opportunities to exchange knowledge, and forging collaborations with fellow attendees. Together, we can explore current frontiers and advance the future of electron microscopy, laying the groundwork for the next generation of research and technology in imaging and analysis.

Conference co-chairs, on behalf of the Scientific Committee:

Nan Yao *(Princeton University*)
Daniel McNally *(Nature Research)*

General Information

Conference Name Badges
Please wear your name badge at all times for the entire duration of the conference. If you misplace yours, please go to the registration desk.

Scientific Session Protocol
Photography, audio or video recording by audience members of the scientific sessions is not permitted.

Our conferences have an opt-out social media policy. If a speaker or presenter opts out, requesting to keep their presentation closed and in confidence to the conference attendees, honor their request and do not post or share information from their presentation.

Also note that abstracts may not be re-printed, and poster presentations are also considered personal communications. Information in these may not be publicly shared without prior permission of the presenter.

In cases where speakers and presenters are fine with social media, please use and follow the hashtag:

#FEMPL3

Remember to turn off cell phones or set to vibrate or silent during the general sessions.

Filming
There will be a film crew from Nature Conferences taking footage throughout the duration of the program. If you do not wish to be recorded or photographed, please see Alexandra Glasner at the registration desk.

Poster Session
The poster session will be taking place the evening of **Thursday, October 17th from 5:30 – 7:00 p.m. at the Friend Center Convocation Room**.

Board number assignments appear in the Poster Presenter List section of this book.

For poster presenters: we will advise when and where you can put up your poster upon registration check-in. Posters must be taken down at the close of the poster session or else they will be discarded.

All poster presenters will also give a Flash Talk on Wednesday, October 16th. There are 2 sessions listed in the conference program. Flash Talk assignments can also be found in the Poster Presenter List section of this book.

Conference App - Whova

All attendees have access to the conference app called Whova. You should have received an email with instructions for downloading and creating your account.

At the registration desk, there will be QR codes posted, which link to the Whova app. You will be able to check in yourself and then take your name badge. (We can also check you in manually.)

Certificates of Attendance

If you need a Certificate of Attendance – or a Certificate of Poster Presentation, please email alexandra.glasner@us.nature.com after the conference's conclusion.

WiFi

Princeton University provides wireless internet service to campus visitors through either the Visitor Wireless service or Eduroam. Visitors who are in the Eduroam community should connect to Eduroam. All other visitors should use the visitor wireless service.

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DAILY PERMIT IS REQUIRED TO PARK ON CAMPUS

To obtain permit, register vehicle that will be parked on campus **prior to arrival**.

There is **no fee** for daily permits.

HOW TO REGISTER
Scan or click QR code to complete registration form. **Include license plate information for vehicle that will be parked on campus.**

WHERE TO PARK

Once vehicle is registered, visitors may park in **Stadium Drive Garage** all day.

Additional **highlighted parking areas** are available **Monday through Friday after 4 pm and on weekends**.

For overnight parking, contact The Service Point.
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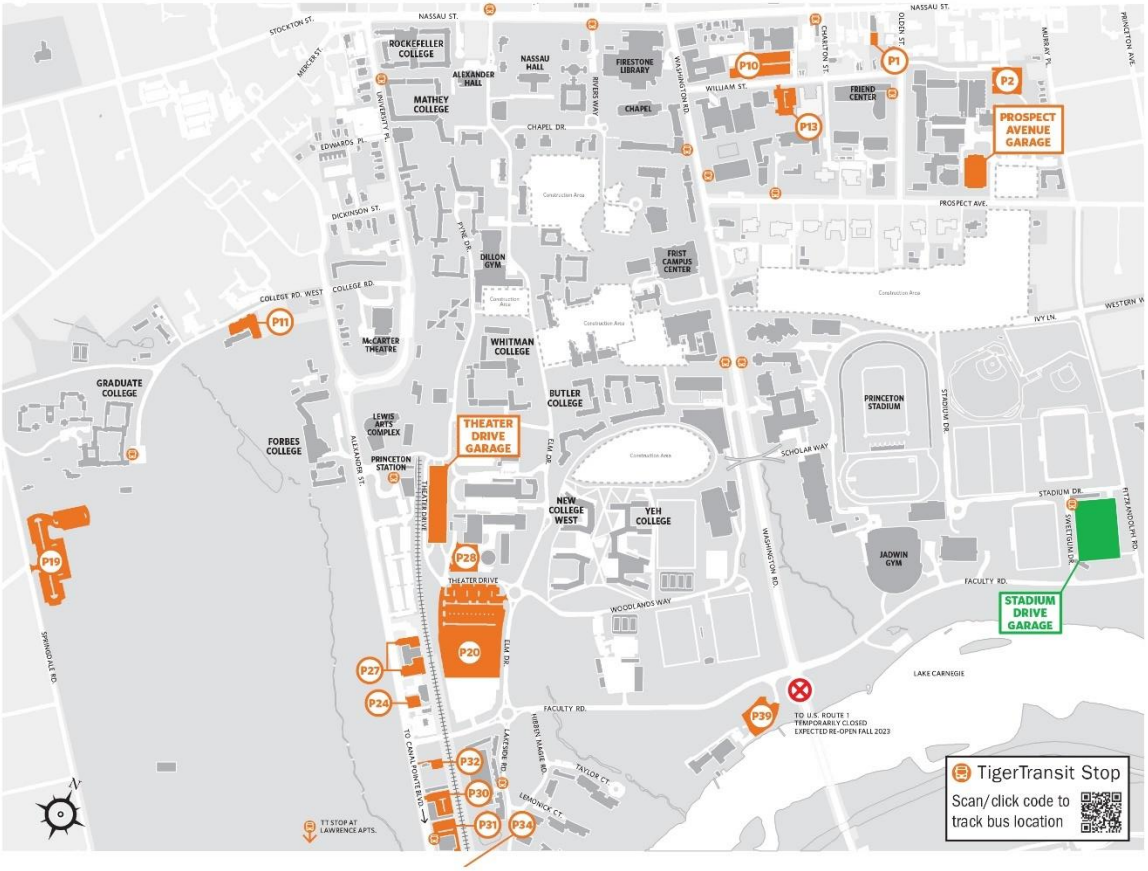

UPDATED: SEPTEMBER 2023

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CONFERENCE PROGRAM

DAY 1: Wednesday, October 16, 2024

8:00 – 8:50 a.m.	Registration check-in, and Breakfast
8:50 – 9:00 a.m.	**Welcome remarks** **Craig B. Arnold** (Princeton University, USA)

SESSION I: 2D Materials

Moderator: Nan Yao (Princeton University, USA) & Daniel McNally (*Nature Materials*, USA)

9:00 – 10:00 a.m.	**KEYNOTE** **Kostya Novoselov** (National University of Singapore, Singapore)
10:00 – 10:30 a.m.	*Deep sub-angstrom resolution without an aberration corrector* **Pinshane Huang** (The Grainger College of Engineering, USA)
10:30 – 11:00 a.m.	**BREAK**
11:00 – 11:30 a.m.	*Four-dimensional scanning transmission electron microscopy for strain-engineered nanomaterials* **Yimo Han** (Rice University, USA)
11:30 – 12:00 p.m.	*Probing phonon and magnon dynamics with fast electrons* **Yimei Zhu** (Brookhaven National Laboratory, USA)
12:00 – 12:30 p.m.	**Flash Talks – Session A**
12:30 – 2:00 p.m.	**Lunch**

SESSION II: Machine learning and computational approaches

Moderator: Florian Ullrich (*Nature*, Germany)

2:00 – 2:30 p.m.	*Cryo-EM for molecular biology in the cell* **Bronwyn Lucas** (University of California, Berkeley, USA)
2:30 – 3:00 p.m.	*Introducing the Iliad – the first fully integrated advanced analytical (S)TEM* **Paolo Longo** (Thermo Fisher, USA)
3:00 – 3:30 p.m.	**BREAK**
3:30 – 4:00 p.m.	*MemBrain v2: an end-to-end tool for the analysis of membranes in cryo-electron tomography* **Tingying Peng** (Helmholtz AI, Germany)
4:00 – 4:45 p.m.	**Flash Talks – Session B**
5:00 – 6:30 p.m.	**Welcome Reception**
6:30 p.m.	**Informal social gathering at Ivy Inn Princeton** (248 Nassau St. Princeton)

DAY 2: Thursday, October 17, 2024

8:00 – 9:00 a.m. Registration check-in, and Breakfast

SESSION III: Methods development and sample preparation

Moderator: Arunima Singh (*Nature Methods*, USA)

9:00 – 10:00 a.m. **KEYNOTE**
Tools and technologies for cryo electron tomography
Bridget Carragher (Chan Zuckerberg Imaging Institute, USA)

10:00 – 10:30 a.m. *Innovation of cryo-EM specimen preparation by graphene and other nanomaterials*
Hong-Wei Wang (Tsinghua University, China)

10:30 – 11:00 a.m. Break

11:00 – 11:30 a.m. *Using microfluidics to advance sample preparation for single-particle cryo-EM*
Rouslan Efremov (VIB, Belgium)

11:30 – 12:00 p.m. *Natural product discovery enabled by microED*
Hosea Nelson (Caltech, USA)

12:00 – 1:30 p.m. Lunch

SESSION III cont'd: Methods development and sample preparation

Moderator: Arunima Singh (*Nature Methods*, USA)

1:30 – 2:00 p.m. *In situ structural biology: from cells to molecules*
Peijun Zhang (University of Oxford, UK)

2:00 – 2:30 p.m. *Beyond ribosomes: in situ structural biology of challenging targets in C. reinhardtii*
Jessica Heebner (Thermo Fisher, USA)

SESSION IV: In situ methods – catalysis

Moderator: Benjamin Martindale (*Nature Catalysis*, USA)

2:30 – 3:30 p.m. **KEYNOTE**
Visualizing single atom dynamics in catalysis
Pratibha Gai (University of York, UK)

3:30 – 4:00 p.m. Break

4:00 – 4:30 p.m. *Operando electrochemical liquid-cell STEM at dynamic energy materials interfaces*
Yao Yang (University of California, Berkeley, USA)

4:30 – 5:00 p.m. *A catalysts life and its circumstances*
Beatriz Roldan Cuenya (Fritz Haber Institute of the Max Planck Society, Germany)

5:00 – 5:30 p.m. *Aberration-corrected electron microscopy of atomically dispersed catalysts: challenges and opportunities*
Jingyue Liu (Arizona State University, USA)

5:30 – 7:00 p.m. **Poster Session & Reception**

DAY 3: Friday, October 18, 2024

8:00 – 9:00 a.m. Registration check-in, and Breakfast

SESSION V: In situ methods

Moderator: Stacey Chin (*Nature Materials*, USA)

9:00 – 9:30 a.m. *Probing the structural dynamics of active molecular materials by a combination of cryo and liquid EM*
Joe Patterson (University of California, Irvine, USA)

9:30 – 10:00 a.m. *Using liquid EM to probe biomineralization in collagen and at biomaterial interfaces*
Kathryn Grandfield (McMaster University, Canada)

10:00 – 10:30 a.m. *Hydrogel structure elucidation and property prediction by liquid phase TEM*
Nathan Gianneschi (Northwestern University, USA)

10:30 – 10:40 a.m. **Closing Remarks**
Richard Register (Princeton University, USA)

10:40 – 12:00 p.m. Meet the Editors and Lunch

Speaker Bios (in ALPHA Order by Last Name)

Bridget Carragher

Bridget Carragher received her Ph.D. in Biophysics from the University of Chicago in 1987. She worked in a variety of positions, both in industry and academia before moving to the New York Structural Biology Center in 2015 to lead the Simons Electron Microscopy Center (SEMC), together with Clint Potter. While at SEMC, Bridget and Clint directed the National Resource for Automated Molecular Microscopy (NRAMM), the National Center for CryoEM Access and Training (NCCAT), the National Center for In-situ Tomographic Ultramicroscopy (NCITU), and the Simons Machine Learning Center (SMLC). They also founded the company NanoImaging Services in 2007. Bridget moved to her current role as Founding Technical Director of the Chan Zuckerberg Imaging Institute in January 2023.

Beatriz Roldán Cuenya

Prof. Dr. Beatriz Roldán Cuenya is currently the director of the Interface Science Department as well as interims director of Inorganic Chemistry Department at the Fritz Haber Institute in Berlin (Germany). She began her academic career by completing her MSc in Physics in Spain in 1998 and a PhD in Physics in Germany in 2001. Her postdoctoral research took her to the Department of Chemical Engineering at the University of California Santa Barbara (USA). In 2004 she joined the Department of Physics at the University of Central Florida as Assistant Professor becoming a full professor in 2012. In 2013, she moved back to Germany and became a Chair professor of Solid State Physics at the Ruhr-University Bochum. She then joined the FHI in 2017. Prof. Dr. Beatriz Roldan Cuenya is the author of 237 peer-reviewed publications and 6 patents. She serves in the editorial board of the Journal of Catalysis and the Chemical Reviews journal. She is a member of the Academia Europaea as well as of the Germany National Academy of Sciences Leopoldina. Recently she received the Manchot Research Professorship from TU Munich (2023), the 2022 Paul H. Emmet Award of the North American Catalysis Society, the Röntgen Medal (2022), the Faraday Medal from The Electrochemistry Division of the UK Royal Society of Chemistry (2022), the AVS Fellow Award (2021) and the International Society of Electrochemistry-Elsevier Prize for Experimental Electrochemistry (2021).

Rouslan Efremov

Rouslan Efremov studied Physics at the Moscow Institute for Physics and Technology (Russia), then did PhD at the institute for structural biology at Forschungszentrum (Research Center) Jülich (Germany) and postdocs at Mitochondrial Biology Unit of MRC (Cambridge, UK) and at Max Planck Institute for Molecular Physiology (Dortmund, Germany). Since 2013, Rouslan is the group leader at VIB (Flemish Institute for Biotechnology) and from 2015 a professor at VUB (Free University Brussels) (Belgium).

Pratibha Gai

Professor Pratibha L. Gai is Fellow of the Royal Society (National Academy of Sciences) and Royal Academy of Engineering, UK. She is at the Department of Chemistry at University of York, UK and has served as a founding Co-Director of the York Nanocentre. She is an Honorary Fellow of Girton College, University of Cambridge, UK. Previously she held senior positions at DuPont Central Research Laboratory, USA and concurrently as adjunct Professor at University of Delaware, USA; and led in-situ electron microscopy (EM) and catalysis group at University of Oxford, after PhD in physics from the Cavendish Laboratory, University of Cambridge. With Prof. E. D. Boyes, she co-invented the world's first in-situ atomic resolution environmental transmission electron microscope (atomic resolution-ETEM) to visualize and analyse dynamic chemical processes in reacting gas and temperature environments at the atomic level and in real-time, which is used globally by researchers, electron microscope manufacturers and chemical companies. She has carried out the development and applications of novel analytical in-situ Environmental Scanning TEM (ESTEM) with single atom resolution for visualizing and analysing dynamic single atom catalysis in real time, in collaboration with E. D. Boyes. Her other honours include L'Oreal-UNESCO International Women in Science Award as the 2013 Laureate for the continent of Europe and Gabor prize of the Institute of Physics. She has authored scientific papers, books and patents with practical applications. She is an Honorary Fellow of the Royal Microscopical Society and Fellow of the Royal Society of Chemistry, Institute of Physics, Institute of Materials and Microscopy Society of America. She has been appointed a Dame in the UK National Honours for services to chemical sciences and technology.

Nathan Gianneschi

Nathan C. Gianneschi received his B.Sc(Hons) at the University of Adelaide, Australia in 1999 under Louis Rendina. In 2005 he completed his Ph.D at Northwestern University, with Chad Mirkin. Following a Dow Chemical postdoctoral fellowship at The Scripps Research Institute with Reza Ghadiri, in 2008 he began his independent career at the University of California, San Diego where, until June 2017, he was Teddy Traylor Scholar and Professor of Chemistry & Biochemistry, NanoEngineering and Materials Science & Engineering. In July of 2017, Gianneschi moved his research group to Northwestern University where he is currently Jacob & Rosaline Cohn Professor of Chemistry, Materials Science & Engineering, and Biomedical Engineering. The Gianneschi group takes an interdisciplinary approach to nanomaterials research with a focus on multifunctional materials with interests that include biomedical applications, programmed interactions with biomolecules and cells, and basic research into nanoscale materials design, synthesis and characterization. For this work he has been awarded the NIH Director's New Innovator Award, the NIH Director's Transformative Research Award and the White House's highest honor for young scientists and engineers with a Presidential Early Career Award for Scientists and Engineers. Prof. Gianneschi was awarded a Dreyfus Foundation Fellowship, is a Kavli Fellow of the National Academy of Sciences, a Fellow of the Royal Society of Chemistry, and is an Alfred P. Sloan Foundation Fellow.

Kathryn Grandfield

Kathryn Grandfield is an Associate Professor and the Associate Graduate Chair in the Department of Materials Science & Engineering at McMaster University, Canada. She holds the Canada Research Chair in Microscopy of Biomaterials and Biointerfaces and is the Past-President of the Microscopy Society of Canada. Dr. Grandfield studied Materials Science and Engineering at McMaster University before attaining a Ph.D. in Engineering Sciences at Uppsala University, Sweden then a postdoctoral fellowship at the University of California, San Francisco. She is the recipient of several early career awards including the Early Researcher Award from the Ministry of Science, Research, and Innovation. Her research within the Canadian Centre for Electron Microscopy focuses on the development of multiscale, multidimensional and in situ correlative microscopies including FIB-SEM, electron tomography, atom probe tomography, and liquid phase TEM for advancing the study of biomaterials and biomineralized tissues.

Yimo Han

Dr. Han is an assistant professor of Materials Science and NanoEngineering at Rice University. She earned her B.S. in Physics from Tsinghua University and her Ph.D. in Applied Physics from Cornell University. Following this, she completed postdoctoral training in the Department of Molecular Biology at Princeton University. Her research is dedicated to advancing electron microscopy methods to explore low-dimensional nanomaterials. She is the recipient of the NSF CAREER award, Roy E. Campbell Faculty Development Award, and was listed on the 2022 MIT TR 35 China list.

Jessica Heebner

Jessica is a Scientist III on the Applications Development Team at Thermo Fisher Scientific. She holds a B.Sc. in Biology from the University of Alabama (2010) and a PhD in Biomedical Science and Clinical and Translational Science from Penn State College of Medicine (2023) where she focused on high-throughput image analysis workflows for cryo-electron tomography of developing neurons. Her research interests center around deep learning tools, image segmentation, and high resolution microscopy. She is passionate about sharing her knowledge with others and teaching image segmentation to help drive others' research forward.

Pinshane Huang

Pinshane Y. Huang is an Associate Professor and Racheff Faculty Scholar in the Department of Materials Science and Engineering at the University of Illinois, Urbana-Champaign, where she is also the Associate Director of the Materials Research Laboratory. Pinshane holds a Ph.D. and an M.S. in Applied and Engineering Physics from Cornell University, and B.A in Physics from Carleton College. Her research is focused on transmission electron microscopy and spectroscopy of two-dimensional and nanoelectronic materials. Her awards include a Presidential Early Career Award for Scientists and Engineers (PECASE), a Packard Fellowship, a Sloan Fellowship, as well as Air Force Young Investigator and NSF CAREER awards. Her research has been featured in Nova, National Geographic, BusinessWeek, CBS News, Discover Magazine, and the Guinness Book of World Records.

Jingyue Liu

Professor Jingyue (Jimmy) Liu is now a Professor of Physics and Senior Scholar in the Global Institute of Sustainability at Arizona State University (ASU). He received his B.Sc. (1982) in metal physics from the University of Science and Technology Beijing and his Ph.D. (1990) in condensed matter physics from ASU under the guidance of Professor John M. Cowley. From 1990 to 1994, Dr. Liu was funded by the Shell Development Company to conduct research on heterogeneous catalysts in the Center for Solid State Science at ASU. In 1994, Dr. Liu joined Monsanto Corporate Research in St. Louis, Missouri as a group leader and was appointed Corporate Science Fellow in 2000 and Senior Science Fellow in 2004 for sustained outstanding contributions to Monsanto's businesses. In 2006, Dr. Liu joined the University of Missouri-St. Louis as Professor of Physics and Chemistry and Director of the Center for Molecular Electronics. In 2007, he became the inaugural Director of the Center for Nanoscience at UM-St. Louis. Professor Liu retuned to ASU in 2011 as Professor of Physics and Senior Scholar in the Global Institute of Sustainability. He received the prestigious 2001 Edgar M. Queeny Award, Monsanto's highest recognition for achievement in innovative science and technology. He was elected a Fellow of the Microscopy Society of America in 2012. Professor Liu's current research interest includes single-atom and cluster catalysis, energy generation and storage, and advanced electron microscopy techniques.

Paolo Longo

Paolo Longo is the Director of Product Marketing in the High-End TEM group within the Material Science Business Unit • Developments and strategies around key component technologies • Primary responsibility for the product marketing of the various High-End TEM platforms Prior to joining Thermo Fisher Scientific, Paolo was at Gatan Inc. for over 10 years in various roles supporting initially the GIF (Gatan Imaging Filter) product line and lately all TEM and SEM products for both Gatan and EDAX as Director of Global applications. During his time at Gatan, Paolo was key to promote and successfully establish direct detection solutions for EELS data collection in the analytical TEM market. Paolo has a rich background in analytical (S)TEM which also helps to strengthen the core applications expertise of the entire TEM product portfolio within Thermo Fisher Scientific. In 2008, Paolo obtained his PhD in Physics from the University of Glasgow (UK), where he worked on the chemical and compositional characterization of semiconductor transistors using a combination of EELS STEM based techniques.

Bronwyn Lucas

Bronwyn is an Assistant Professor of Biochemistry, Biophysics and Structural Biology in the Department of Molecular and Cell Biology and the Center for Computational Biology at the University of California Berkeley and a Faculty Biophysicist at the Lawrence Berkeley National Laboratory. The Lucas Lab uses and develops new cryo-EM image analysis methods to leverage the growing databases of structural models to locate and characterize molecular structures inside cells, with the goal to uncover the molecular organization of biochemical pathways. Bronwyn originally heralds from Australia where she did her undergraduate and Honours research in Molecular Biology at the University of Adelaide. She moved to the USA for her PhD in Molecular Biology at the University of Rochester studying the regulation of mRNA localization and turnover by RNA-binding proteins with Lynne Maquat. As a postdoc with Nikolaus Grigorieff at Janelia Research Campus and the University of Massachusetts Medical School she pioneered the application of 2D Template Matching, a new approach to visualize and characterize macromolecular structure directly in the cell using cryo-EM. Now, in her own lab, she is synthesizing these two areas to develop new tools combining cryo-EM, bioinformatics and computer vision techniques to visualize RNA-protein complex assembly directly in cells.

Hosea Nelson

Prof. Nelson earned a B.S. in Chemistry from University of California at Berkeley in 2005 and a Ph.D. from the California Institute of Technology in 2013. After postdoctoral training at University of California at Berkeley, Prof. Nelson joined the UCLA faculty in 2015. In 2021 he joined Caltech's Division of Chemistry and Chemical Engineering. Prof. Nelson's research program is focused on the development of enabling technologies for chemical synthesis. His group focuses on this goal through two primary avenues of research. 1) In their structural chemistry subgroup they develop new electron microscopy techniques that enable the characterization of complex molecules, often unattainable using traditional methods. 2) Their synthetic subgroup focuses on the development of new chemical reactions that will enable the efficient and rapid synthesis of bioactive compounds.

Kostya Novoselov

Prof Sir Konstantin 'Kostya' Novoselov FRS was born in Russia in August 1974. He is best known for isolating graphene at The University of Manchester in 2004, and is an expert in condensed matter physics, mesoscopic physics and nanotechnology. Every year since 2014 Kostya Novoselov is included in the list of the most highly cited researchers in the world. He was awarded the Nobel Prize for Physics in 2010 for his achievements with graphene. Kostya is a director of the Institute of Functional Intelligent Materials and holds a position of a Tan Chin Tuan Centennial Professor at the National University of Singapore. He is also part time Langworthy Professor of Physics and the Royal Society Research Professor at The University of Manchester. He graduated from the Moscow Institute of Physics and Technology, and undertook his PhD studies at the University of Nijmegen in the Netherlands before moving to The University of Manchester in 2001. Later Professor Novoselov joined the National University of Singapore in 2019. Professor Novoselov has published more than 400 peer-reviewed research papers. He was awarded with numerous prizes, including Nicholas Kurti Prize (2007), International Union of Pure and Applied Science Prize (2008), MIT Technology Review young innovator (2008), Europhysics Prize (2008), Bragg Lecture Prize from the Union of Crystallography (2011), the Kohn Award Lecture (2012), Leverhulme Medal from the Royal Society (2013), Onsager medal (2014), Carbon medal (2016), Dalton medal (2016), Otto Warburg Prize (2019), John von Neumann Professor from the John von Neumann Computer Society (2022) among many others. He was knighted in the 2012 New Year Honours.

Joe Patterson

Joe Patterson is an Assistant Professor in the Chemistry Department at the University of California, Irvine (UCI). He obtained his undergraduate and PhD at the University of York and Warwick respectively in the UK. He did postdoctoral research at both the University of California, San Diego and the Eindhoven University of Technology. His main research interest is the development of cryo and liquid electron microscopy to provide a better understanding of molecular self-assembly.

Tingying Peng

Tingying Peng (b. 1984) is a junior research group leader at Helmholtz Munich and conducts research on AI for microscopy image analysis. The mission of her research group is to create new AI methods to help life scientists and pathologists analyze microscopic images more quantitatively and efficiently, allowing them to extract more knowledge. Her group has worked on various microscopy imaging types, including histopathological images for computational pathology, classic brightfield and fluorescence images, and more advanced ones, such as Cryo-ET, 3D light-sheet microscopy and, extended depth-of-field (EDOF) microscope with “Electrically Tunable Lenses”.

Hong-Wei Wang

Dr. Hong-Wei Wang is currently Professor of School of Life Sciences, Tsinghua University. He received his B.S. degree in Biological Sciences & Biotechnology from Tsinghua University in Beijing, China in 1996. He did his thesis work under the supervision of Prof. Sen-Fang Sui, earning a Ph.D. degree from Tsinghua University in 2001. Subsequently, he worked as a Postdoctoral Fellow under the supervision of Prof. Eva Nogales, advancing to Research Scientist in 2006 at Lawrence Berkeley National Laboratory. He joined Yale University as a Tenure-Track Assistant Professor of Molecular Biophysics and Biochemistry in 2009 and returned to his alma mater as a Professor of Life Sciences in December, 2010. He served as dean of School of Life Sciences from 2016-2021. Prof. Wang’s current research interests include methodology development for more efficient and high resolution cryo-EM, the mechanism and regulations of nucleic acid quality control and the coordination mechanisms of cytoskeleton and membrane systems. So far, he has published more than 120 SCI papers in Cell, Science, Nat. Commun., JACS and other journals and made many pioneering achievements in the development and application of cryo-EM methodology. The cryo-EM facility of Tsinghua University which he is responsible for building has been one of the world's largest cryo-EM platforms at the international level, and has produced a number of scientific research achievements with important impact in the past dozen years; his academic and disciplinary contributions to the field of cryo-EM have been widely recognized by international peers, and he was elected as the president of the 2019 3DEM GRC Conference.

Yao Yang

Yao Yang is an assistant professor of chemistry at Cornell University. He received PhD at Cornell University (2021) and the Miller postdoctoral fellowship at UC Berkeley (2021-2024). His group focuses on developing operando electrochemical liquid-cell scanning transmission electron microscopy (EC-STEM) and correlative synchrotron based X-ray methods to probe chemical dynamics of energy materials at solid-liquid interfaces across multiple spatiotemporal scales.

Peijun Zhang

Dr. Peijun Zhang is a Professor of Structural Biology in the Nuffield Department of Medicine at the University of Oxford and the founding director of eBIC (the UK National Electron Bio-imaging Centre) at the Diamond Light Source. She obtained B.S. in Electrical Engineering and M.S. in Solid State Physics from Nanjing University, and Ph.D. in Biophysics and Physiology from University Virginia. She was a post-doctoral fellow and subsequently a staff scientist at the National Cancer Institute, NIH, and then an Assistant Professor and tenured Associate Professor at the University of Pittsburgh School of Medicine. She joined Oxford and Diamond Light Source in 2016. Professor Zhang is a leading expert in the fields cryoEM and cryo-electron tomography of macromolecular complexes and assemblies, especially investigating these in situ in the native cellular context. Her research is aimed at an integrated, atomistic understanding of molecular mechanisms of viral and bacterial infections by developing and applying novel technologies for high-resolution cryoEM and cryoET. Her current research efforts focus on HIV-1 and SARS-CoV-2 infections using multi-scale correlative and integrative structural biology and bacterial chemotaxis signalling pathways using time-resolved cryoEM and cryoET.

Yimei Zhu

Yimei Zhu is a Senior Physicist and Group Leader at Brookhaven National Laboratory (BNL) and an Adjunct Professor at Stony Brook University and Columbia University. He received his PhD from Nagoya University and studied as a Research Associate at the University of Virginia. Joining BNL in 1988 as an Assistant Scientist, he ascended to the position of Tenured Senior Physicist in 2002. His research focuses on advanced electron microscopy, exploring correlations among charge, orbital, spin and lattice and their dynamics. He and his group investigate structure-property relationships, roles of interfaces and defects in strongly correlated quantum systems and energy materials at high spatiotemporal resolution. He is also interested in methodology and instrumentation development, particularly in ultrafast MeV electron diffraction and time-resolved microwave imaging with electrons. Zhu has been honored with numerous awards, including the Vernon Cosslett Medal by the International Federation of Societies for Microscopy at the recent 20th International Microscopy Congress, the Distinguished Scientist Award from the Microscopy Society of America, and the Peter Duncumb Award from the Microanalysis Society. He is the recipient of two R&D 100 Awards, and four Microscopy Today Innovation Awards. He has served on various government panels, committees, and editorial boards, published 700 peer-reviewed scientific journal articles, holds many patents, and delivered over 300 invited talks at international conferences. He is Fellow of American Physical Society, American Association for Advancement of Science, Materials Research Society, Microscopy Society of America, and Microanalysis Society.

Speaker Abstracts (in order of presentation)

KEYNOTE

Kostya Novoselov

National University of Singapore, Singapore.

Talk abstract unavailable at time of print.

DEEP SUB-ANGSTROM RESOLUTION WITHOUT AN ABERRATION CORRECTOR

Pinshane Y. Huang[1], Chia-Hao Lee[1], Kayla Nguyen[1], Yi Jiang[2], Priti Kharel[1], Yue Zhang[1], Arend van der Zande[1]

[1]University of Illinois at Urbana-Champaign, Urbana, Illinois, USA; [2]Argonne National Laboratory, Lemont, Illinois, USA.

Sub-angstrom resolution has long been confined to aberration-corrected electron microscopy, where it is a powerful tool for understanding the structure and chemistry of materials at the atomic scale. Here, we achieve 0.44 angstrom spatial resolution without an aberration corrector in a conventional scanning transmission electron microscope, nearly quadrupling the resolution set by the microscope optics. This high resolution is enabled using a high dynamic range detector for full-field ptychography, combined with reconstruction using mixed-states, which we believe account for effective losses of coherence resulting from the instrumental instabilities. Counterintuitively, we find that, rather than being detrimental, geometric aberrations can benefit electron ptychography because they produce structured beams that are more dose-efficient for ptychography than focused, aberration-free probes. This work indicates new frontiers to advance electron ptychography by expanding the types of electron microscopes and optical conditions that are compatible with sub-angstrom materials characterization. Most importantly, our results demonstrate that expensive aberration correctors are no longer required to achieve atom-by-atom imaging, a significant step towards democratizing access to high-end electron microscopy.

FOUR-DIMENSIONAL SCANNING TRANSMISSION ELECTRON MICROSCOPY FOR STRAIN-ENGINEERED NANOMATERIALS

Yimo Han, Chuqiao Shi

Department of Materials Science and NanoEngineering, Rice University, Houston, Texas, USA.

Strain plays a critical role in nanomaterials as it influences a wide range of physical and chemical properties. Recent advances in four-dimensional scanning transmission electron microscopy (4D-STEM) have enabled unprecedented resolution, precision, and capabilities in visualizing strain at the nanoscale, particularly in materials with complex geometries or confined to lower dimensions. In this presentation, we introduce how 4D-STEM is employed to study strain in both two-dimensional (2D) van der Waals ferroelectrics (FEs) and catalytic core-shell nanoparticles, offering insights into strain-engineering strategies for enhanced material performance.

We will first discuss our work on 2D SnSe, a van der Waals FE material, where we mapped domain-dependent strain and stacking orders at nanometer resolution [1]. Our 4D-STEM analysis revealed up to 4% lattice strain across domain walls and identified unique FE to antiferroelectric (AFE) transitions. These findings provide a deeper understanding of how strain affects domain wall properties, enabling potential applications in nanoelectronics, including memory devices and sensors. In addition, we will cover the study of strain preservation in catalytic core-shell nanoparticles [2]. In this work, we discovered that sharp-edged nanoparticle morphologies can stabilize surface strain more effectively than round-edged counterparts, leading to significantly enhanced catalytic activity, particularly in Suzuki-type homocoupling reactions. This work demonstrates how morphology control can serve as a novel approach to preserving strain and improving the performance of nanoscale catalysts. Finally, we will explore the integration of machine learning with 4D-STEM to analyze and predict strain behavior in various nanomaterials [3]. By coupling machine learning with 4D-STEM data, we aim to accelerate the study of strain dynamics, opening new possibilities for optimizing materials and engineering their properties.

[1] C. Shi, et al. *Nature Communications*, 14, 7168 (2023)

[2] C. Shi, et al. *Science Advances*, 10, eadp3788 (2024)

[3] C. Shi, et al. *npj Computational Materials*, 8, 114 (2022)

PROBING PHONON AND MAGNON DYNAMICS WITH FAST ELECTRONS

Yimei Zhu

Department of Condensed Matter Physics and Materials, Brookhaven National Laboratory, Upton, New York, USA.

Electron microscopy plays an increasing pivotal role in physical science research, particularly in understanding charge, spin and lattice correlations. In this presentation, I will demonstrate how ultrafast electron diffraction can be used to probe photoinduced phonon dynamics through quantitative diffuse scattering analysis, tracking the evolution of both optical and acoustic phonon populations in a two-dimensional charge-density-wave (CDW) system, $TiSe_2$. We reveal that one-dimensional CDW-related defects emerge within 1 ps after photoexcitation. This defect growth is not governed by the amplitude of the order parameter but is instead mediated by a non-thermal population of longitudinal optical phonons.

Additionally, I will introduce a novel electron-pulse-based imaging method for directly visualizing magnon dynamics. By integrating microwave into TEM, we create and stabilize topological spin textures in permalloy, featuring vortex-antivortex pairs and various types of domain walls within a magnetic single-layer geometry. We visualize the generation, propagation, reflection, and interference of spin waves under RF-field excitation, demonstrating that spin waves are preferentially generated at topological anti-vortices and the spin wave emission is associated with the oscillatory motion of specific domain walls. This work opens new avenues for spintronic and multiferroic applications, including spin transfer torque oscillators through selective coupling and reconfigurable spin wave interactions.

CRYO-EM FOR MOLECULAR BIOLOGY IN THE CELL

Bronwyn Lucas[1,2], Matthew Giammar[1,2], Shloka Jaisingh[1,2], Kexin Zhang[3], Nikolaus Grigorieff[3]

[1]University of California Berkeley, Berkeley, California, USA; [2]Lawrence Berkeley National Laboratory, Berkeley, California, USA; [3]University of Massachusetts Chan Medical School, Worcester, Massachusetts, USA.

Cryogenic electron tomography (cryo-ET) has revolutionized cell and structural biology by visualizing macromolecular structure in the native cell context. However, >99% of the molecules in the cell are inaccessible by cryo-ET because the relatively low resolution of cellular tomograms limits annotation to unusually large and/or abundant complexes. I will describe how we are using an alternate strategy to complement cryo-ET, high-resolution template matching of 2D cryo-EM images (2DTM), for confident and precise localization of single molecules in cryo-EM images of cells. 2DTM can be used to classify related complexes with single molecule probabilities and generate high-resolution *in situ* reconstructions without model bias. I will additionally describe the development of a new method called molecular *in situ* atomic coordinate scan (MOSAICS) that extends 2DTM to characterize small differences between template and cellular target structures. MOSAICS can identify differences of <10 kDa between populations of 100 molecules, two orders of magnitude fewer than required for high-resolution 3D reconstruction, extending structural analysis to rare complexes. I will demonstrate how we have applied MOSAICS to reveal the order of ribosome biogenesis factor binding during 60S ribosomal subunit assembly in the nucleus. 2DTM and MOSAICS present unconventional ways to characterize the structure of biological molecules that can provide detailed information about rare complexes. The ongoing parallel developments in structure determination and prediction are generating vast databases of structural models of proteins. 2DTM and MOSAICS are single molecule tools for the new 'structureomic' era that can leverage these databases to visualize molecular networks in cells and study the molecular biology of the cell… in the cell.

INTRODUCING THE ILIAD – THE FIRST FULLY INTEGRATED ADVANCED ANALYTICAL (S)TEM

Paolo Longo, Sorin Lazar, Maria Meledina, Jain Noopur, Peter Tiemeijer, Maarten Wirix

Thermo Fisher Scientific, Eindhoven, The Netherlands.

Over the last decades we have witnessed a tremendous development in advanced transmission electron microscopy. To name a few, probe correctors have become more powerful and now in combination with ultra-brightness electron sources 60 – 70pm spatial resolution in STEM can be routinely reached with nearly 1nA probe current, improvements in energy resolution have led to phonon spectroscopy, EDS detector systems have become 6x more efficient and sensitive with the latest generation leading to low-dose data collection and in-situ analytical studies, detectors are now capable to count electrons dramatically reducing the contribution of instrumental noise and that has led to the revolution in cryo-EM.

Very recently with the market introduction of the Iliad (S)TEM microscope platform, Thermo Fisher Scientific has brought, for the very time, full microscope hardware and software integration that now extends and includes the Iliad EELS spectrometer and energy filter and the NanoPulser the Ultrafast Electrostatic Beam Blanker ESBB. Multimodal integration of EELS, EDS and the NanoPulser ensures the most advanced and effective electron dose optimization strategies. EELS data collection is extended with the ease-of-use of Velox software ecosystem making the most challenging experiments now accessible to every user including novices. Nearly every component in the Iliad platform can be fully accessed via Python scripting using Autoscript and that opens the possibility of creating new data collection workflows as well as the adoption of Artificial Intelligence based data collection and processing strategies.

MEMBRAIN V2: AN END-TO-END TOOL FOR THE ANALYSIS OF MEMBRANES IN CRYO-ELECTRON TOMOGRAPHY

Tingying Peng[1,3,*], Lorenz Lamm[1,2,3], Simon Zufferey[2], Ricardo D. Righetto[2], Wojciech Wietrzynski[2], Kevin A. Yamauchi[4,5], Alister Burt[6], Ye Liu[1,3], Hanyi Zhang[1,3], Antonio Martinez-Sanchez[7], Sebastian Ziegler[8,9], Fabian Isensee[8,9], Julia A. Schnabel[1,3,10], Benjamin D. Engel[2,*]

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MemBrain v2 is a deep learning-enabled program aimed at the efficient analysis of membranes in cryo-electron tomography (cryo-ET). It consists of the three main modules „MemBrain-seg", „MemBrain-pick", and „MemBrain-stats". MemBrain-seg is a generalizable membrane segmentation network that can be used out-of-the-box for a wide variety of tomograms. We achieve this by combining a well-curated and diverse training dataset with strong data augmentations, as well as a custom loss function that aims to capture the correct membrane topology. MemBrain-pick is our interactive framework for localizing proteins on membrane surfaces. To this end, we convert membrane segmentations to mesh representations and perform learning directly on these meshes, leading to improved data efficiency compared to common voxel-based approaches. MemBrain-pick works interactively with membrane surface annotation tools to enable going back-and-forth smoothly between annotation and training processes. MemBrain-stats leverages the outputs of MemBrain-pick and MemBrain-seg to generate several membrane statistics, like protein concentrations, geodesic nearest neighbor analysis, and Ripley's statistics.

KEYNOTE

TOOLS AND TECHNOLOGIES FOR CRYO ELECTRON TOMOGRAPHY

Bridget Carragher, David Agard, Angel-Mallak Ali, Ruiming Cao, Anchi Cheng, Yongbaek Cho, Utz Ermel, Garrett Greenan, Matthias Haury, Norbert Hill, Joshua Hutchings, Saugat Kandel, Dari Kimanius, Gorica Margulis, Elizabeth Montabana, Pavel Olshin, Mohammadreza Paraan, Noeli Paz Soldan Cruz, Ariana Peck, Davis Perez, Julia Peukes, Clinton Potter, Jonathan Schwartz, Daniel Serwas, Shu-Hsien Sheu, Hannah Siems, Armando Soria, Carmela Villegas, Yue Yu, Kevin Zhao, Shawn Zheng

Chan Zuckerberg Institute for Advanced Biological Imaging (CZ Imaging Institute), Redwood City, California, USA.

The mission of the Chan Zuckerberg Imaging Institute (CZII) is to enable deep insights into the architecture of complex biological systems, at the molecular level, through the development and application of novel imaging technologies. The initial grand challenge for CZII is to develop technologies and methodologies to image the molecular architecture of the cell to near atomic resolution using cryo electron tomography. We will describe our plans and progress on the platforms, technologies and driving biological projects that have been initiated towards these goals.

INNOVATION OF CRYO-EM SPECIMEN PREPARATION BY GRAPHENE AND OTHER NANOMATERIALS

Hong-Wei Wang[1], Nan Liu[2], Hailin Peng[3], Kaili Jiang[4]

[1]Beijing Frontier Research Center for Biological Structures, School of Life Sciences, Tsinghua University, Beijing, China; [2]School of Biological Sciences, The University of Hong Kong, Hong Kong, China; [3]College of Chemistry and Molecular Engineering, Peking University, Beijing, China; [4]Department of Physics, Tsinghua University, Beijing, China.

Specimen preparation is a critical but challenging step in high-resolution cryo-EM structural analysis of macromolecules. In the past decade, graphene, as a two-dimensional nanomaterial, has gained much recognition as the supporting substrate to optimize cryo-EM specimen preparation. It improves macromolecule embedding in ice, reduces beam-induced motion, while imposes negligible background noise. Various types of graphene-coated cryo-EM grids were implemented to improve the robustness and efficiency of specimen preparation. Graphene functionalization by different means has been proved specifically useful in addressing challenges related to the air-water interface (AWI), such as preferential orientation and sample denaturation. Graphene sandwich specimen preparation sets a new direction to explore in cryo-EM analysis of biological specimens. More recently, we developed method to use carbon nanotubes (CNTs) as a one-dimensional nanomaterial to interact with macromolecules, effectively shielding them from air-water interfaces. Our findings revealed that macromolecules fully retained within the vitreous ice are assembled around CNTs in various orientations. Furthermore, biofunctionalized CNTs exhibit strong specificity in binding target macromolecules, enabling the direct purification of targets from cell lysates for cryo-EM specimen preparation. I will discuss the current challenges and future prospects of graphene and CNT application in cryo-EM analysis of biological specimens

USING MICROFLUIDICS TO ADVANCE SAMPLE PREPARATION FOR SINGLE-PARTICLE CRYO-EM

Gangadhar Eluru[1,2], Stefania Torino[1,2], Steven De Gieter[1,2], Mugdha Dhurandhar[1,2], Stephan Schenck[1,2], Janine Brunner[1,2], Rouslan Efremov[1,2]

[1]Center for Structural Biology, VIB, Brussels, Belgium; [2]Structural Biology Brussels, Department of Bioengineering Sciences, Vrije Universiteit Brussel, Brussels, Belgium.

Single particle cryo-EM has emerged as a powerful technique for solving structures of proteins and protein complexes. Despite its remarkable success, cryo-EM still holds untapped potential, such as enabling the visualization of transient states and transition kinetics, as well as reducing the amount of biological material required for grid preparation by several orders of magnitude. In this presentation, two microfluidics-based instruments designed to optimize sample preparation for single-particle cryo-EM will be introduced. The first instrument integrates a droplet-based microfluidic mixer with a laser-driven, on-demand droplet dispenser, enabling the preparation of time-resolved cryo-EM samples. The second device miniaturizes on-chip protein purification and cryo-EM grid preparation. The operational principles of these microfluidic devices will be described along with showcase application examples. The remaining challenges and directions for future development will be discussed.

NEW REACTIONS OF DICOORDINATED CARBOCATIONS AND METHODS FOR STRUCTURAL CHARACTERIZATION

Hosea M. Nelson

Caltech, Pasadena, California, USA.

In this talk I will discuss our recent efforts to utilize phenyl and vinyl carbocations in C–H functionalization reactions. We will describe how these high-energy dicoordinated carbocations can be generated under mild conditions and utilized in the selective C–C bond forming reactions of simple hydrocarbons. Moreover, we will discuss our efforts to understand the mechanism of these reactions through computational chemistry, kinetics, electron microscopy, and isotopic labeling studies. We will also discuss our efforts to apply electron microscopy to problems in organic chemistry through the use of MicroED and other CryoEM modalities.

IN SITU STRUCTURAL BIOLOGY: FROM CELLS TO MOLECULES

Peijun Zhang

Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom.

Recent development in cryo-electron microscopy (cryoEM) has revolutionized the field of structural biology, allowing protein structures to be determined at the atomic resolution in a close-to-native, frozen-hydrated state. For studying macromolecular complexes that are intrinsically flexible and dynamic, and often function in higher-order assemblies that are difficult to purify, cryoET with subtomogram averaging (cryoET STA) has emerged as a potent tool to obtain structures of these at near-atomic resolution. The study of such complexes and assemblies in situ in the native cells with cryoFIB/SEM and correlative imaging, opens a new frontier in structural cell biology. I will present our recent *in situ* studies of HIV-1 and SARS-COV-2 infection, COVID vaccine and antiviral treatment, as well as the basic architecture of native chromatin fibers in intact T-cells.

BEYOND RIBOSOMES: IN SITU STRUCTURAL BIOLOGY OF CHALLENGING TARGETS IN C. REINHARDTII

Jessica Heebner[1], Ron Kelley[1], Martin Obr[1], Sagar Khavnekar[1], Xianjun Zhang[1], Saikat Chakraborty[1], Ricardo Righetto[2], Florent Waltz[2], Alicia Michael[2], Wojciech Wietrzynski[2], Grigory Tagiltsev[3], John Briggs[3], Juergen Plitzko[3], Ben Engel[2] and Abhay Kotecha[1*]

[1]Thermo Fisher Scientific, Hillsboro, Oregon, USA; [2]Biozentrum, University of Basel, Basel, Switzerland; [3]Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Martinsried, Germany.
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Understanding high resolution protein structure in the context of the whole cell is the vision of visual proteomics. With the advent of high-throughput cryo-electron tomography and cryo-FIB, paired with cutting edge computational techniques, achieving such an ambitious goal is no longer a far-reaching dream. The new generation of cryo-FIB from Thermo Fisher Scientific uses plasma ions, which reduce redeposition and ion beam damage, substantially improving throughput. In preparation for a large-scale effort towards visual proteomics of the model organism *Chlamydomonas reinhardtii*, we have prepared enough lamellae to allow for acquisition of more than 1800 tomograms of different cellular compartments.

While some proteins were in such high abundance that template matching alone was sufficient to obtain a sub-nanometer structure, as in the case of Rubisco where we were able to determine a 7Å structure from within the pyrenoid compartment, other targets proved more of a challenge and necessitated a combination of computational techniques to achieve results.

The scale of this dataset is exciting, but the huge number of molecular complexes within living cells makes it difficult to identify, confirm the identity of, and determine each structure by just one group. Achieving a full visual proteome of *C. reinhardtii* will necessitate a large collaborative effort. To that end, we would like to create an open access database for *C. reinhardtii* to accelerate annotation and curation, enable further cell biology research, and develop new computational tools for in situ cryo-ET. Along with sharing the raw data, reconstructed tomograms, denoised datasets, and structural determinations, we will provide high quality segmentations of selected datasets created using 2.5D (Dragonfly) and 3D (MemBrain Seg) UNets. This project has the potential to provide invaluable insights into cellular processes and will hopefully lay the foundation for other large-scale studies of other species.

KEYNOTE

VISUALIZING SINGLE ATOM DYNAMICS IN CATALYSIS

Pratibha L. Gai[1], Edward D Boyes[2]

[1]Nanocentre, Department of Chemistry, University of York, York, United Kingdom;
[2]Nanocentre, School of Physics, Engineering and Technology, University of York, York, United Kingdom.

Catalysis is central to the global chemical industry and economy. Many heterogeneous catalytic reactions involve gases catalyzed over solid surfaces at elevated temperatures. They play a critical role in the production of energy, healthcare, pollution control, industrial products and food. Understanding and controlling the reactions at the atomic level, at which they take place, are crucial for developing improved catalysts and more sustainable chemical processes. Increasingly, it is believed dynamic single atoms and atom clusters can lead to enhanced catalyst performance. Conversely, they can contribute to deactivation mechanisms limiting reaction effectiveness and the consistency essential for commercial operations. All depend on understanding and controlling single atom dynamics at their heart. With positive and negative outcomes illustrated.

The atomic resolution Environmental Transmission Electron Microscope (ETEM), developed by the authors, is widely used for real time atomic level in-situ visualization and analysis of dynamic gas-solid catalyst reactions at operating temperatures under controlled reaction conditions of gas environment, temperature and time. It is recently advanced to environmental scanning TEM (ESTEM) with single atom sensitivity and full analytical capabilities. The groundbreaking instrumentation developments and methods provide a much better understanding of how catalysts work. By correlating atomic scale observations with bulk scale experiments, deeper insights into key catalytic reaction mechanisms are possible, helping the development of efficient catalytic systems with valuable economic, environmental, and societal benefits. Advances in in-situ technologies, with some of the challenges and opportunities in tracking reacting single atoms, are highlighted. There are considerable synergies with developments of minimally invasive but highly informative analytical methods in other fields of microscopy.

OPERANDO ELECTROCHEMICAL LIQUID-CELL STEM AT DYNAMIC ENERGY MATERIALS INTERFACES

Yao Yang

Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York, USA.

Energy Materials lie at the interface between electrochemistry and materials science and represent one of the most promising approaches for enabling renewable energy technologies to mitigate carbon emissions through the electrochemical reduction of CO_2 and green H_2 production. One of the key challenges is understanding how to achieve and sustain catalytic activity under operating conditions for extended time periods, and such fundamental understanding calls for the use of multimodal *operando* analytical methods.

In this talk, I will first introduce my recent work on *operando* correlative studies for tracking the dynamic evolution of Cu nanocatalysts and identifying the complex nature of active Cu sites for CO_2 electroreduction. I will focus on the development of multimodal *operando* methods to elucidate *operando* (operating) structure-property relationships of energy materials with an emphasis on electrochemical liquid-cell STEM (EC-STEM) and correlative synchrotron X-ray-based methods. *Operando* electrochemical 4D-STEM in liquid, driven by machine learning, has shown great potential to interrogate complex structures of active sites of energy materials at solid-liquid interfaces. I will then introduce the instrument development on the first-of-its-kind *operando* heating/cooling EC-STEM to investigate the dynamic growth of heterogeneous nanomaterials in liquid across a wide range of controlled temperatures.

A CATALYSTS LIFE AND ITS CIRCUMSTANCES

Beatriz Roldan Cuenya

Department of Interface Science, Fritz Haber Institute of the Max Planck Society, Berlin, Germany.

Climate change concerns have spurred a growing interest in developing environmentally friendly technologies for green energy generation and storage in the form of chemical bonds. The latter includes green H_2 production from water splitting and the re-utilization of CO_2 via its thermal or electrocatalytic reduction into value-added chemicals and fuels. Moreover, alternative methods for ammonia synthesis either with green H_2 or direct electrocatalytic NH_3 synthesis are also being sought in order to reduce carbon emission while providing a transport carrier vector for green hydrogen. Thus, it is in this context important to develop low cost, highly efficient and durable catalysts with tunable selectivity. This requires understanding the evolution of their structure and surface/bulk composition under reaction conditions, i.e. while the active sites are formed or become poisoned during their deactivation.

This talk will offer new mechanistic insights into the electrocatalytic reduction of CO_2 and nitrate/nitrite as well as the oxygen evolution reaction using as model pre-catalysts size- and shape-controlled nanoparticles (Cu, Cu_2O nanocubes, Cu_2O octahedra, Cu_2O spheres and Fe-doped NiO_x octahedra). I will illustrate the need of a multi-technique operando microscopy and spectroscopy approach, when possible combined, to gain understanding into the active state formation of complex electrocatalytic materials and its evolution. Examples will be given on the correlation between the dynamically evolving structure and composition of the catalysts under operando reaction conditions and their activity and selectivity.

These results are expected to open up new routes for the reutilization of CO_2 through its direct conversion into industrially valuable chemicals and fuels such as ethylene, methanol and ethanol, and the generation of green H_2 and ammonia via electrolysis.

ABERRATION-CORRECTED ELECTRON MICROSCOPY OF ATOMICALLY DISPERSED CATALYSTS: CHALLENGES AND OPPORTUNITIES

Jingyue (Jimmy) Liu

Arizona State University, Tempe, Arizona, USA.

To develop new materials with desired functionalities, the fundamental knowledge of how atoms are arranged and bonded to each other, especially at interfaces and surfaces, becomes essential. The electron microscope is the primary tool for directly imaging atoms and even their three-dimensional arrangement of a variety of materials including metals, ceramics, composites, or biological materials. Aberration-corrected electron microscopy (ACEM) and its associated spectroscopy and diffraction techniques played a critical role in developing atomically dispersed metal catalysts including the recent advance of single-atom catalysts. For example, atomic-resolution high-angle annular dark-field (HAADF) imaging not only reveals the presence of metal atoms, clusters or particles in a supported metal catalyst but also shows the atomic arrangement of these species and their relationship with respect to the surface atomic structure of supports, providing critical information for understanding the nature of active sites during catalytic reactions.

There exist, however, major challenges in reliably and correctly determining the coordinates of metal atoms of interest, chemically identifying the observed metal atoms in multi-element clusters, and evaluating the oxidation states of each individual metal atoms. For atomically dispersed metal catalysts, the catalytically active species are usually located on, or embedded in, the top surface layer of a support, making them less stable and susceptible to electron-beam-induced effects. Furthermore, the high-surface-area support materials are frequently non-conducting refractory oxides (e.g., silica, alumina, cordierite or zeolite), activated carbon, or semiconducting metal oxides. Under electron-beam irradiation, the surface structures of these support materials are often not stable and can deteriorate, leading to major challenges in quantitative analysis of the three-dimensional arrangements of the metal atoms and the structural relationship between the metal atoms and their support surfaces. This presentation discusses these challenges, explores potential mitigation strategies that provide reliable and useful information about catalytically active sites and their microenvironments, and outlines opportunities in applying ACEM methods to solving critical issues in developing atomically dispersed catalysts including single-atom, heteroatom dimer, and cluster catalysts.

PROBING THE STRUCTURAL DYNAMICS OF ACTIVE MOLECULAR MATERIALS BY A COMBINATION OF CRYO AND LIQUID EM

Joe Patterson

University of California, Irvine, Irvine, California, USA.

Active molecular materials use competing chemical reaction systems to create complex and dynamic hierarchical structures. This results in a system in which the structural dynamics are very sensitive to the steady state established by these competing reactions. This sensitivity allows active materials to respond to changes in their environment and endows them with properties such as self-healing, homeostasis and motility.

The transient and dynamic nature of these materials presents challenges in characterization. Liquid EM is a powerful technique for observing the dynamics of nanoscale structures and has provided us with a wealth of new knowledge about the chemistry of nanomaterials in solution. However, Liquid EM faces several challenges which are exacerbated for active molecular materials, namely, beam sensitivity and low signal to noise. To overcome these challenges, we couple Liquid EM with Cryo EM which enables us to obtain high contrast images in low dose imaging conditions. Although Cryo EM cannot extract the dynamics of individual particles, these two techniques together provide us with a robust method to investigate this new exciting class of materials and discovery new dynamics processes.

USING LIQUID EM TO PROBE BIOMINERALIZATION IN COLLAGEN AND AT BIOMATERIAL INTERFACES

Kathryn Grandfield[1], Liza-Anastasia DiCecco[1], Ruixin Gao[2], Jing Zhang[1], Eli Sone[2]

[1]McMaster University, Hamilton, ON, Canada; [2]University of Toronto, Toronto, ON, Canada.

Biomineralization is a crucial process in the formation and maintenance of hard tissues and the integration of biomedical devices, yet its mechanisms remain elusive due to the limitations of traditional electron microscopy techniques which often involve static, dehydrated, or frozen samples. This talk will present new liquid transmission electron microscopy (liquid-EM) methodologies that overcome these limitations by enabling the visualization of mineralization processes in their native liquid environments. Thin-film enclosures and flow cells were used to explore collagen mineralization in the presence of a calcium phosphate and polyaspartic acid solution, capturing the progressive crystallization of mineral particles and eventual attachment along collagen fibrils. The findings, which capture non-classical crystallization in real time, align well with findings using cryo-TEM. Further, a new method combining the nanofabrication of titanium lamellae by focused ion beam microscopy with *in situ* liquid-EM to investigate calcium phosphate-titanium interfacial mineralization will be introduced. This technique revealed multiphasic calcium phosphate particles nucleating, adhering, and assembling on and around titanium surfaces. This highlights the potential of liquid-EM to explore nanoscale interactions between biomaterials and liquids as a platform for enhancing implant osseointegration and developing new treatments for mineralized tissue diseases. Other applications of advanced electron microscopy for understanding biomineralization will be highlighted.

HYDROGEL STRUCTURE ELUCIDATION AND PROPERTY PREDICTION BY LIQUID PHASE TEM

Nathan Gianneschi

Northwestern University, Evanston, Illinois, USA.

Talk abstract unavailable at time of print.

Poster Abstracts
(in alphabetical order by presenter's last name)

SEM/EDX AS NON-INVASIVE TECHNIQUES REFLECTING ON MARINE POLLUTION: AN OPPORTUNITY OR A FALSE HOPE

Mohamed Abouleish[1], Roa Daher[1], Fadi Yaghmour[2]

[1]American University of Sharjah, Sharjah, Sharjah, United Arab Emirates; [2]Environment and Protected Areas Authority, Sharjah, Sharjah, United Arab Emirates.

Investigation of marine pollution and specifically the reflection on marine organisms' health is important for achieving a healthier environment in the future. Pollution effect on marine organisms is difficult to detect until the organisms show signs of health deterioration or are dead. Therefore, there is a need for a non-invasive technique that would enable the continuous monitoring of the health of the marine organisms.

Sea Turtles are an important part of the marine ecosystem, and they could be used as biomonitoring tool for reflecting on marine pollution. There are different methods (invasive and non-invasive) that could be used to reflect on pollution effect on the health of sea turtles. In this research, we use a non-invasive approach to reflect on marine pollution, specifically heavy metals in Sea Turtles. This research investigates the usage of ICP-OES and XRF/SEM-EDS to reflect on the levels of heavy metals in the clippings of beaks and claws from the sea turtles, and studies the correlation between the two methods. This approach presents a less invasive technique compared to testing the organs of the organisms.

The results of this research demonstrated that 11/12 studied heavy metals were detected at varying concentrations between the beaks and claws, except for Cadmium. Mercury was only detected in the claws. The results were compared to the levels of heavy metals found in other organs in the literature. SEM images of the beaks and claws displayed different characteristics that could contribute to the accumulation of the heavy metals. Comparison of the two methods of analysis (ICP-OES and EDX), showed some variation in results, which requires more investigation in the future. The usage of such an approach will provide an early diagnosis of marine pollution and therefore contribute to a healthier environment and reflect on environmental sustainability.

MODEL BIAS IN CRYO-EM

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Model bias constitutes a considerable challenge in many scientific domains, leading to consistent but erroneous estimations due to inherent inaccuracies in the model. A 2013 publication in PNAS on the 3-D structure of an HIV molecule sparked significant controversy within the cryo-electron microscopy (cryo-EM) community regarding validation techniques. The debate, which led to four follow-up PNAS articles, centered on whether the collected datasets contained informative biological data or were merely noise images. The ongoing discussion highlights that model bias remains a crucial problem in cryo-EM.

Model bias can manifest during two key stages in the computational workflow of cryo-EM: the detection and extraction of particle images, known as particle picking, and the reconstruction of the 3-D density map. Bias in particle picking can arise from using unsuitable templates, particularly under low signal-to-noise ratio conditions, a typical circumstance in cryo-EM. Furthermore, an imprecise 3-D initial model can introduce bias into the 3-D reconstruction algorithm.

This study provides empirical evidence and statistical analysis of bias in the particle picking and 3-D reconstruction stages. On the empirical front, we illustrate how widely used particle picking algorithms can discern patterns, even when presented with pure noise input (data sets without any particle images). Subsequently, these patterns result in 3-D structures based solely on our assumptions and convictions rather than the data collected. From a statistical standpoint, we analyze the 'Einstein from Noise' experiment—an archetypal instance of model bias in template matching techniques. We offer a thorough statistical examination of the issue and outline several potential solutions.

Our research underscores a pivotal challenge in processing scientific datasets when the ground truth is unknown, particularly pertinent to physicists and biologists working with low signal-to-noise ratio data sets, as in cryo-EM. Grasping these limitations and potential biases is of utmost importance. In a wider context, this research offers a cautionary guideline to the scientific community, underscoring the necessity of meticulously interpreting observations affected by noise.

NICKEL AS AGGREGATES, OXIDES, AND DOPANTS IN CERIUM OXIDE AEROGELS WITH VARIOUS CERIUM OXIDIATION STATES

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With continuously improving hardware for Scanning Transmission Electron Microscopy Electron Energy Loss Spectroscopy (STEM-EELS), scientists can now detect and isolate few-percent loadings of specific chemical species and their oxidation states in morphologically complicated systems where most component objects are on the few-nanometer scale, i.e. larger than atomic-scale but smaller 10s of nanometers typical for energy dispersive spectroscopy. Here, we are specifically interested in the use of cerium oxide aerogels with nickel added during synthesis ($Ni_xCe_{1-x}O_2$) for improved yield in the water-gas shift (WGS) reaction converting H_2O and CO into CO_2 and H_2. The motivation being the introduction of earth-abundant and relatively chemically benign Ni to replace Fe-Cr oxides which can leak water-soluble Cr_{6+}, which is toxic and thus limits widespread adoption in broadly deployed catalytic systems. To this end, we find $Ni_xCe_{1-x}O_2$ aerogels beneficially impact catalytic conversion of CO into H_2 with suppressed methanol production in the WGS reaction up to $5\%_{at}$ Ni. While initial catalytic yields are very promising, the exact form of the added nickel, whether as metal-like nickel aggregates, atomically-dispersed dopants, or nickel oxide particles has a seemingly profound effect on the catalytic pathways on the highly-porous, covalently-bonded CeO_x aerogel scaffolds, which also apparently can prevent aggregation and oxidation of elemental Ni into undesirable NiO_x species. Using STEM-EELS on $Ni_xCe_{1-x}O_2$ aerogels, we identify ~1nm Ni-rich aggregates within a homogenously-dispersed Ni-containing background, as well as larger NiO_x particles with methods including direct spectra summing, principle component analysis, and Varimax rotation on a STEM-EELS spectrum image (SI) data collected on a Nion UltraSTEM200X operating at both 60 and 200kV with a Quantum Detector MerlinEELS camera and Gatan Enfinium EELS optics. We employ machine learning techniques to inform our understanding of the system, to which we also apply density-functional-theory techniques, which can both corroborate and drive experimental techniques.

NOBLE DOME: A NOVEL AIR-FREE TRANSFER SYSTEM FOR SCANNING ELECTRON MICROSCOPY AND FOCUSED ION BEAM

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This paper introduces an innovative solution for loading air-sensitive samples in and out of SEM instruments. Traditional loading methods expose samples to atmosphere during transfer, hindering investigations of reactive materials. The presented prototype, Noble Dome, is a glove box which directly attaches to the SEM/FIB, enabling complex sample manipulation and transfer into a vacuum chamber in an oxygen-free environment.

Air-sensitive samples include metals commonly used as battery materials such as lithium and sodium, which are highly reactive with moisture and oxygen. Another compelling application space for Noble Dome is TEM prep on 2D materials, where the large ratio of surface area to volume causes rapid sample damage by surface oxidation with even a small amount of air exposure. Noble Dome is large enough to contain an entire TEM rod, significantly simplifying the workflow for air-free lamella preparation.

In order to test Noble Dome's ability to protect samples from atmosphere, we imaged and collected EDS spectra on the same location of a lithium metal sample in a pristine state, after sitting in Noble Dome for 30 minutes, and after sitting in atmosphere for 30 minutes and compared the results. We note that there was very little change in the surface topography and oxygen counts between the sample in its pristine state and the sample after sitting in Noble Dome for 30 minutes. However, there is a marked increase in surface oxidation and topography after the sample sat in atmosphere for 30 minutes.

Other solutions currently on the market for loading air sensitive samples into a SEM/FIB involve manipulating the sample in a glove box which is separate from the instrument, loading the sample into an inert gas transfer shuttle which is closed by remote control, removing the shuttle from the glovebox, loading the shuttle into the SEM/FIB, and then opening the sample again via remote control. These solutions are not ideal because they're very expensive, they require a separate glove box, they have a variety of limitations on sample size, stage travel, and signal strength, are high tech solutions prone to costly malfunction, and the workflow for TEM prep is complex and high-risk for sample loss. These drawbacks highlight the demand for a more practical and efficient approach.

Noble Dome was invented, prototyped and tested by faculty at University of Oregon. This device offers an innovative approach for air-free transfer in and out of electron microscopes, opening new application spaces for electron microscopy.

TRACKING THE STRUCTURAL EVOLUTION OF THOUSANDS OF NANOPARTICLES IN REACTIVE ENVIRONMENTS USING REAL TIME ELECTRON MICROSCOPY

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Platinum (Pt) catalysts are essential in many industrial applications for their high activity and selectivity. The stability of supported Pt nanoparticles is key to maintaining catalyst effectiveness, but these particles can degrade under reactive conditions due to various destabilizing mechanisms. Understanding these processes is crucial for improving catalyst performance. In this study, we investigate the destabilization mechanisms of Pt nanoparticles using a combination of in situ transmission electron microscopy (TEM) and unsupervised machine learning detection techniques. This approach allows for real-time analysis of thousands of nanoparticles' transformation and movement. Our observations reveal significant Pt sintering at 800°C, with no such behavior at 700°C, no evident Ostwald ripening, and limited particle displacement. Furthermore, there is no apparent size or distance dependence among the sintered particles. Detailed TEM analysis shows the formation of platinum silicide and the presence of silicon nanocrystals on the silicon nitride window after annealing. The findings suggest that the observed selective sintering of Pt nanoparticles is primarily driven by dewetting effects due to surface energy differences between silicon islands and the silicon nitride substrate. By combining direct experimental observations of surface coverage change with computational modeling of thermodynamic and kinetic behaviors, we present a comprehensive explanation of the dewetting effect related to the interaction between Pt nanoparticles and the silicon nitride substrate. This study offers valuable insights into nanoparticle stability and evolution, potentially guiding the development of more durable and efficient catalytic systems.

STRUCTURAL INFORMATION OF MOLECULES DETERMINED BY NC-ATOMIC FORCE MICROSCOPY WITH CO FUNCTIONALIZED TIP

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The high-resolution non-contact atomic force microscopy (nc-AFM) is a powerful and non-destructive tool that uses an atomically sharp tip to probe the surface of a sample. It measures the atom-atom interactions between the tip atom and the sample atoms with pN precision. nc-AFM's unique capabilities include a non-destructive approach to probing soft and weakly bonded materials, such as small organic molecules, and obtaining three-dimensional structural information about the sample molecules. It can also provide a detailed structure of chemical bonds and confirm the adsorption of molecules on the surface. These capabilities precisely determine the chemical structure of unknown compounds, making nc-AFM a valuable tool for solving practical problems in petroleum-related studies.

In this study, we utilized nc-AFM to examine specially designed model compounds as representatives for archipelago structures in asphaltenes and heavy oils. We obtained experimental nc-AFM images of BPM and BPE molecules using a CO-functionalized tip in constant height scanning mode. We were able to differentiate the structure between BPM and BPE molecules due to their distinct adsorption conformation and bonding. nc-AFM was crucial in identifying various adsorption conformations of three different types of linkers in the BPM molecules and their steric structure as unique characteristics. With the steric fingerprint information of the linkers, we could address essential questions regarding the identification of molecular structures in chemistry and industrial applications. One such example is the long-standing query of determining the molecular structures of petroleum pitch. The role of nc-AFM in providing this information is significant as it enables the identification of unknown molecules with similar linkers. While nc-AFM might not resolve all the atoms and bonding details for nonplanar molecules, the fingerprint-like features of each bonding and adsorption configuration type can serve as a standard sample database for structure and conformation determination. Therefore, nc-AFM forms a firm basis for applying nc-AFM to unravel unknown structures in petroleum-related studies and to address a wide range of practical problems in the future.

REVEALING THE ATOMIC STRUCTURES OF HIGHLY AIR-SENSITIVE 2D MATERIALS

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The field of two-dimensional (2D) crystals and artificial structures rapidly expands and provides new opportunities in many physics, chemistry, and engineering areas. The physical properties of most 2D crystals in this large class remain unexplored or poorly explored, especially those beyond the air-stable materials, such as graphene and semiconducting transition-metal dichalcogenides (TMDs). Many 2D crystals and van der Waals structures are generally sensitive to the environment, though their bulk phases may be stable in air. Here, we demonstrated a robust method for atomic-resolution visualization of air-sensitive monolayers and twisted-bilayer of 2D TMDs obtained through chemical and mechanical exfoliation and fabrication. We observed the high crystalline quality of exfoliated 2D TMDs samples using high-resolution scanning transmission electron microscopy (STEM) (no serious beam damage issue). Three examples (WS_2, WTe_2, and $MoTe_2$) will be presented. We first identified the atomic structures of the three monolayer 2D TMDs and then investigated the moiré patterns of the twisted-bilayer of 2D WTe_2 and $MoTe_2$. We observed the twisted-bilayer WTe_2 with twist angles of approximately 5° and 2° and no noticeable lattice reconstruction. The twisted-bilayer MoTe2 exhibited a twist angle of about 3.7° and showed lattice reconstruction confirmed by the selected-area electron diffraction analysis. The direct visualization procedure described in this work deepens the understanding of the intrinsic microstructure of monolayer and twisted-bilayer 2D TMDs, which is essential for understanding and manipulating their quantum properties. The authors acknowledge the use of Princeton's Imaging and Analysis Center (IAC), which is partially supported by the Princeton Center for Complex Materials (PCCM), a National Science Foundation (NSF) Materials Research Science and Engineering Center (MRSEC; DMR-2011750).

IN SITU STUDY OF NOBLE METAL NANOPARTICLE ETCHING UNDER ACIDIC CONDITIONS

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Due to their exceptional chemical stability and catalytic activity, noble metal nanoparticles such as Pt and Pd are widely used in catalysis, fuel cells, and various industrial applications. However, etching and dissolution in acidic environments can significantly compromise their performance and durability, particularly under operational conditions. This study presents in situ investigations of the etching behavior of Pt and Pd nanoparticles in acidic environments using advanced characterization techniques such as transmission electron microscopy (TEM) and spectroscopy techniques. We employ real-time liquid phase TEM to observe the morphological and chemical changes in Pt and Pd nanoparticles exposed to varying acid concentrations and e-beam doses. Our findings reveal the mechanisms of platinum dissolution, including surface roughening, pit formation, and eventual particle fragmentation. The etching process is shown to be influenced by factors such as particle size, crystallographic orientation, and the nature of the acid. These insights into the etching mechanism of Pt and Pd nanoparticles are crucial for designing more robust Pt and Pd-based catalysts and enhancing their stability in acidic conditions, thereby improving the efficiency and longevity of catalytic systems. The authors acknowledge the use of Princeton's Imaging and Analysis Center (IAC), which is partially supported by the Princeton Center for Complex Materials (PCCM), a National Science Foundation (NSF) Materials Research Science and Engineering Center (MRSEC; DMR-2011750).

MORPHOLOGICAL CHARACTERIZATION OF CO_2-SENSING NEURONS AT NANOSCALE RESOLUTION

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Insects rely on olfactory cues to forage, seek mates, and avoid predators. Among behaviorally relevant odorants, carbon dioxide (CO_2) holds special ethological significance, such as mediating host-seeking arousal in mosquitoes and stress-induced aversion in fruit flies. Although the functional significance of CO_2-sensing neurons has been extensively studied, their morphological and morphometric features remain uncharacterized. Here, we employ serial block-face scanning electron microscopy to elucidate the three-dimensional (3D) morphology of CO_2-sensing neurons in *Drosophila melanogaster* and *Aedes aegypti* at nanoscale resolution.

Our 3D models of CO_2-sensing neurons in *D. melanogaster* reveal a distinctive morphological feature in the sensory dendrites—a flattened, sheet-like structure, unlike the narrow, cylindrical branches typical of odor-sensing neurons. Unexpectedly, a small subset of these neurons further exhibits ring-shaped dendritic morphologies, where the dendritic sheet appears to curve inward and fuse. In *A. aegypti,* our models show a similar flattening at the dendritic level, where a cylindrical process folds upon itself to form elaborate lamellae. These shared features suggest that the flattened dendritic morphology is critical to CO_2 sensing.

MULTICOLOR ELECTRON MICROSCOPY USING LANTHANIDE NANOPARTICLES AS PROTEIN TAGS

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In crowded cellular environments, nanoscale cellular features (ultrastructure) play a crucial role in the precise localization of proteins, which is essential for various cellular functions including signaling, cell division, cell migration, and biosynthesis. To study these phenomena, it is necessary to visualize proteins within their ultrastructural context. However, no single microscopy method offers the required specificity and resolution to image both proteins and ultrastructure at the nanoscale. We propose leveraging an electron-matter-light phenomenon, cathodoluminescence (CL), to introduce protein sensitivity in electron microscopy (EM). CL is the emission of light from a material upon excitation by electrons. Since CL can be induced by a focused electron beam, both CL and EM images have the same nanoscale resolution.

We are developing multicolored cathodoluminescent nanoprobes for visualizing proteins in EM. The probes are nanoparticles (NPs) that contain lanthanide ions, which we functionalize for protein labeling. We have engineered their architecture to achieve bright, small (sub-15 nm) particles, which makes them optimal for tagging proteins. By labeling different proteins with probes of distinct colors, we can simultaneously image multiple proteins in EM. We validate the effectiveness of NPs as cathodoluminescent probes by imaging them alongside their ultrastructural context in HEK293 cells.

MOLECULAR RESOLUTION ELECTRON IMAGING OF ICE-WATER INTERFACIAL DYNAMICS

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The phase transformation between liquid water and ice is among the most fundamental processes that shape the Earth's ecosphere and define human activities. Understanding their molecular origins is critical to developing next-generation cryopreservation agents and protocols, deciphering glacial evolution and associated geological history, and improving transportation safety through anti-icing material innovations. However, the ice-water interface has never been imaged at molecular resolution. This presentation discusses our recent breakthroughs in directly observing ice crystals and the ice-water interface at Ångstrom-level spatial resolution. We developed a frozen liquid-cell approach to prepare single-crystalline ice I_h sections up to microns in area. These samples are of high purity and stable under relatively high electron flux (up to ~100 e/Å^{2}s), allowing us to routinely achieve a lattice resolution < 2 Å during electron imaging. Consequently, this platform enables studies of near-equilibrium ice structure and dynamics at unprecedented spatial resolution. With it, we discovered subdomain-rich regions near the defective crystal edges despite the structure appearing single-crystalline according to diffraction criteria. These subdomains connect via low-angle grain boundaries with flat energy landscapes as a function of tilt angles (according to simulations), showing the high tolerance of ice to defect structures. When we tuned the sample temperature and electron flux rate, we observed radiolysis-controlled bubble generation and dissolution in ice single crystals near a steady state of bubble dynamics. Furthermore, rich beam-induced melting and recrystallization dynamics were observed with lattice resolution at the ice-water interface, providing critical insights into the crystal orientation-dependent phase transformation on the nanoscale and local molecular fluctuations. Moving forward, finer control of temperature, electron irradiation profile, and imaging detection could eventually lead to real-time observation of ice nucleation in water and address long-standing questions about ice nucleation pathways.

PRECISE DETERMINATION OF IMAGING HEIGHTS IN CO-FUNCTIONALIZED ATOMIC FORCE MICROSCOPY EXPERIMENTS USING DENSITY FUNCTIONAL THEORY-BASED SIMULATIONS

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CO-functionalized atomic force microscopy (AFM) involves attaching a single CO molecule to a metallic Cu tip, which bonds the C atom to Cu. This CO molecule acts as a molecular probe, enabling the direct observation of atomic and subatomic features of a specimen in real space. CO tip AFM has achieved significant milestones, such as imaging complex molecular and surface structures, distinguishing various functional groups and atomic species, and even observing electron orbital signatures. However, a fundamental question remains unresolved: determining the precise distance between the CO tip and the sample during imaging. Researchers have theoretically estimated tip-sample distances using different models, ranging from less than 3 Å to over 15 Å. The uncertainty in tip height prevents us from precisely discerning how the tip interacts with the sample and identifying the exact nature of what is being probed. Our research used density functional theory (DFT) calculations to interpret experimental measurements and accurately determine these imaging heights.

In our experiment, we utilized a CO-functionalized AFM tip mounted on a qPlus sensor to image CO molecules adsorbed on a Cu(111) surface at various tip heights. We conducted DFT-based AFM simulations of the surface-bound CO molecules. By correlating the appearance of bond-like features between CO molecules, we inferred that the absolute tip height at this point was approximately 2.9 Å. Based on this reference point, we could deduce the actual imaging heights for all experimental images.

Our work illustrates that AFM imaging can be performed not only in the attractive regime but also in the repulsive regime, where Pauli repulsion dominates, both of which allow for the precise determination of imaging heights. We elucidate why AFM images often exhibit height-dependent distortions at small tip heights. In addition, we find that imaging in the repulsive regime reveals additional fine details of the specimen. These findings not only enhance our understanding of AFM images but also have the potential to significantly advance the capabilities of AFM in the characterization of complex molecular systems, offering hope for future breakthroughs in the field.

QUANTITATIVE ANALYSIS OF ATOMIC RESOLVABILITY AND RADIATION DAMAGE IN APOFERRITIN COMPLEXES USING CRYOGENIC-ELECTRON MICROSCOPY AND Q-SCORE EVALUATION

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Electron radiation damage is well-known to limit the resolvability in density maps reconstructed from images of single particles in cryogenic-electron microscopy (Cryo-EM). However, it is not clear whether this damage affects all side chains equally. In this study, we analysed a set of images of vitrified apoferritin particles that could yield a map with a final resolution of 1.69Å. To assess the effects of radiation damage at the side chain level, we computed the Q-scores of maps and models generated from later sets of frames in the movies corresponding to higher cumulative doses. Our analysis revealed that the map resolution, as computed from the Fourier Shell Correlation (FSC), decreased with increasing cumulative doses of the corresponding maps. Furthermore, the Q-scores (i.e. resolvability) of the side chain densities declined at a faster rate for negatively charged residues compared to positive and non-polar residues. We observed that aromatic residues exhibited greater structural preservation under similar cumulative dose conditions, while in the case of negative charged residues, the density resolvability could arise from inherent scattering properties. To isolate the scattering effect from the dose effect in two different tests, we modified the original map by 1) phase randomization and 2) equalizing amplitudes at resolution lower than 1.69A. This approach allowed us to isolate the impact of radiation damage from the scattering effects of negatively charged atoms. The observed declines in Q-score and structural visibility in the maps with higher cumulative doses were indeed attributed both to dose-induced damage and to scattering differences at low resolution, particularly involving negatively charged oxygen atoms. We demonstrated that signal falloff occurs more rapidly with increased dosage, particularly in the critical 2.1 Å and 3.2 Å resolution ranges where protein side chains, ions, and waters undergo radiolytic processes. This suggests that inherent limitations in cryo-EM's structural fidelity may exist, independent of radiation effects, potentially due to the intrinsic scattering properties of the sample or other factors such as side chain dynamic motions. Understanding these limitations is crucial for refining Cryo-EM techniques and achieving more accurate structural interpretations in complex biological systems.

EFFICIENT SEGMENTATION OF CRYOGENIC ELECTRON TOMOGRAPHY DATA WITH VISION FOUNDATION MODELS

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Cryogenic electron tomography (cryoET) is a powerful technique for visualizing subcellular structures in their native state at high resolution. Quantitative analysis of cryoET data is time-consuming but essential for extracting meaningful 3D visualizations and objective biological insights. Current automated segmentation methods using the popular 3D U-Net architecture show limited performance when labeled training data are scarce. Here we introduce CryoViT, a label-efficient method that leverages a pre-trained vision foundation model for feature extraction and fine-tunes a lightweight 3D convolutional neural network for segmentation. To evaluate CryoViT's performance in segmenting mitochondria, we collected 112 cryoET tomograms of mitochondria from embryonic stem cell-derived human retinal ganglion cells (RGCs). We found that CryoViT achieved a Dice Similarity Coefficient (DSC) of 0.82 ± 0.16, outperforming the 3D U-Net's score of 0.60 ± 0.27. Even when trained on just one-tenth of the dataset, CryoViT surpassed the performance of the 3D U-Net trained on the full dataset. The model was also robust to domain shifts, reaching a DSC of 0.81 ± 0.17 when segmenting mitochondria in unseen tomograms of Huntington's disease neurons. Furthermore, we applied CryoViT to segment mitochondrial electron-dense granules, cristae, and cytoplasmic microtubules, showcasing its versatility. Using CryoViT, we observed changes in the mitochondrial granule distribution within RGCs that were induced to calcify ectopically to mimic a disease called optic disc drusen (ODD), the most common cause of RGC calcification. This finding provides insights into mitochondrial calcium regulation and its role in ODD pathology. Our results highlight the potential of using AI to overcome the data analysis bottleneck in cryoET studies and accelerate biological discoveries. CryoViT is available at https://github.com/sanketx/CryoVIT.

SPATIOTEMPORAL ANALYSIS IN CANCER RESEARCH: UTILIZING AI TO PREDICT TUMOR GROWTH AND SPREAD PATTERNS

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Spatiotemporal analysis in cancer research, utilizing artificial intelligence to forecast tumor development, and metastatic distribution, is recognized as a high-impact innovation to advance a spatiotemporal approach towards cancer studies. This implies that in addition to capturing spatial information for instance tumor morphology and its micro environment the technique also captures information in time dimension, or in other words the progression of the disease and builds predictive models from this to determine how a tumor will progress and spread. The benefit of this technique therefore is that it offers an improved understanding of the behaviour of cancer hence improved early and targeted intercessions. The background of this study stems from the consideration of the fact that in most traditional mathematical models of the tumor growth, the interactions between geometry, micro-environment and temporal development are not fully considered. These interactions play important role to unravel the pathophysiological processes that underpin cancer progression and for designing relevant therapies. New developments in AI which include deep learning and neural network have offered capabilities of analyzing large and complex datasets and models that can help the researchers to identify structures or patterns, and trends, that were unrecognizable before. The approach used in this study entails utilizing the spatiotemporal ML models in cancer datasets, where datasets contain imaging scans, biopsy, and patients' records. Various analyzing methods like convolutional neural networks (CNNs) and recurrent neural networks (RNNs) are used to process and analyze these data which contain spatial and temporal information for tumor growth models. The models are trained with large set of data to determine future behaviour of tumor like its growth rate, direction of growth, possibility of metastasize and location of metastasis etc. It then calculates and compares the results of the prediction with the actual observations of different patients to establish validity and applicability. Thus, by using all of these modern techniques of AI, the study will contribute to the development of tools that will be helpful for oncologists to make accurate predictions which will have a positive impact on the improvement of cancer treatment plans.

STATISTICAL DETERMINATION OF THE SURFACE COVERAGE AND DISTRIBUTION OF SURFACE IMMOBILIZED MOLECULAR COMPLEXES

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The immobilization of molecular catalysts on the semiconductor surface is attractive because it combines the benefits of homogeneous and heterogeneous catalysis. The molecular nature of the catalyst allows tuning of activity and selectivity by modifying the metal or ancillary ligands while the heterogeneous nature has the advantage of enabling facile separation from products. This approach is especially appealing for photo-electrocatalytic applications such as CO_2 reduction or H_2 evolution reactions, where high selectivity and rapid electron transfer are important. The characterization of the hybrid photoelectrode has relied on spectroscopy, which provides averaged information about the structure but rarely information regarding the extent and homogeneity of surface coverage. Electrochemistry or inductively coupled plasma mass spectrometry (ICP-MS) can provide a measure of the total average surface coverage, but they cannot provide insight into the location and distribution of the catalysts.

Here, we demonstrate that the combination of high angular dark field scanning transmission electron microscopy (HAADF-STEM) and image analysis based on a convolutional neural network (CNN) provides a statistically robust determination of the surface coverage and distribution of immobilized molecular catalysts. Using this method, we could characterize 1st to 3rd-row transition metal atoms in the immobilized molecular catalysts on silicon support, or 3rd-row transition metal on TiO_2 deposited on the Si support. Further, we investigate how changes in the functionalization conditions, attachment group, and structure of the molecular catalyst affect the surface coverage and distribution, providing insight into the chemical mechanism of surface immobilization. The method could be generally valuable for correlating the surface coverage and distribution to a catalytic system's activity, selectivity, and stability.

NANOSCALE SCULPTING OF HBN WITH AN ELECTRON BEAM AND CHARACTERIZATION VIA PL MICROSCOPY AND IONIC TRANSPORT

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Creating sub- to few-nanometer defects and nanopores in hexagonal boron nitride (hBN) opens opportunities for engineering quantum emitters and for nanofluidic and sensing applications. Using the electron beam in the aberration-corrected scanning transmission electron microscope, we demonstrate modification, thinning, and drilling of features in few-layer hBN membranes (~ 5-to-20-nm-thick). The atomic composition is monitored with electron energy loss spectroscopy, which also facilitates drift correction. We report effects of electron-beam energy and exposure times on defect size and structure. While previous studies focused on beam energies ≤ 80 keV to avoid material damage, we show that drilling is favorable at a higher beam energy of 200 keV. The drilling rate at 200 keV is about 10 times larger than at 80 keV (~ 1.2 vs. 0.1 nm/min), and smaller pores are achievable with minimized damage to the surrounding material. Thinned hBN nanoscale features demonstrate enhanced emission via photoluminescence spectroscopy. For sub-nm hBN pores, ionic transport measurements confirm the complete opening of the 2D hBN pores made through the AC-STEM drilling.

COEXISTENCE OF ORDER AND DISORDER IN SOLID-ELECTROLYTE INTERPHASE OF LI-METAL ANODES

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Understanding the structure of matters is critical as it directly influences their properties, and battery interfaces are no exception. These interfaces can dictate Li-ion diffusivity and mechanical strengths, which are essential for stable operations of batteries. We investigated the structures of battery interfaces using cryogenic scanning electron beam diffraction (cryo-SEND). These observations were coupled with a cryogenic lift-out technique to extract ~ 100 nm thick battery slices, preserving both battery interfaces and liquid electrolytes. We identified two types of battery interfaces, commonly referred to as solid-electrolyte interphases (SEI). The first SEI is composed of an amorphous structure with short-range ordering, located adjacent to Li metal grains. The second type is comprised of a mixture of another amorphous structure and LiF crystals with defects, which precipitates near a copper current collector. By Comparing SEIs across a variety of liquid electrolytes, we found that the SEI structure strongly influences Li metal growth and removal, which are key processes for stable Li-metal batteries.

Our findings are significant as battery interfaces are found to be primarily amorphous, in contrast to the previous descriptions of SEIs as a combination of amorphous and crystalline components. These earlier descriptions were first attempted by cryogenic-transmission electron microscopy and may have been influenced by the extremely beam electron sensitivity of battery interfaces. Consequently, we propose our SEND approach as a method to uncover the structural information on battery interfaces without damaging them.

In this presentation, we introduce a method to prepare battery slices for desirable electron transmission and discuss a methodology to characterize structures of battery interfaces with extremely low electron dose, allowing us to provide insights into achieving stable battery interfaces. In the second part of this presentation, we examine electron beam sensitivity of battery interfaces that has been largely overlooked in prior studies.

SCALABLE ELECTRON TOMOGRAPHY FOR BRAIN MAPPING

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One of the grand quests in neuroscience is to build complete maps of the brain, charting all of its cells and the connections between them. Such wiring diagrams, called connectomes, promise to shed light on how networks of neurons can give rise to thoughts, memories, and actions. Mapping brain connectivity at the level of individual connections between neurons (*synapses*) is a formidable technical challenge because it requires nanometer-resolution imaging over large 3D volumes. As a result, comprehensive, whole-brain maps of wiring and connectivity – known as "connectomes" – are only available for very small organisms such as the roundworm *C. elegans* (302 neurons) and the fruit fly *Drosophila melanogaster* (~100,000 neurons). Comprehensively mapping even these small nervous systems required executing difficult techniques with extremely high levels of quality control, which become impractical for mapping many individual brains, or brains of larger organisms such as mice (~100 million neurons) and humans (~100 billion neurons).

Many recent breakthroughs in connectomics have been enabled by automated, wide-field transmission electron microscopy (TEM). However, these techniques are bottlenecked by the precarious process of thin-sectioning, in which thousands of successive ultra-thin (<50 nm) sections need to be collected without fail. A promising alternative approach is electron tomography, which can image samples up to 20 times thicker by combining information from many tilt angles to provide a 3D reconstruction of the sample. Here, we will demonstrate how a limited-tilt, serial section electron tomography approach can provide next-generation capabilities for connectomic imaging. We will share recent developments for an automated electron tomography system that combines upgraded cameras, optics, sample stages, vacuum and control systems with the capability to rapidly tilt the sample for electron tomography to supercharge imaging speeds. We will demonstrate how this new system can achieve large-scale brain mapping with a simple, robust, and inexpensive sample preparation, opening new possibilities for comparative studies in mammalian brains.

CRYO-EM AND AI REVEAL THE MOLECULAR BASIS OF LAMININ POLYMERIZATION AND LN-LAMININOPATHIES

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Basement membranes (BM) are dynamic extracellular matrices that form a sheet-like lattice on cell surfaces underlining epithelia and endothelia in multicellular animals. Laminin (Lm) polymerization is the major step in the BM assembly. The Lm polymer node, which constitutes the repeating unit of the Lm lattice, is a flexible heterotrimeric glycoprotein consisting of Lm alpha, betta and gamma subunits joined together through their N-termini. Genetic alternations of Lm subunits result in failures of Lm polymerization manifesting as a wide spectrum of human disorders that we collectively define as LN-lamininopathies. Understanding the process of Lm polymerization and its defects requires the knowledge of the Lm polymer node structure. However, due to (i) its intrinsic flexibility (~80% protein loop content), (ii) time- and concentration-dependent instability, (iii) relatively small size (~50-60 kDa subunits), (iv) the presence of preferred orientations manifesting as the directional resolution anisotropy, and (v) near structural identity of monomers within an asymmetric trimer (RMSDs ~1-3 Å) that hampers model building, the Lm polymer node has been elusive to structure determination. We have successfully overcome the above listed issues by developing novel sample preparation and data collection protocols, and by exploiting deferential N-glycosylation patterns of Lm subunits for model building. Consequently, we determined a 3.7 Å cryo-EM structure of the functional Lm heterotrimer. The structure revealed the molecular basis of calcium-dependent formation of Lm lattice and provided insights into polymerization defects manifesting in human disease. In addition, we employed AF2 to model twenty-three structures of pathogenic Lm polymer nodes and demonstrated that the *in-silico* models display a remarkable accuracy and precision by validating a subset of computed models against the experimentally derived cryo-EM structure. Our data collectively reveal the molecular mechanisms underlying LN-lamininopathies.

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SHORT RANGE ORDER WITH SYMMETRY BREAKING AT THE NANOSCALE FOR TUNABLE METAMATERIALS

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Chirality is a geometrical property described by continuous mathematical functions [1–3]. However, chirality in chemistry commonly manifests as a binary property: chiral molecules are either right-handed or left-handed. Small molecules are typically known as D/L or R/S enantiomers, while larger molecules with helical shapes are often denoted as Δ/Λ or M/P enantiomers. While theoretically possible, a family of stable chemical structures with the same shape and progressively tunable chirality is yet unknown. The transition from discrete chiral phases and shapes to a palette of chemical compounds with continuously tunable chirality would be transformative for the development of chiral photonics, chiral metamaterials, biochemical separations, and chiral catalysis.

Here we show that micron-sized particles can be self-assembled from anisotropic building blocks of cadmium-cystine complexes with widely variable geometry, chirality measures, and optical asymmetry while retaining consistent bowtie shapes. The self-limited self-organization of anisotropic building blocks driven by a competition between electrostatic and van der Waals interactions makes possible high synthetic reproducibility and computational predictability of the geometries for different concentrations of reagents, ionic components, enantiomeric compositions, media polarity and other parameters. The tunability of fabricated structures hinges on the disorder and flexibility of bonds present at the nanoscale. Correlated application of Electron microscopy, X-ray diffraction (XRD), and IR spectroscopy reveal four levels of hierarchical organization within the cadmium cystinate bowties. I will further discuss challenges and prospects in determining short range order in self-assembled structures where minor changes in bond angles can lead to massive structural changes at the micron scale.

TOWARDS HIGH CONTRAST ATOMIC RESOLUTION IN CRYOGENIC PTYCHOGRAPHY SINGLE-PARTICLE ANALYSIS (CRYO-EPTY SPA)

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Rapid advances in cryo-electron microscopy (cryo-EM) have revolutionized biological macromolecule visualization, enabling near-atomic resolution imaging in a frozen-hydrated state. However, the high electron irradiation sensitivity of biological samples results in a low signal-to-noise ratio and weak contrast in EM images. While cryo-EM single particle analysis (SPA) improves contrast, it compromises low-frequency information and inverts high-frequency information in the phase contrast transfer function (PCTF), complicating particle identification and alignment. Cryogenic electron ptychography (cryo-EPty) shows promise for high-contrast, low-dose, 3D imaging of biological samples, leveraging the entire diffraction pattern to achieve dose efficiency and, when combined with SPA, potential atomic-level resolution. Electron ptychography, particularly Multi-Convergence-Angle (MCA) Ptychography, addresses these limitations by utilizing multiple condenser apertures to modify the convergence angles during scanning. This method enhances image clarity and precision, providing both strong contrast and high resolution.

In cryo-EPty SPA, 2D phase images of rotavirus double-layer particles (DLPs) and apoferritin were successfully reconstructed, with high-resolution 3D maps achieved at sub-nanometer levels. The resolution was further enhanced by increasing convergence semi angle (CSA), indicating the potential for atomic-level resolution.

In MCA-ptychography, SCA-ptychographic results at smaller convergence angles provided strong contrast but limited resolution, whereas larger angles achieved higher resolution with weaker contrast. MCA-ptychography combined these advantages, maintaining robustness at low electron doses while delivering strong contrast and high resolution.

Combining cryo-EPty with SPA offers an alternative method for high-resolution 3D reconstructions of biological samples, potentially reaching atomic resolution. MCA-ptychography, by incorporating multiple convergence angles, provide enhanced 2D projection images and improving 3D reconstruction methods. Integrating MCA-ptychography with cryo-EM will further advance structural biology, facilitating more precise imaging for disease research and drug development.

IMAGEING THE CRITICAL ROLE OF STATIONARY ATOMS DURING SOLIDIFICATION OF PT-NANOCLUSTERS

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Solidification of liquids is a complex process where atoms transition from random motion to an ordered crystalline structure. The onset of nucleation is crucial for determining the structure and properties of the resulting solid, but it is challenging to capture, describe, and control. High-resolution transmission electron microscopy was used to image Pt nanoclusters. Using MEMS chips to control the temperature, Pt nanoclusters were molten in situ, allowing the investigation of the atomic mechanisms of solidification. We discovered that molten nanodroplets contain a small number of stationary Pt atoms, while the others move freely in the liquid. The electron beam can significantly increase the number of stationary atoms, which impacts the solidification process. When the number of stationary atoms is low, classical one-step nucleation mechanisms occur. However, an increase in stationary atoms drastically alters the solidification pathways. When the liquid nanodroplets are surrounded by a corral of stationary Pt atoms, crystallization is effectively halted. Surprisingly, these corralled nanodroplets remain liquid at temperatures several hundred degrees Celsius below the normal crystallization temperature of Pt nanoparticles before transforming into an amorphous solid instead of the usual face-centered cubic phase. Our study demonstrates the crucial role of stationary atoms in the solidification of liquids and the subsequent formation of solid phases.

OVERCOMING CHALLENGES IN CRYO-EM FOR DIVERSE PROTEIN TYPES: ADVANCES IN SAMPLE PREPARATION AND IMAGING

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Cryo-electron microscopy (cryo-EM) is essential for studying diverse protein types, each presenting distinct challenges. Soluble proteins, especially large or flexible complexes, often adopt multiple conformations, complicating the creation of homogenous samples necessary for high-resolution structures. Membrane proteins, due to their hydrophobic nature, require stabilization in membrane-mimicking environments like detergents or lipid nanodiscs, making sample preparation particularly challenging. Their inherent flexibility further complicates achieving clear images. Small proteins under 100 kDa have traditionally been difficult for cryo-EM due to low electron density, but recent advances in detectors and image processing have significantly improved their visualization. Amyloid filaments, with their elongated, rigid structures and structural polymorphism, require careful separation and individual analysis of different filament types. Ensuring sufficient protein concentration and preventing preferred orientation on grids are critical challenges across all these protein types. Additionally, protein complexes, often large and heterogeneous, pose difficulties in maintaining structural integrity and resolving individual components due to variability and flexibility.

Despite these challenges, advancements in cryo-EM sample preparation and imaging techniques have significantly broadened its applicability, allowing for detailed structural insights across different protein classes. Continued innovation is crucial to overcoming these obstacles and advancing our understanding of protein structure and function.

SEM: ADVANCING FROM 2D TO 3D IMAGING THROUGH AI

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Scanning Electron Microscopy (SEM) is the standard method of choice for many high-resolution imaging applications in the physical sciences. SEM provides 2D images, but often, understanding the sample requires a 3D image. For obtaining 3D information, methods such as Atomic Force Microscopy (AFM) are available, which, although very high resolution, take a long time. Attempts to make SEM images 3D include techniques such as shape from shading, stereo imaging, photogrammetry, and focus variation. However, these methods are not always reliable and cannot be effectively used for quantitative analysis.

We propose a method enabled by artificial intelligence (AI). In this method, we will train a machine learning algorithm to yield the 3D SEM image of a sample surface from several 2D images taken at different angles. The machine learning algorithm will be trained with a number of training data points. Each training data point consists of several 2D SEM images from the sample of interest and 3D surface profile data obtained using confocal microscopy and AFM. To ensure diverse training data, various surface profiles are created using a laser material processing system that enables the creation of surface profiles with different diversities with high control and precision. We will use different types of samples to make this algorithm more universal.

DEVELOPING A DIGITAL TWIN FOR SCANNING ELECTRON MICROSCOPY

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The use of Scanning Electron Microscopy (SEM) is prevalent across many scientific and industrial applications. We propose to develop a digital twin for SEM with the following intent and objectives: The digital twin, empowered by physics-based simulations (using electron beam tracing and Monte Carlo simulation of electron interactions within the sample's atomic structure), will allow users to realistically simulate the SEM imaging process in a virtual environment.

One major benefit of this digital twin is the avoidance of time-consuming and expensive trial and error processes for optimizing imaging practices. Many scenarios of imaging can be automated and simulated virtually, and the best one can be selected before trying it on the physical machine. This can significantly enhance efficiency and reduce costs. Another significant advantage is training on SEM. SEM machines are expensive and can be damaged due to misuse, posing restrictions on training, especially for young students and scientists. With these digital twins, training can be conducted virtually, without any risk to the actual machine. Once adequately trained, users can switch to the physical use of the machine with confidence.

Additionally, the digital twin allows for the virtual creation of SEM images of virtual samples, whose inner and exterior structures are known. These pairs of images and structures (CAD models) can be used as training data for machine learning algorithms aimed at automated image segmentation. With this method, every image created virtually is inherently labeled, allowing for the easy production of training data without the cost of using a physical machine.

In summary, our proposed digital twin for SEM, supported by physics-based simulations, offers several benefits, including optimizing imaging practices, safe and effective training, and the generation of labeled training data for machine learning applications.

IN SITU FORMATION OF METAL-ORGANIC FRAMEWORKS AND OPTIMIZATION OF THEIR STRUCTURES IN RESPECT TO MINIMIZING THEIR INTERACTION WITH THE ELECTRON BEAM OF THE TEM

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Two-dimensional polymers, as layer-stacked conjugated metal-organic frameworks (2D c-MOFs) spark enormous interest nowadays due to their structure-dependent properties. By linking different node molecules with metal atoms, a manifold of structures and hence, a large variety of properties is possible. Understanding their atomic structure is crucial for correlating structure and properties. In this study, we investigate the key properties contributing to the electron beam resilience of thoughtfully tailored c-MOF structures and explore the effects of chemical composition, pore size, and conductivity on the electron beam stability. We found that the hydrogen free c-MOF Cu_3(BHT) (BHT = benzenehexathiol), expresses high electron beam resilience unlocked through its high electrical conductivity. Leveraging this high electron beam resilience, we achieve sub-angstrom resolution in high-resolution transmission electron microscopy (HRTEM) experiments, at an accelerating voltage of 80 kV using our spherical and chromatic aberration-corrected TEM (SALVE (Sub-Angstrom Low-Voltage Electron Microscopy)). Furthermore, utilizing this highly stable c-MOF enables us to perform in situ HRTEM studies, capturing the heat-induced structural dynamics of Cu_3(BHT). In addition, we study the formation process of Cu_3(BHT) and perform in situ liquid-cell transmission electron microscopy (LC-TEM) experiments. Surprisingly, the growth here results in the formation of Cu_4(BHT) instead of the expected Cu_3(BHT). We explain this difference by considering radiolysis taking place during the interaction of the electron beam with the solutions inside the liquid cell.

SHEDDING LIGHT ON THE MICROBIAL DARK MATTER: ELECTRON MICROSCOPY OF SR1, A RARE AND UNCULTURED PARASITIC BACTERIAL MEMBER

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The Candidate Phyla Radiation (CPR) is a vast and enigmatic group within the bacterial domain characterized by their minimalistic genomes, and cell sizes. Despite their ubiquity and potential ecological significance, the majority of CPR bacteria (also known as Patescibacteria superphylum) remain uncultured, largely due to their dependencies on host organisms and unique environments. This has limited our understanding of their biology and interactions within microbial communities.

SR1/ Absconditabacteria are particularly elusive members of this group due to their parasitic behaviors. These have extremely reduced genomes (<1.5 Mbp) and ultra-small cell sizes (<0.5 uM). There are only 2 known members of SR1, *Ca.* Absconditicoccus praedator and *Ca.* Vampirococcus lugosii, both are known to attach and extract nutrients from its prey exhibiting a vampiric lifestyle, feeding on the cytoplasm of host bacteria.

Our SR1, found in the enrichments collected from the sulfidic Zodletone Springs in Oklahoma, is the first known member that is seen to be attaching to a Deltaproteobacteria- namely, a *Desulfomicrobium* sp.

Given the challenges associated with cultivating SR1 bacteria, electron microscopy offers a vital tool for directly observing the physical interactions between the two bacteria. Detailed visualization of the unique cellular structures and parasitic mechanisms of SR1 members provide tangible clues that complement genomic data, offering deeper insights into their biology and interactions.

In this project, we have employed Scanning Electron Microscopy techniques that lead us to the hypothesis- “SR1 bacteria, after attaching to their host *Desulfomicrobium* sp., undergo cell division to form stacked cell structures that subsequently invade the host's periplasmic space, utilizing this niche to extract nutrients and sustain their parasitic lifestyle”. To test this hypothesis, we will further integrate visual data from TEM, and Cryo-EM, alongside various microbiological techniques, to comprehensively analyse the parasitic behaviour and interactions of SR1 bacteria with their host.

A LASER PHASE PLATE FOR CRYO-ELECTRON MICROSCOPY

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Cryo-electron microscopy can visualize biomolecules at high resolution in a near-native state, but the technique is limited by the low contrast of specimens together with their sensitivity to electron radiation. To maximize image contrast, a phase plate can be employed to apply an additional delay between the scattered and unscattered beams in a diffraction plane of the microscope. While this approach is widely used in optical microscopy, phase plates developed for electron microscopy have been prone to being charged by or scattering the electron beam, leading to unstable behavior and resolution loss.

To overcome these limitations, we have developed a laser-based phase plate (LPP). A laser beam, amplified and focused by a Fabry-Pérot optical cavity, intersects the unscattered electron beam to impart the desired phase shift. The LPP provides a stable phase shift over hours while keeping material objects far from the electron beam path to avoid scattering and decoherence.

The reliability of the LPP paves the way for several future directions in cryo-electron microscopy and tomography. Improved contrast at low spatial frequencies enables detection of small particles inaccessible to conventional (defocus-based) phase contrast imaging, as well as superior correction of beam-induced motions. Additionally, the LPP eliminates the need for specimen defocus and, in combination with a spherical aberration corrector, permits near-perfect contrast transfer over many orders of magnitude in resolution (from ~100 nm to ~0.1 nm). Simultaneous visualization of both small and large structural features promises to benefit cryo-electron tomography by providing high-resolution information together with larger-scale context.

We show our progress on single-particle reconstructions and tomography with the LPP and discuss prospects for the new imaging modalities enabled by the LPP based on a combination of experiments and simulations.

OPTIMIZING MINERAL CARBONATION: INSIGHT INTO SURFACE FUNCTIONALIZATION INDUCED ENHANCED CARBONATION OF MAGNESIUM OXIDE-RICH MINE TAILINGS

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The growing demands of industrialization and development highlight the critical need for an efficient and scalable Carbon Capture and Storage (CCS) approach. Mineral carbonation of MgO emerges as a promising solution due to its significant theoretical capacity for CO_2 adsorption. However, the slow kinetics of the carbonation process remain a major obstacle. Therefore, a deep understanding of the structural and chemical changes that occur during carbonation is essential for effective material design. In this study, we carry out a detailed ex-situ structural and chemical analysis of the MgO carbonation process, using samples sourced from various mine tailings, with the help of electron microscopy techniques including TEM, STEM and EELS. Our findings reveal that treating MgO with polar solvents substantially increases its carbonation efficiency, providing a promising pathway for improvement. Additionally, we observe that the carbonation rate of MgO is influenced by particle size and that the addition of Si-based compounds further accelerates the reaction kinetics. Ab-initio Density Functional Theory (DFT) calculations shed light on the mechanisms behind the enhanced carbonation of polar solvent-treated MgO, showing a stronger interaction between CO_2 and the treated MgO (100) surfaces. These findings present an alternative strategy for improving MgO carbonation, potentially offering a more effective method for sequestering atmospheric CO_2 using MgO-rich mine waste.

NON-AVERAGED 3D STRUCTURE OF A SINGLE BIOMOLECULE BY CRYO-ELECTRON TOMOGRAPHY

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Although the atomic structure of biomolecules can be determined by current techniques such as X-ray crystallography and cryo-electron microscopy (cryo-EM), achieving a low-resolution 3D structure of some biomolecules with flexible structures and continuously changing conformations—such as lipoproteins, antibodies, and nucleotide nanostructures—remains challenging. This difficulty arises because current techniques typically require an averaging process, wherein a small population of homogeneous particles must be pre-selected from a large pool and then averaged to produce a static 3D structure. While averaging can improve the signal-to-noise ratio and achieve high-resolution structures of rigid-body portions, it can also result in the loss of density in flexible portions/domains and produce an anisotropic resolution distribution on the averaged 3D map. Notably, the structures of most unselected particles remain elusive.

In recent years, we have enhanced the cryo-electron tomography (cryo-ET) technique for determining the 3D structure of a single biomolecular particle without averaging from different particles, called individual-particle electron tomography (IPET) (Zhang et al. in PLoS One, 2012;7(1)). However, due to the extremely weak signal from a single biomolecule caused by the electron dose limit necessary to prevent radiation damage, only the low-resolution of a single biomolecule can be achieved (up to 2 nm), which is barely sufficient to reveal the tertiary structure of nucleotide nanoparticles during their processes of self-folding and intramolecular conformational changing.

In this study, we demonstrate the implementation of the IPET technique in investigating the inner and intramolecular conformational changes of tetra-nucleosome arrays during phase transition processes (Zhang, M., et al., Nat Commun, 2024, 15, 4395 (2024); Mol Cell. 2022, 82(16):3000-3014.e9). We found that the crucial parameter determining the structure adopted by chromatin arrays is the angle between the entry and exit of the DNA and the corresponding tangents to the nucleosomal disc. Additionally, we discovered that the transition involves the exposure of nucleosome hydrophobic surfaces, leading to modified inter-nucleosome interactions. These results provide insights into the initial stages of intra-array compaction and suggest a critical precursor to condensation in the regulation of chromatin organization, and a physical mechanism by which chromatin may transition from interphase to metaphase structures.

LAYER-BY-LAYER ATOMIC STRUCTURE IN VAN DER WAALS FERROELECTRICS VIA MULTISLICE ELECTRON PTYCHOGRAPHY

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Two-dimensional (2D) van der Waals (vdW) materials are noted for their robust intralayer bonding and weak interlayer interactions. This unique combination allows for flexible interlayer sliding under lattice strain, leading to emergent properties, including strong electronic correlations, novel topological states, and tunable ferroic behaviors. Our previous study has discovered unique strain and stacking in vdW ferroelectric (FE) materials. However, visualizing the layer-by-layer atomic structure remains challenging.

In this work, we utilize multi-slice electron ptychography (MEP) to investigate layer-by-layer lattice structures of 2D vdW FEs with sub-angstrom in-plane resolution and nanometer-scale resolution along the z-axis. The relative sliding of atoms between different layers leads to the coexistence of FE and antiferroelectric (AFE) stacking orders, resulting in a unique FE-AFE domain wall both laterally and vertically. By applying polarization mapping to the 3D ptychographic reconstructions across these FE and AFE domains, we identified a distinctive sequence of alternating charged and uncharged 90º domain walls along the z-axis. Additionally, the ferroelastic strain varies between the top and bottom layers, inducing a polarization vortex along the z-axis. The results offer insights into the structural engineering and switching mechanisms of vdW FE materials, paving the way for their applications in electronic devices.

FAST AND ROBUST ALIGNMENT OF DEFORMABLE IMAGES IN FILTERED SLICED WASSERSTEIN DISTANCE

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Image alignment is a central task in many image processing pipelines. In cryo-EM, it is a critical step for the 3-D reconstruction of molecules from tomographic projection images. One advantage of cryo-EM is that the molecules do not need to be locked in a single confirmation, and instead exist in a continuum of states. However, these different states produce heterogeneous images which are challenging for conventional alignment algorithms based on Euclidean distance. In this work, we present a fast algorithm for aligning images using optimal transport. Our method is based on the sliced Wasserstein distance and computes the 1-D Wasserstein distance between radial line projections of the input images. Using this approach, we develop an algorithm that can align two $L \times L$ images in $O(L^2 \log L)$ operations. We show that our method is robust to rotations, translations and deformations in the images.

PRECISE CANCER THERANOSTICS VIA BIO-RESPONSIVE SELF-ASSEMBLY NANOCOMPOSITES

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The ultrasensitive biorecognition for the early diagnosis / monitoring of some important disease like cancers has attracted much attention in the relevant fields, especially as a hot topic in the relative areas involving in patient care and treatments. In this study, we have explored the possibility of the application of some supramolecular probes combining with the bio-responsive self-assembly nano-scaled probes in targeting bio-recognition and high-sensitive biosensing / bioimaging of the diseased locations. Especially, we have developed some novel strategies for the fast and highly sensitive recognition of the targeted biomolecules and cancer cells / exosomes by combining the active agents and functionalized nanointerfaces with multiscale theranostics. Our observations demonstrate that the *in situ* self-assembly of the specific supramolecules with the nano-probes could provide a multifunctional nanostructure or interface for the rapid identification and imaging of cancer cells / exosomes, with a broad detection range and low detection limit. It is evident that different types of cancer cells could be readily distinguished on the bio-responsive spectra-electrochemical nano-interfaces, which have the promising application to be adopted as a significant way to detect various kinds of mutant cells / tissues or related exosomes *in vivo* and advance the clinic diagnosis and treatment of some important diseases. Moreover, some ultrasensitive and intelligently multi-functional nanoscale biomarkers based on the *in vivo* self-assembly bio-synthetic nanoclusters through utilizing the peculiarities of the microenvironment and relevant redox homeostasis from cancer cells or bacteria have been also explored for the real-time multi-scale monitoring and multi-modality imaging of cancer cells or affected tissues for precise theranostics.

ATOMIC-SCALE CONTROL AND DETECTION OF FERROMAGNETIC PHASE TRANSFORMATION BY USING ATOMIC-SCALE PROBE

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Controlling and detecting ferromagnetic phase transformations at high spatial resolutions are crucial for advancing our understanding of spintronics and high-density information storage applications. Traditional methods for achieving these transformations typically involve thermal treatment or chemical agents, which can significantly alter the thermodynamic phase diagram of bulk compounds. However, these methods have a fundamental limitation for local modification, as the entire sample is subjected to the same environment. Alternative approaches, such as using light excitation, biasing, or scanning tip-based methods, have been proposed and demonstrated to manipulate thermodynamic stability at the microscale. Nevertheless, achieving control at the nanoscale remains challenging due to the intrinsic length scale constraints of these methods. In this work, we propose a unique method using a high-resolution electron beam to control and detect the transition from non-ferromagnetic to ferromagnetic phases at the atomic level. We demonstrate that an atomic probe can initiate the phase transition between the rock salt and spinel structures in NiFe2O4. The electron beam allows for precise control of this transition, enhancing the material's properties at high spatial resolutions. The transition between ferromagnetic and non-ferromagnetic phases can be both controlled and imaged at the atomic scale. Furthermore, the ferromagnetic signal can be detected at the nanoscale using electron magnetic circular dichroism (EMCD), enabling the manipulation and detection of ferromagnetic phases with high spatial precision. Our study also provides insights into the mechanisms behind the ferromagnetic transition. Imaging of light elements revealed that the oxygen network in the rock salt films undergoes structural distortions, and transitional metal cations migrate through various lattice sites. These movements are facilitated by the presence of cation vacancies and lead to the formation of the ferromagnetic spinel phase when the rock salt films are exposed to an electron beam. This atomic-scale engineering enables potential applications in magneto-optic-based information storage and related devices.

REVEALING FLUIDIC TRANSPORT UNDER EXTREME CONFINEMENT AT THE NANOSCALE

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Fluids under extreme confinement exhibit distinctly new properties compared to their bulk analogs. Understanding the structure and intermolecular bonding of confined water lays the foundation for creating and improving applications at the water-energy nexus. However, probing confined water experimentally at the length scale of intermolecular and surface forces has remained a challenge. Here, we report a combined experiment/theory framework to reveal changes in H-bonding environment with nanoscale resolution and the underlying molecular structure of confined water inside individual carbon nanotubes. H-bonding is directly probed through the O-H stretch frequency with vibrational electron energy-loss spectroscopy and compared to spectra from molecular-dynamics simulations based on density-functional-theory. Experimental spectra show that water in larger carbon nanotubes exhibit the bonded O-H vibrations of bulk water, but at smaller diameters, the frequency blueshifts to near the 'free' O-H stretch found in water vapor and hydrophobic surfaces without bimodal distribution, indicating a total disruption of the H-bond network of confined water. The matching simulations reveal that, in addition to steric confinement, vibrating tube as a molecular-level flexible channel plays a key role in breaking up the H-bond network through fluctuation/breathing mode, resulting in an orientationally-dispersed, non-H-bonded phase. Furthermore, the temperature-dependence of the vibrations is investigated, providing insights into phase transitions and the confined-water density. This research demonstrates the potential of the experiment/theory framework to unveil molecular-level structure and bonding in confined fluids.

IN-SITU CRYO-EM STRUCTURAL STUDIES OF EOSINOPHIL GRANULES

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Eosinophils (EOS) are granulocytic white blood cells that participate in innate and humoral immune responses and inflammatory disorders. Upon activation, EOS release basic cytotoxic proteins as active effectors from their secretory granules to the extracellular space. Among them, major basic protein-1 (MBP-1) is noteworthy because it is the most abundant intragranular protein and forms crystalline cores within each granule. Little is known about the formation and assembly of these nanocrystalline cores. To investigate the dynamic and heterogeneous organization of crystalline MBP-1 within comprehensive cellular landscapes, we developed a correlative single-cell and single-granule profiling workflow. We applied cryo-focused ion beam (cryo-FIB) milling, montage cryo-electron tomography (MPACT), and micro-electron diffraction (micro-ED) to directly study human EOS. In this work, we report on the *in-situ* structure of crystalline MBP-1 within intact cytosolic granule. Our results provide insights into the degranulation process and highlight the importance of *in-situ* macromolecular structure determination.

ELECTRON MICROSCOPY CHARACTERIZATION OF 2D TITANIUM CARBIDE AND CARBONITRIDE MXENES

Yihui Zhang[1], Teng Zhang[2,3], Rajeev Kumar Rai[1], Francesca Urban[2,3], Stepano Ippolito[2,3], Danzhen Zhang[2,3], Yury Gogotsi[2,3], Eric Stach[1]

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MXenes, two-dimensional transition metal carbides and carbonitrides, have attracted global research interest due to their unique properties and tailoring capabilities. Their exceptional electronic conductivity makes them particularly promising for electronics and communications applications. However, synthesizing carbonitrides with precisely controlled N/C ratios and layer numbers has been challenging. Recent advances by Zhang et al.[1] have opened new avenues for fundamental studies and property fine-tuning of these materials. Despite substantial research efforts, fundamental aspects of MXenes' structure, defects, and surface terminations remain unexplored. Advanced scanning transmission electron microscopy (STEM) and electron energy loss spectroscopy (EELS) techniques with sub-atomic resolution are crucial for elucidating these properties. While MXenes encompass diverse chemistries and structures, few studies have addressed the relationship between chemical composition, atomic defects, chemical bonding, and stability under thermal exposure and electron beam irradiation.

This work presents the first systematic experimental study employing STEM and EELS to investigate freestanding monolayers of $Ti_3(C_{1-y}N_y)_2T_x$ ($0 \leq y \leq 0$) where T represents surface terminations of O, OH, or F, with additional -Cl termination. We also explore the stability of $Ti_3(C_{1-y}N_y)_2T_x$ under thermal exposure and electron beam irradiation using *in situ* heating STEM/EELS from room temperature to high temperature. Our results reveal the fundamental relationship between nitrogen concentration, titanium vacancy concentration, and surface termination. We quantitatively and qualitatively analyze the Ti $L_{2,3}$ edge and C/N/O K edges from EELS, providing insights into the nature of chemical bonds in these carbides/carbonitrides. This comprehensive study contributes to the broader understanding of this promising class of 2D materials by elucidating their fundamental properties. Our findings highlight the potential for improving MXene stability and expanding their applications through a deeper understanding of their atomic-scale structure and behavior under various conditions.

[1] *J. Am. Chem. Soc.* 2023, 145, 41, 22374-22383

Speaker Abstracts

Abstracts appear in this book in presentation order. Below is an alphabetical list of speakers by last name and the corresponding page for their printed abstracts.

Speaker	Page No.
Bridget Carragher	31
Beatriz Roldan Cuenya	39
Rouslan Efremov	33
Pratibha Gai	37
Nathan Gianneschi	43
Kathryn Grandfield	42
Yimo Han	26
Jessica Heebner	36
Pinshane Huang	25
Jingyue Liu	40
Paolo Longo	29
Bronwyn Lucas	28
Hosea Nelson	34
Kostya Novoselov	24
Joe Patterson	41
Tingying Peng	30
Hong-Wei Wang	32
Yao Yang	38
Peijun Zhang	35
Yimei Zhu	27

POSTER NUMBER & FLASH TALK SESSION ASSIGNMENTS

Poster Session Location: **Friend Center Convocation Room**
Princeton University
Princeton, NJ USA

POSTER SESSION:	Thursday, October 17th, 5:30 – 7:00 p.m.
FLASH TALK SESSION A:	Wednesday, October 16th, 12:00 – 12:30 p.m.
FLASH TALK SESSION B:	Wednesday, October 16th, 4:30 – 5:00 p.m.

1. Posterboards will be numbered and located in the Friend Center Convocation Room.
2. We will provide binder clips to hang up your poster.
3. Please ensure that your poster dimensions are **30" x 40" maximum**.
4. **Poster numbers are subject to change**. Updated lists will be sent out periodically (if needed), but the final list will be at the Registration Desk onsite for you to double check.

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*Some authors are presenting multiple posters. The **Notes** column contains the first few words of the poster title to distinguish which poster corresponds to which Poster Number.*

Flash Talk Session	Poster Number	First Name	Last Name	Notes
A	1	Mohamed	Abouleish	
A	2	Tamir	Bendory	
A	3	Todd	Brintlinger	
A	4	Valerie	Brogden	
A	5	Pengcheng	Chen	
A	6	Cheng-Yu	Chen	
A	7	Guangming	Cheng	*"Revealing the atomic..."*
A	8	Guangming	Cheng	*"In situ study..."*
A	9	Jonathan	Choy	
A	10	Jeremy	Conway	
A	11	Jingshan	Du	
A	12	Radoslav	Enchev	
A	13	Dingxin	Fan	
A	14	Sarina	Hasan	
A	15	Cathy	Hou	
B	16	Sungho	Jeon	
B	17	Rachael	Keneipp	
B	18	Hyeongjun	Koh	
B	19	Aaron	Kuan	
B	20	Arek	Kulczyk	

B	21	Prashant	Kumar	
B	22	Yu	Lei	
B	23	Christopher	Leist	
B	24	Bo	Liang	
B	25	David	Mücke	
B	26	Sayali	Mulay	
B	27	Petar	Petrov	
B	28	Rajeev	Rai	
B	29	Gang (Gary)	Ren	
B	30	Chuqiao	Shi	
B	31	Emanuel	Marcus	*"SEM: Advancing..."*
B	32	Emanuel	Marcus	*"Developing a digital..."*
B	33	Eric	Verbeke	
B	34	Xuemei	Wang	
B	35	Kun	Xu	
B	36	Xintong	Xu	
B	37	Jae	Yang	
B	38	Yihui	Zhang	

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Delegates are listed below in ALPHA order by Last Name.

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Bendory	Tamir	Tel Aviv University
Benjamin	Martindale	Nature Catalysis
Bouvette	Jonathan	Princeton University
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Carter	Liz	Angstrom Scientific Inc.
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Gavin	Dustin	-
Gianneschi	Nathan	Northwestern University
Gilles	Marc Aurèle	Princeton University
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Grandfield	Kathryn	McMaster University
Han	Yimo	Rice University
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He	Xu	Princeton University
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Kok	Hui Taou	Princeton University
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Kuan	Aaron	Yale School of Medicine
Kulczyk	Arek	Rutgers University
Kumar	Prashant	Nanyang technological university
Lei	Yu	University of Warwick
Leist	Christopher	Ulm University
Liang	Bo	Emory University School of Medicine
Ling	Xiaobin	Umass Chan Medical School
Liu	Fangyuan	Yale University
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Petry	Sabine	Princeton University

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