BioXFEL

AGENDA

All Times are given in Eastern Standard Time (EST)
30 minute talks include a 10 minute discussion
20 minute talks include a 5 minute discussion

Time	Session
	SUNDAY, JANUARY 31ST, 2021
10:00 am – 3:00 pm	**Fundamentals of Negotiating Workshop**
	MONDAY, FEBRUARY 1ST, 2021
11:00 am – 3:20 pm	**Scientific Advisory Board Meeting**
10:00 am – 2:30 pm	**Interview Skills Workshop**

8TH BIOXFEL INTERNATIONAL CONFERENCE

Time	Session
	TUESDAY, FEBRUARY 2ND, 2021
11:00 am – 11:10 am	**Conference Welcome** **Conference Co-Chairs: Sabine Botha (ASU) & Matthias Frank (LLNL)**
11:10 am – 12:00 pm	**Science Keynote: 10 Years of Serial Femtosecond Crystallography** **Henry Chapman (DESY)**
12:00 pm – 12:45pm	**Lunch Break**
12:45 pm – 2:05 pm	**From Sample to Structure** **Session Chairs: Arwen Pearson (U Hamburg) & Iris Young (UCSF)** Session sponsor: MiTeGeN
12:45 pm – 1:15 pm	**Chris Kupitz (SLAC)** XFEL experiments: from sample preparation to injection
1:15 pm – 1:45 pm	**Alexandra Tolstikova (DESY)** Data analysis in serial crystallography: current state and future perspectives.
1:45 pm – 2:05 pm	**Chenghua Shao (PDB)** 50 Years of the protein data bank and impact of XFEL/SSX.
2:05 pm – 2:30 pm	**Break**
2:30 pm – 4:00 pm	**Facilities & Remote Operations Panel Discussion** Petra Fromme (ASU), Allen Orville (Diamond), Kristina Lorenzen (XFEL), Kensuke Tono (SACLA), Chris Milne (SwissFEL), Fred Poitevin (LCLS) **Session Chairs: Adrian Mancuso (XFEL) & Christina Schmidt (XFEL)**
4:30 pm – 5:30 pm	**Poster Session**

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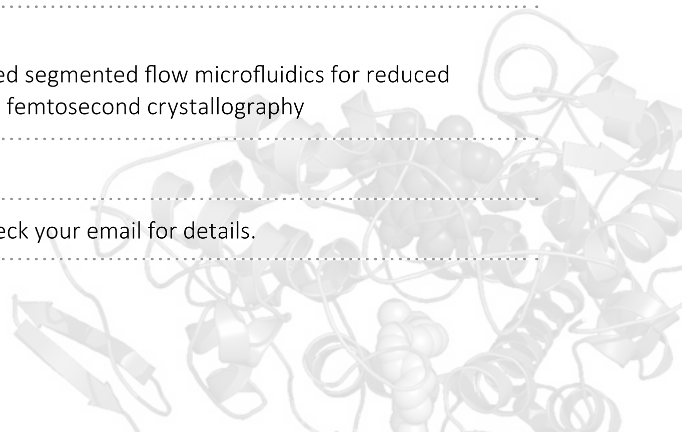
AGENDA, continued

All Times are given in Eastern Standard Time (EST)

	WEDNESDAY, FEBRUARY 3RD, 2021
11:00 am – 12:30 pm	**Fundamental Insights & New X-ray Technology** **Session Chairs: William Graves (ASU) & Anais Chretien (XFEL)**
11:00 am – 11:30 am	**Martin Hogbom (Stockholm)** Toward geometric structures of high-valent metal-oxygen intermediates in di-metal protein cofactors by XFEL crystallography.
11:30 am – 12:00 pm	**Ilme Schlichting (MPI-H)** Protein structure and dynamics using X-ray free-electron lasers - pitfalls
12:00 pm – 12:30 pm	**Franz Kaertner (CFEL)** Compact terahertz driven electron and X-ray sources
12:30 pm – 1:30 pm	**Lunch Break**
12:30 pm – 1:00 pm	**Educational Opportunities for Students and Postdocs: Lunch**
1:30 pm – 2:30 pm	**Educational Keynote: Reimagining medicine through the lens of D&I** **Anastacia Awad (Novartis)** **Session Chairs: Bill Bauer (HWI) & Abigail Kosgei (Rice)**
2:30 pm – 4:55 pm	**Scientific Advances, Methods, & Data** **Session Chairs: Keith Moffat (U Chicago) & Rebecca Jernigan (ASU)**
2:30 pm – 3:00 pm	**Doeke Hekstra (Harvard)** Info to come
3:00 pm – 3:30 pm	**TJ Lane (DESY)** Revealing allosteric pathways via large-scale crystallographic studies
3:30 pm – 3:45 pm	**Break**
3:45 pm – 4:15 pm	**Aaron Brewster (LBL)** Toward metallo-chemical insights using per-pixel modeling of SFX images
4:15 pm – 4:35 pm	**Deepshika Gilbile (UC Davis)** Versatile cyclic olefin copolymer (COC) fixed-targets for hydrated, room-temperature serial protein crystallography.
4:35 pm – 4:55 pm	**Diandra Doppler (ASU)** Electronically stimulated hybridized segmented flow microfluidics for reduced sample consumption during serial femtosecond crystallography
5:00 pm – 6:00 pm	**Poster Session**
7:00 pm	**Social** Join us for Trivia Night! Check your email for details.



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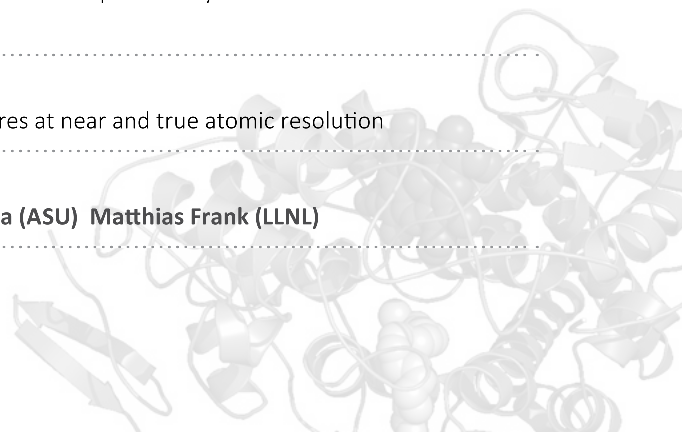
AGENDA, continued

All Times are given in Eastern Standard Time (EST)

THURSDAY, FEBRUARY 4TH, 2021

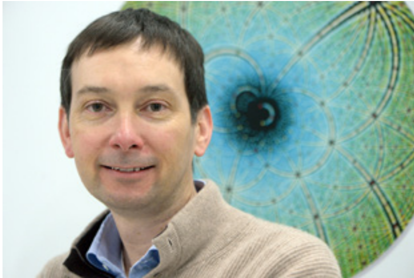
Time	Session
11:00 am – 11:50 pm	**Time-resolved Crystallography, COVID-19** **Session Chairs: Sarah Bowman (HWI) & Mukul Sonker (ASU)**
11:00 am – 11:30 am	**Marius Schmidt (UWM)** New results from time-resolved crystallographic experiments at XFELs
11:30 am – 11:50 pm	**Jeffrey McKinney (UB)** Photoswitching of long-range vibrational modes in orange carotenoid protein
11:50 pm – 1:00 pm	**Lunch Break**
1:00 pm – 1:50 pm	**Time-resolved Crystallography, COVID-19 Part II**
1:00 pm – 1:20 pm	**Swagatha Ghosh (Gothenberg)** Snapshots of structural intermediates during CO-dissociation process in *ba3*-type cytochrome c oxidase
1:20 pm – 1:50 pm	**Brenda Hogue (ASU)** Viroporin structure and function – targets for therapeutic development
1:50 pm – 2:20 pm	**Petra Fromme (ASU)** Serial fs crystallography of the SARS-CoV2 riboendonuclease NendoU
2:20 pm – 2:30 pm	**Sponsor Talk: Rayonix** rayonix
2:30 pm – 2:45 pm	**Break**
2:45 pm – 4:05 pm	**Recent Advances in SPI and Cryo-EM** **Session Chairs: Carolin Seuring (Centre for Structural Systems BiologyH) & Roshanak Etamedpour (UWM)**
2:45 pm – 3:15 pm	**Holger Stark (MPI-Gottingen)** High-resolution structure determination of dynamic macromolecular complexes by cryo-EM.
3:15 pm – 3:35 pm	**Eduardo Cruz Cho (UWM)** Selection of XFEL single-particle diffraction patterns by geometric machine learning
3:35 pm – 4:05 pm	**Wah Chiu (Stanford)** Macromolecular cryo-EM structures at near and true atomic resolution
4:05 pm – 4:15 pm	**Closing Remarks** **Edward Snell (HWI) Sabine Botha (ASU) Matthias Frank (LLNL)**



KEYNOTE SPEAKER

HENRY CHAPMAN

Center for Free Electron Laser Science, DESY
Centre for Ultrafast Imaging, University of Hamburg
Department of Physics, Uppsala University



Ten years of serial femtosecond crystallography

At around 4 pm on the 12th of December, 2009, a diverse, international, and multidisciplinary group of scientists and engineers witnessed the first femtosecond snapshot protein crystal diffraction pattern at the AMO beamline of LCLS. During a week-long run, with wits and determination (and what in hindsight was a great deal of luck), the team collected over 3 million diffraction patterns and kicked off the field of serial femtosecond crystallography, or SFX as it came to be known. I will discuss what brought this group together to do an experiment which was never on the radar of first FEL experiments, and some of the outcomes of the continuous development of the technique since that time.

BIO:

Henry Chapman is a director of the Center for Free-Electron Laser Science at DESY and the University of Hamburg in Germany, and a visiting professor at Uppsala University. He carried out his PhD in X-ray optics at the University of Melbourne, Australia, work for which he was awarded the Bragg Gold Medal from the Australian Institute of Physics. After a postdoc at Stony Brook University, collaborating with David Sayre, he moved to LLNL to work on EUV lithography. He later brought together a group to demonstrate "diffraction before destruction" imaging at the FLASH FEL in Hamburg. Henry develops methods in coherent X-ray imaging and in exploiting the short pulse durations and extreme intensities of free-electron lasers to obtain room-temperature macromolecular structures.

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KEYNOTE SPEAKER

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ANASTACIA AWAD, PHD BIO

BIO:

As Head of Diversity & Inclusion (D&I) for the Novartis Institutes for BioMedical Research (NIBR), Anastacia Awad brings a scientific lens and strong focus on equity, inclusion, and diversity to lead strategic efforts enabling scientific innovation. She has established a focused, multi-year NIBR D&I approach that aims for broader impact in key scientific, collaborative, and social areas, including clinical trial diversity, inclusive networks, and micro-moments in talent & culture.

Anastacia sits on several leadership teams at the global, divisional, and country levels to advance the global Novartis D&I strategy for 110,000 employees worldwide, through integration of D&I into core business practices. She heads a global approach to strengthen over 60 thriving employee resource groups (ERGs) as communities of belonging and accelerators of inclusive culture. She also co-leads an enterprise-wide effort to expand racial & ethnic equity efforts globally.

Previously at NIBR, Anastacia led efforts in gender equity, allyship and advocacy, mentorship and sponsorship, early talent development, and academic outreach. She developed and implemented accelerated scientific training programs for emerging scientists and their mentors. She also worked closely with academic and industry partners to shape biomedical education and training of future scientific leaders, including those from historically under-resourced communities. She served on numerous national boards, consortia, and committees including the Diversity Advisory Committee for Keystone Symposia. Lastly, Anastacia convened a global mentoring community group to enhance mentoring & sponsoring capabilities.

Prior to joining NIBR, Anastacia earned a BS in Biology at Florida A&M University and a PhD in Genetics & Molecular Biology at the University of North Carolina at Chapel Hill. Her postdoctoral work at Memorial Sloan-Kettering Cancer Center focused on understanding molecular networks in primary brain tumors.

SPEAKER

CHRIS KUPITZ

XFEL Experiments: From Sample Preparation to Injection.

Time at an XFEL is precious, and so is your sample. This presentation will focus on crystallization methods and developments to transition from macrocrystals to microcrystals, optimizing your protein microcrystallization, and matching injection method with sample parameters to reduce waste at the experiment. There have been advancements in microcrystallization techniques primarily for use at XFELs. Some of these techniques include free interface diffusion, crystallization via concentrator, and stirring to induce rapid crystallization. Developments in the field of protein crystal characterization include equipment, such as SONICC, which are not common in most labs, but also techniques such as the crystal dissolution method, which can be done in any wetlab space.

It is not enough to prepare microcrystals in isolation without also considering the downstream applications. Sample delivery is a key component of a successful beamtime that can either be dictated by your sample crystallization or the reverse. For example, if you have a low sample volume, using a slow flow injection technique would be necessary. Or if you're interested in ultrafast time points, a technique like GDVN or MISC is required, with crystallization conditions adapted to the injectors. This presentation will prepare you to use a holistic approach in planning experiments, detailing the interdependence of microcrystallization and delivery, and the techniques for doing so.

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SPEAKER

ALEKSANDRA TOLSTIKOVA

Deutsches Elektronen Synchrotron DESY, Notkestrasse 85, Hamburg 22607, Germany

Data analysis in serial crystallography: current state and future perspectives.

Serial crystallography (SX) is based on merging data from single diffraction patterns collected from randomly-oriented crystals, which differs significantly from the conventional rotation crystallography and therefore requires specific data analysis methods. After ten years of continuous development, SX data processing software has reached a point where it can routinely process huge amounts of data acquired in SX experiments and produce reliable structure factors for protein structure determination. However, we are yet to achieve the desired speed and level of automatization compared to what has been achieved in conventional crystallographic software. This is in large part due to the large number of analysis parameters which require expert user input, as well as multiple practical challenges such as perpetual issues with detector geometry and detector calibration which necessitate expert user supervision and quality control during data processing.

However, continued reliance on manual data processing is not sustainable. As facilities move to new detectors and higher acquisition rates, storing large amounts of raw data becomes unsustainable and real-time data reduction and analysis are becoming a necessity. In this presentation, I will give an overview of the current state and main challenges of SX data analysis, present recent developments in Cheetah software for SX data reduction and analysis, and discuss our current efforts and future possibilities for moving towards near real-time processing.

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SPEAKER

CHENGHUA SHAO

Chenghua Shao, Jasmine Y. Young, Ezra Peisach, Zukang Feng, John D. Westbrook, Yuhe Liang, Irina Persikova, and Stephen K. Burley

RCSB Protein Data Bank, Institute of Quantitative Biomedicine, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, USA.

50 Years of the Protein Data Bank and Impact of XFEL/SSX.

Approaching the 50th anniversary, the Protein Data Bank (PDB) is the single global repository for experimentally determined three-dimensional structures of biological macromolecules and their complexes with ligands, etc. The Worldwide Protein Data Bank (wwPDB) is the international collaboration that manages the PDB archive according to the FAIR Principles: Findability, Accessibility, Interoperability, and Reusability. The PDB archive currently holds and freely disseminates more than 170,000 experimentally-determined structures of biological macromolecules. Consistent year-on-year growth in the number of structures coming into the PDB from X-ray Free-Electron Laser (XFEL) facilities has been observed during the past few years. Current PDB holdings include ~500 XFEL structures. About half of the XFEL structures were deposited in the past two years, together with >130 Serial Synchrotron Crystallography (SSX) structures (determined using diffraction data collected at the conventional light sources).

Together with wwPDB PDBx/mmCIF Working Group and experts from the XFEL user community, the wwPDB has developed new PDBx/mmCIF metadata extensions that support XFEL metadata collection using the wwPDB OneDep system for deposition, validation, and biocuration of new structures coming into the PDB. Similar metadata are also collected on Serial Synchrotron X-Ray Crystallography (SSX) structures.

Our presentation will briefly review the PDB archive growth in the past 50 years from a broad perspective of molecular contents and features. We will also focus on XFEL/SSX structures statistics, features, and classification in the PDB archive and go on to demonstrate XFEL/SSX structure deposition, validation and biocuration using the wwPDB OneDep system (https://deposit.wwpdb.org/). We will cover also metadata specific to XFEL/SSX experiments such as light source, sample delivery, and reflection data processing. The wwPDB is committed to working closely with federal funders and XFEL/SSX users to ensure faithful preservation and representation of their structures, experimental data, and metadata in the PDB archive.

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SPEAKER

Remote Facilities Panel

FRED POITEVIN

Associate Staff Scientist, Dpt of LCLS Data Analytics, LCLS, SLAC National Accelerator Laboratory

CHRIS MILNE

Leading Scientist, Femtosecond X-ray Experiments Instrument European XFEL GmbH Holzkoppel 4 the group leader of the Alvra Experimental Station at SwissFEL

KRISTINA LORENZEN

Head of Laboratory, XFEL

PETRA FROMME

Center Director and Professor, Biodesign Center for Applied Structural Discovery Regents Professor, School of Molecular Sciences Arizona State University

ALLEN ORVILLE

Wellcome Investigator and Royal Society Wolfson Fellow Principal Scientist, XFEL Hub at Diamond Light Source

KENSUKE TONO

XFEL Utilization Division, Japan Synchrotron Radiation Research Institute

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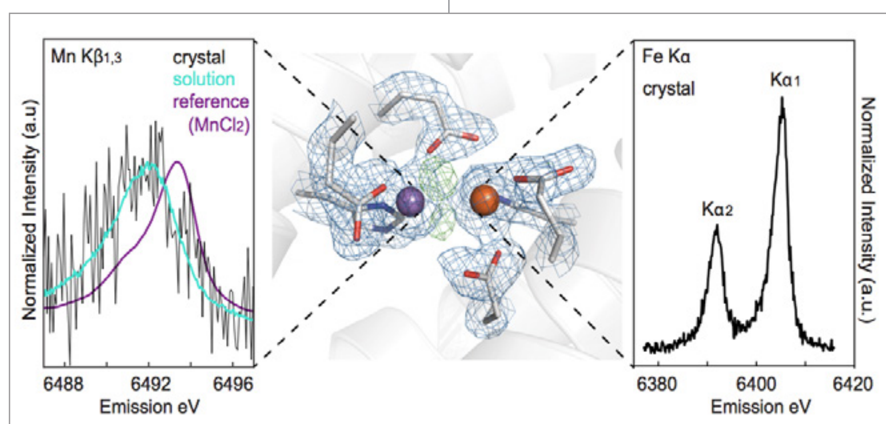
SPEAKER

MARTIN HÖGBOM

Department of Biochemistry and Biophysics, Stockholm University, 10691 Stockholm, Sweden.

Toward geometric structures of high-valent metal-oxygen intermediates in di-metal protein cofactors by XFEL crystallography.



The archetypical di-metal carboxylate proteins bind two ferrous (Fe(II)) ions to produce an oxygen-activating cofactor used for some of the most chemically challenging oxidations observed in nature. The two most well-known examples are methane monooxygenase and the aerobic ribonucleotide reductase R2. Proteins from the same superfamily utilizing heterodinuclear Mn/Fe cofactors have also been discovered[1]. Upon reaction with molecular oxygen, a number of high-valent metal-oxygen intermediates are generated. These intermediates are highly interesting for both basic and applied science but obtaining their global geometric structures has proven very challenging.

In close collaboration with scientists at the LCLS, LBNL and the university of Minnesota, a conveyor-belt sample injector-based experimental regime has been developed that allows micrometer-sized crystals to be incubated with oxygen for a defined period of time before exposure to the free-electron laser X-ray beam[2]. This setup allows varying the time for intermediate trapping while the use of femtosecond XFEL crystallography eliminates the effect of X-ray photoreduction on obtained data. Simultaneous XES also allows in situ oxidation state determination of probed intermediates.

This setup and its use to obtain high-resolution global geometric structures of high-valent intermediates will be discussed, as well as our recent progress defining radiation undamaged structures of methane monooxygenase[3] and ribonucleotide reductase R2 protein[4].

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Acknowledgments:

This work was funded by the Knut and Alice Wallenberg Foundation, the Swedish Research Council and the European Research Council (ERC).

References:

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2 F.D. Fuller et al. Nature Methods, 14(4):443-449 (2017)
3 Srinivas V. et al. J Am Chem Soc, 142:14249-14266 (2020)
4 Lebrette H. et al. in preparation

ILME SCHLICHTING

Max Planck Institute for Medical Research, Jahnstr. 29, 69120 Heidelberg, Germany; E-mail: Ilme.schlichting@mr.mpg.de

Protein structure and dynamics using X-ray free-electron lasers - pitfalls.

X-ray crystallography has tremendous impact on biology, having yielded the structures of thousands of proteins and given detailed insight into their working mechanisms. The requirement for macroscopic crystals, which can be difficult to obtain, as well as the often severe radiation damage caused by the ionizing X-rays during data acquisition - in particular when using small crystals - has been relieved with the advent of X-ray free-electron lasers (XFELs). With their highly brilliant short X-ray pulses XFELs enable room temperature measurements, allowing to perform time-resolved experiments at atomic resolution at the chemical timescale of femtoseconds. More than ten years have passed since the Linac Coherent Light Source opened its doors to users. Since then a great deal of technical development has taken place, allowing to gain new scientific insight. More XFEL facilities have come online, including the first MHz facility. Time to reflect on lessons learned, pitfalls, and to think about future challenges. The talk focusses on pump probe experiments.

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SPEAKER

FRANZ X. KÄRTNER

Ultrafast Optics and X-rays Group, Center for Free-Electron Laser Science, Deutsches Elektronen Synchrotron (DESY), Department of Physics and The Centre for Ultrafast Imaging, Universität Hamburg, Germany

Compact Terahertz Driven Electron and X-ray Sources.

The use of very high frequencies, in the THz region and more specifically 100 - 500 GHz, enables operation of accelerators at higher field strength with lower energetic driver pulses[1]. This opens up the possibility of compact low emittance electron sources and high-brightness fully coherent X-ray sources. In this contribution, we summarize the progress made in the ERC Synergy Grant AXSIS: Attosecond X-ray Science – Imaging and Spectroscopy in source technology[2]. We discuss the scaling and design for THz driven electron guns, linear accelerators and diagnostic instrumentation for a versatile electron and X-ray source technology[3,4]. The high acceleration fields and field gradients possible in terahertz devices enable novel electron bunch manipulations, bunch diagnostic and promise ultimately fully coherent X-ray production from compact sources. Latest experimental results in the implementation of electron and X-ray sources based on this technology will be discussed[5-8]. Experimental results and limitations on laser based high energy terahertz generation including the optical laser technology providing 1 Joule picosecond and nanosecond pulses at ultimately 1 kHz operation frequency will also be presented[9-10]. An overview on the recently constructed AXSIS-Laboratory at DESY is presented, and, the design and implementation of the first fully THz driven electron accelerator and X-ray source is discussed.

References:

1 E. A. Nanni et al., "Terahertz-driven Linear Electron Acceleration," Nat. Com. 6, p. 8486 (2015).

2 F. X. Kärtner et al., AXSIS: Exploring the frontiers in attosecond X-ray science, imaging and spectroscopy, Nucl. Instrum. Methods Phys. Res. A 829, 24–29 (2016).

3 W. R. Huang et al, "Terahertz-driven, all-optical electron gun," Optica 3, p. 1209 (2016).

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5 D. Zhang et al., "Segmented THz electron accelerator and manipulator (STEAM)," Nat. Photonics 12: (6) 336 (2018).

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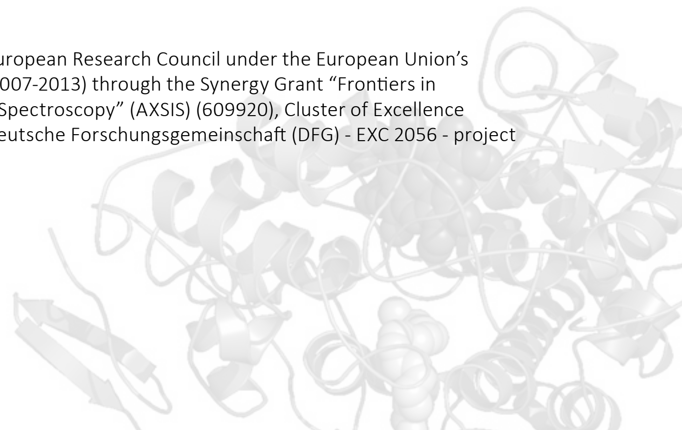
Acknowledgment:

This work has been supported by the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013) through the Synergy Grant "Frontiers in Attosecond x-ray Science: Imaging and Spectroscopy" (AXSIS) (609920), Cluster of Excellence "Advanced Imaging of Matter" of the Deutsche Forschungsgemeinschaft (DFG) - EXC 2056 - project ID 390715994.



SPEAKER

DOEKE HEKSTRA

Boris Krichel[1], **G**

1 Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Gerd;

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STILL TO COME

SPEAKER

THOMAS J. LANE

Center for Free Electron Laser Science, Deutsches Elektronen Synchrotron,
22607 Hamburg, DE

Revealing allosteric pathways via large-scale crystallographic studies.

Correlated motions in proteins, where changes in one part of the structure induce specific mechanical responses in distal regions, underpin enzyme catalysis, cellular signaling, and might provide new possibilities for drug targeting. Such dynamics are difficult to discover, however, as few tools exist for resolving structural correlations at atomic resolution.[1] Here we report a surprising success story conducting such a measurement.

In the summer of 2020, a large collaboration led by DESY aimed to respond rapidly to the COVID pandemic by studying the binding of drug candidates to the SARS-CoV-2 Main Protease (Mpro). Samples were crystallized in the presence of ligands from a repurposing library in the hope that a compound known to be safe and medically active could be found and immediately deployed. The majority of crystals studied have no bound ligand, yet even in these apo datasets, significant structural variability between crystals was observed. Remarkably, these structural fluctuations from crystal-to-crystal reproduce dynamics similar to what would be seen in solution: specifically, they qualitatively reproduce long MD simulations[2] (Figure 1). Further, they appear reproducible, consistent with a smaller screen preformed at the Diamond Light Source in the UK.[3]

The active form of Mpro is a homodimer (monomer is inactive) but the active site is far from the dimer interface.[4] By measuring correlations in the atomic displacements of Cα atoms across our "crystal ensemble", we discovered key contacts on the dimer interface that are highly correlated with the active site despite being 30+ Å away. It appears that small crystal-to-crystal variations occur during crystal growth and handling. These differences result in variations in the crystal lattice, in turn inducing changes to the protein structure. By analyzing these structural differences statistically, we discovered this active site-dimer interface correlation, suggesting how dimerization enables function and pinpointing new sites to target for drug discovery.

We predicted three key locations (residues) on the dimer interface that were highly correlated with the active site. Our team later discovered two of the three have been reported to significantly modulate enzyme activity in studies of Mpro from SARS-CoV-1,[5,6] the virus behind the SARS outbreak in 2003, which is nearly identical to Mpro from SARS-CoV-2 (96.1% identity, EMBOSS Needle[7]). This suggests that our measured covariances have functional implications, and that this strategy could be used to measure protein dynamics more generally.

Much work remains to be done, but a number of intriguing questions exists: can we measure more subtle dynamics besides Cα correlations? How general is this strategy? Can the method be extended to serial datasets? I will speculate on these questions and give a prospective for future studies.

References:

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SPEAKER

AARON BREWSTER

Toward metallo-chemical insights using per-pixel modeling of SFX images

Structure factors from XFEL data are typically estimated using pixel summation, where the value of each pixel in a reflection is summed to yield a single quantity, and then symmetry equivalents from many crystals are averaged together after applying corrections and scale factors. This is complicated by crystal and incident beam characteristics that vary shot-to-shot, by partial exposure of reflections near the Ewald sphere, and by difficult to propagate sources of error and noise. In three recent papers (two published and one upcoming), we have investigated a non-summation approach where we estimate structure factors using models that predict per-pixel intensities. The models include parameters for crystal orientation, unit cell dimensions, mosaicity, beam character including spectrum and intensity, detector properties, and importantly the structure factors themselves which can vary depending on incident wavelength and the absorption properties of the material. The program ***nanoBragg*** uses forward modeling approaches to simulate each reflection and the program ***diffBragg*** provides the first derivatives needed to refine the model parameters using maximum likelihood refinement approaches until the per-pixel spot simulations match the data. This approach takes advantage of much more data than a simple summation and, because it includes a complete model of the beam spectra, small changes in pixel intensity due to absorption differences of photons at different wavelengths in a SASE pulse can be used to estimate valence states of individual atoms, revealing metallo-chemical properties of protein ligands in time-course experiments. Two simulated and one experimental example of using these approaches will be presented.

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SPEAKER

DEEPSHIKA GILBILE

Gilbile, D.,[1] Shelby, M.L.,[2] Lyubimov, A.,[3] Ridgway, E.,[1] Wierman, J.L.,[3] Cohen, A.E.,[3] Russi, S.,[3] Coleman, M.A.,[2] Frank, M.,[2] and Kuhl, T.L.[1]

1. Department of Chemical Engineering, University of California at Davis, Davis, CA 95616, USA;
2. Biosciences and Biotechnology Division, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA.
3. Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, Menlo Park, CA 94025.

Versatile cyclic olefin copolymer (COC) fixed-targets for hydrated, room-temperature serial protein crystallography.

One of the key challenges with serial femtosecond crystallography has been efficient, continuous delivery of hydrated protein crystal sample to the X-ray beam while maintaining a low scatter background. Fixed-targets offer several advantages over the widely adopted jet-based sample delivery techniques, like- clog-free delivery, significantly lower sample consumption, and the ability to control crystal sample distribution on-chip using surface-functionalization strategies. The modular, enclosed fixed-target approach enables one to maintain long-term crystal hydration, while tuning sample layer and support film thicknesses to maximize signal-to-noise. Moreover, fixed-targets can be integrated with microfluidic elements to deliver ligands, caged reactants, or electric fields, paving the way for time-resolved experiments.

This work demonstrates the development of versatile, hot-embossed cyclic olefin copolymer (COC) enclosed fixed-targets with ultra-thin, tunable water barrier films made from COC and/or graphene. These supports are inexpensive to fabricate and maintain protein crystal hydration over several days. The supporting polymer layers contribute minimally to the X-ray scatter background while circumventing obstacles arising from beam damage as observed with Si supports. The enclosing COC thin films can be functionalized and patterned using UV-initiated photografting to create surface charge or binding-affinity driven protein-rich interfacial domains that act as “universal” protein crystal nucleants, thereby enabling precise control of sample distribution on chip.

Initial pseudo-serial crystallography measurements at a synchrotron light source (SSRL 12-1 beamline) using model proteins Lysozyme and Thaumatin indicate that high-resolution, high-completeness (no preferential orientation) data collection is possible using these targets with minimal background contribution from the chip components. Our on-chip crystallization approach allows for sample preparation and crystal screening ahead of beamtime, with the aim of maximizing crystal hit-rates and sample throughput. Additionally, surface-interaction driven specific crystal nucleation provides an alternative strategy for protein crystallization, particularly for proteins that are difficult to express in large quantities and crystallize using traditional approaches.

Acknowledgements:

This work was performed, in part, under the auspices of the U.S. DOE by LLNL under Contract DE-AC52-07NA27344. This work was also supported by National Science Foundation (NSF) BioXFEL STC Grant 1231306, NIH grants R01GM117342 (NIGMS) and R21AI120925 (NIAID). Use of the LCLS, SLAC National Accelerator Lab, is supported by the U.S. DOE, Office of Science, under contract no. DE-AC02-76SF00515.

DIANDRA DOPPLER

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3D Printed Hybrid Co-Flow Injectors for Time Resolved SFX Studies at XFELs.

Serial femtosecond crystallography (SFX) with X-ray free electron lasers (XFELs) is an emerging and growing field in structural discovery; one of its principal achievements is the ability to resolve the structure of intricate membrane proteins at room temperature. A newer development of this technique is studying light-activated proteins by subjecting the samples to a pump probe laser prior to irradiation with the XFEL in varying delay times. In this way, light catalyzed reactions can be probed at different time points to create a “molecular movie” of the reaction mechanism. Serial injection in a liquid jet for this new technique requires high jet speeds and large sample volumes, which are two of its major challenges that are intensified when needing to probe several time points. At the European XFEL (EuXFEL), the pulse structure is very unique: It delivers fs pulses at 10 Hz trains of X-ray pulses, with an intra-train MHz repetition frequency and at SLAC LCLS, fs pulses are delivered at 120 Hz. The gas dynamic virtual nozzles (GDVNs) can achieve the high velocities required for fresh sample to be delivered for each pulse; however, between XFEL pulses, the continuously injected crystal sample is wasted. Some alternative injectors, like the double flow focusing nozzle or the piezoelectric droplet ejector, have been designed to conserve sample, however, they either cannot achieve the required fast sample replenishing or are not suitable to deliver higher viscosity buffers.

To reduce the volume of sample wasted during a SFX experiment at the EuXFEL, we propose a 3D printed T- and Y-junction device with an integrated GDVN that generates a simultaneous flow of sample with an immiscible liquid prior to being injected into the XFEL beam. This device is capable of injecting both lower viscosity aqueous liquid samples as well as higher viscosity samples. In addition, these integrated 3D-printed devices, made of a novel custom resin, have been proven to withstand the high intensity illumination of the pump-probe laser in vacuum, 40 mJ of 532 nm lasing at 120Hz.

The reproducibility of co-flow and jetting parameters of the device have been demonstrated with three buffers of varying viscosities and with a suspension containing photosystem II crystals. The mass flow rates of helium used in all experiments ranges from 10 mg/min to as high as 50 mg/min, with pressures up to 700psi for the liquid flow rates; these parameters were then used to determine the velocities at which the jet is expelled from the nozzle as well as the different experimental regimes in which co-flow will be ideal for a given analyte sample. The co-flow can be achieved with flow rates of sample as low as 1 µL/min at total flow rates of 20 µL/min compensated from oil, maintaining the stable injection of jetting for high viscosity liquid samples while keeping the jet at speeds capable of replenishing sample at the high repetition rate at current XFELs. Furthermore, the co-flow thickness was characterized through experimental and computational modeling where the width could be modulated from 6 to 60 µm according to the flow rates. These devices were then tested for serial crystallography liquid injection experiments at two XFELs. At both the SPB/SFX instrument at the EuXFEL as well as at the CXI instrument at LCLS (SLAC) diffraction of photosystem II was recorded. Future work includes confirmation of jet velocities at varying liquid flow rates while varying the helium gas pressures and application of the co-flow injector for full data set recording including crystal suspensions with high viscosity such as photosystem II.

RETURN TO AGENDA

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New Results from Time-Resolved Crystallographic Experiments at XFELs.

We describe newest results obtained from the SPring-8 Angström Compact X-ray Laser (SACLA) in Japan, and the European X-ray Free Electron Laser (EuXFEL). SACLA is a compact X-ray laser that delivers femtosecond X-ray pulses at a lower 30 Hz repetition rate. The EuXFEL is a superconducting XFEL that started user operations in 2017. The EuXFEL is located, near Hamburg, Germany, and produces femtosecond, ultra-brilliant and spatially coherent X-ray pulses with MHz rates. X-rays arrive in pulse trains at 10 Hz. Each train consists of bursts of X-ray pulses with an intra-train rate of up to 4.5 MHz. Due to the MHz pulse repetition rates, an experiment at the EuXFEL can be challenging. Previously, we investigated the picosecond time range in the photocycle of photoactive yellow protein. We showed that difference electron density maps of excellent quality can be obtained[1]. This opens the door to a wide range of time resolved studies at the EuXFEL.

Here, we show results from the first successful mix-and-inject experiment at the EuXFEL conducted in March 2020. The initial phase of the catalytic cycle of the M. tuberculosis β-lactamase (BLAC) was investigated with single millisecond time resolution using the cephalosporine antibiotic ceftriaxone (CEF) as substrate[2]. We characterize the induced fit and determine a diffusion coefficient of CEF in BlaC crystals from X-ray data. At SACLA we made progress to investigate the photocycle of a bacterial phytochrome which is a red-light regulated enzyme. We were able to photoinitiate the photocycle of the phytochrome photosensory core module (PCM) which consists of PAS, GAF and PHY domains and lacks the effector domain. We describe what happens 5 ns and 33 ms after photoactivation[3]. As we do not observe large scale relaxations of the phytochrome, we are now aiming to conduct single-particle diffraction experiments at the XFEL. To estimate how many diffraction patterns are required for a dataset that can be successfully phased, we performed realistic simulations using a model of the full-length bacterial phytochrome (MW ~180 kDa) including the effector (enzymatic) domain. The simulations show that with the current XFEL beam parameters (1 µm beam size, λ=5 Å, 1020 photons/cm2) about 40,000 diffraction patterns (single-particle hits) would be sufficient to successfully phase the diffraction data up to 10 Å resolution[4].

1. S. Pandey#, R. Bean#, T. Sato#, I. Poudyal, J. Bielecki, J. Cruz Villarreal, O. Yefanov, V. Mariani, T. A. White, C. Kupitz, M. Hunter, M. H. Abdellatif, S. Bajt, V. Bondar, A. Echelmeier, D. Doppler, M. Emons, M. Frank, R. Fromme, Y. Gevorkov, G. Giovanetti, M. Jiang, D. Kim, Y. Kim, H. Kirkwood, A. Klimovskaia, J. Knoska, F. H. M. Koua, R. Letrun, S. Lisova, L. Maia, V. Mazalova, D. Meza, T. Michelat, A. Ourmazd, G. Palmer, M. Ramilli, R. Schubert, P. Schwander, A. Silenzi, J. Sztuk-Dambietz, A. Tolstikova, H. N. Chapman, A. Ros, A. Barty, P. Fromme, A. P. Mancuso, M. Schmidt (2019) Time-Resolved Serial Femtosecond Crystallography at the European XFEL. Nature Methods, https://doi.org/10.1038/s41592-019-0628-z. #contributed equally
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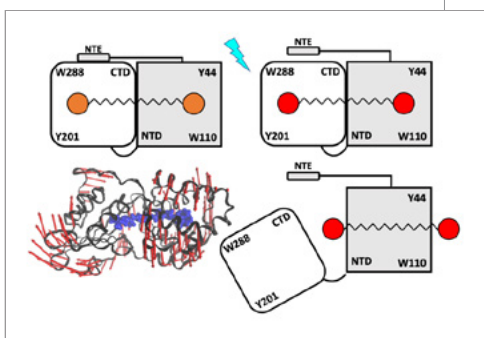
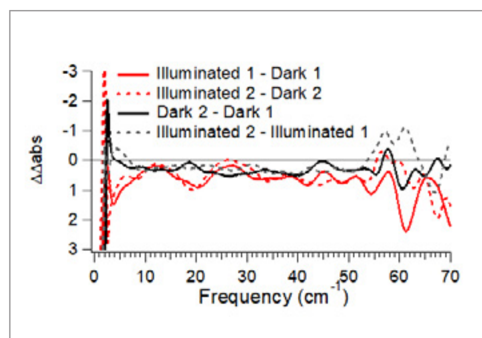
Photoswitching of Long-Range Vibrational Modes in Orange Carotenoid Protein.

Orange carotenoid protein (OCP) plays a role in photoprotection of cyanobacteria. It enacts non-photochemical quenching (NPQ) via interactions with the light harvesting antenna, the phycobilisome (PBS), to prevent oxidative stress in conditions of bright light. OCP is comprised of two globular domains that form a hydrophobic binding pocket for a carotenoid chromophore. Upon activation by blue-green light, the domains separate, the carotenoid is translocated into the N-terminal domain, the absorbance spectrum is red shifted and the N-terminal domain may proceed to interact with the PBS to enact NPQ [1]. The mechanism that drives large scale structural changes upon photoexcitation is not known, however long-range intramolecular vibrations that steer the structure toward functional conformations has been suggested. Characterization of these vibrations requires a technique that is sensitive to specific structural motions, such as anisotropic absorbance [2]. Here, we use anisotropic terahertz microscopy (ATM) to probe intramolecular structural vibrations in dark adapted and illuminated OCP crystals [3]. Recombinant cyanobacterial OCP was purified and crystallized as previously reported [4]. The ATM measurements find distinct vibrational bands that reversibly shift with photoexcitation, which cannot be due to large scale structural changes that are prevented by crystal contacts [5]. The results indicate a reorientation of vibrational displacements upon photoexcitation. We suggest that these changes in intramolecular vibrations may steer the globular structure away from the inactive conformation and toward photoprotective conformations that are involved in carotenoid translocation, PBS interactions and heat dissipation by NPQ. This work was supported by the National Science Foundation (MCB 1616529) and the U.S. Department of Energy (DE-SC0016317)





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SPEAKER

SWAGATHA GHOSH

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Snapshots of Structural Intermediates during CO-dissociation process in ba3-type Cytochrome c Oxidase.

Cytochrome c oxidase (CcO) is an integral membrane protein that participates as terminal acceptor of electrons during cellular respiration. It receives electrons from upstream biological transporters that reduces molecular oxygen to water. The free energy stored in this process is then used for transmembrane transport and ATP synthesis. As CcO plays an inevitable role in mitochondrial energetics and defects in CcO functionality are associated with mitochondrial dysfunction, the enzyme has been under intense investigation. A large amount of biochemical data and equilibrium-states structures of CcO are available to guide the proton translocation pathway of this enzyme, however, the mechanism of redox-linked proton translocation still remains elusive. Using time-resolved serial crystallography (TrSX), we have determined structural changes in ba3- type CcO from Thermus thermophilus at ultrafast time-scales.

TrSX studies on CO- bound CcO show significant structural changes at the catalytic center at 50ns and 2ms after illuminating CO with a green laser. Further, we want to study structural intermediates during oxygen-reduction in micro-crystals of CcO. With that goal in mind, we have developed simple devices for mix-and-inject serial synchrotron crystallography (SSX) for mixing micro-crystals (in lipidic cubic phase) with oxygen and delivering to the X-ray beam. We have performed preliminary experiments and validated our set-up at MAXIV laboratories (Sweden) and collected data-sets of resting state, reduced- and CO-bound (on and off) states of ba3- type CcO from Thermus thermophilus. SSX structure of resting state is solved at 2.3Å, similar to earlier reported structure obtained at X-ray free electron laser (Andersson et. al,2017). Overall, we aim is to create a molecular movie, elucidating the structural mechanisms of redox-linked proton- pumping and gating of proton channel in ba3-type CcO. Our research will provide the molecular framework for understanding electron and proton transfer pathways and mechanisms for addressing fundamental questions on redox reactions and energy conservation in cells. This study could also provide a guide to develop novel therapeutic agents targeting numerous human mitochondrial diseases.

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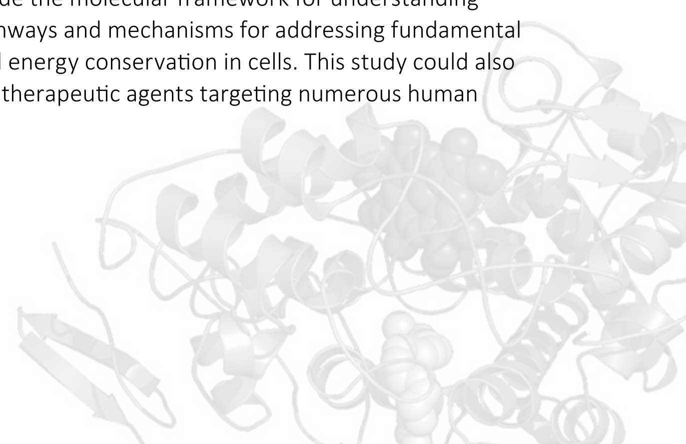
Acknowledgments:

Postdoctoral research fellowship from ERC Horizon 2020 Advanced grant (2017) and ERC Proof of Concept grant (2020)



SPEAKER

BRENDA HOGUE

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Coronavirus Envelope Viroporin Structure-Function Studies

Coronavirus (CoV) envelope (E) proteins are viroporins that play multiple roles during the virus life cycle. Many medically important viruses encode viroporins. Viroporins are small, highly hydrophobic proteins that form oligomeric channels. CoV viroporins play diverse roles that include virus assembly, budding, intracellular trafficking, virion release, and pathogenesis. The proteins localize at intracellular membranes of the endoplasmic reticulum Golgi (ERGIC) where studies suggest a role in modulating curvature during CoV assembly. In cellular and animal models, E proteins trigger an inflammatory response that contributes to disease progression during infection. Ongoing studies are focused on understanding the mechanistic, multifunctional roles of CoV E proteins, particularly their structure and contributions to virus assembly and pathogenesis. E proteins play a key role in assembly of virus-like-particles (VLPs) which form the basis for potential development of a COVID-19 vaccine platform. E proteins are targets for therapeutic development of small molecule inhibitors. Structure-function studies are critical to inform development of both vaccines and therapeutics for control of COVID-19.

Acknowledgements

We acknowledge support from the NSF RAPID: IIBR: Coronavirus Viroporins XFEL Structural Studies, Award 2032199; NSF-STC Biology with X0ray Lasers Award 1231306; NIH R03AI133397, Fast Grant Award. We thank the Staff Scientists at the LCLS and SSRL at SLAC National Accelerator Laboratory and the European XFEL for their contributions.

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BioXFEL

SPEAKER

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PETRA FROMME

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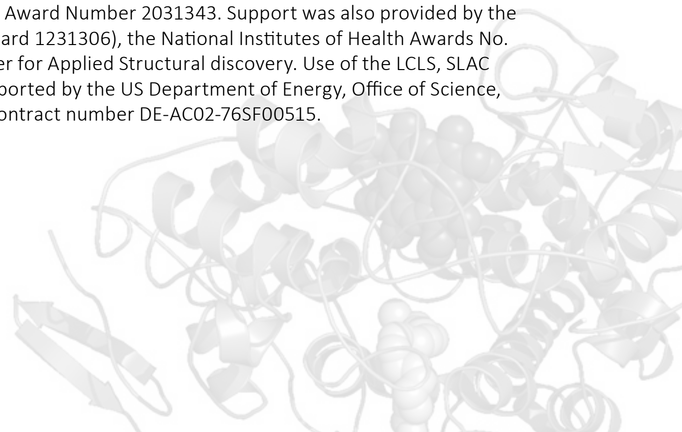
Serial fs crystallography of the SARS-CoV2 riboendonuclease NendoU

The COVID-19 crisis shows a very urgent need to understand the structure and dynamics of the proteins of SARS-CoV-2 (CoV-2), the coronavirus that is the cause of this severe emerging infectious disease. The work presented will describe the first serial fs crystallography studies of the CoV2 enzyme NendoU conducted at LCLS. To investigate the catalytic mechanism of this endoribonuclease (also called the non-structural protein 15, NP15), a dual approach will be used to capture a molecular movie of the catalytic reaction of NendoU: time-resolved-serial femtosecond crystallography (TR-SFX) combined with time resolved cryo-electron microscopy (TR-cryo-EM). The unravelling of the function and mechanism of NendoU is key to the pandemic as it enables the virus to hide from detection by the immune system. In the process of viral RNA replication, negative-strand RNA is formed that contains a poly-uridine (poly-U) tail. The detection of the poly-U-sequence alerts activating host sensors and leads to rapid degradation of the virus in macrophages. NendoU cleaves the poly-U tail, thereby hiding the presence of the CoV-2 RNA from the immune system, so the virus is able to spread undetected in the human body and thereby is a major contributor to the current pandemic. The talk will report on the first SFX studies at LCLS and its combination with functional studies on the substrate specificity of NendoU, with exciting new insights paving the way for time-resolved SFX and TR-CryoEM studies.

Acknowlegements

This work is supported by the NSF RAPID grant RAPID: IIBR: Instrumentation: Time-resolved studies of the SARS-CoV-2 endonuclease NP15, Award Number 2031343. Support was also provided by the BioXFEL Science Technology Center (award 1231306), the National Institutes of Health Awards No. R01GM095583 and the Biodesign Center for Applied Structural discovery. Use of the LCLS, SLAC National Accelerator Laboratory, is supported by the US Department of Energy, Office of Science, Office of Basic Energy Sciences under contract number DE-AC02-76SF00515.



SPEAKER

HOLGER STARK

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High-resolution structure determination of dynamic macromolecular complexes by cryo-EM.

Single particle cryo electron microscopy (cryo-EM) has developed into a powerful technique to determine 3D structures of large macromolecular complexes. Due to improvements in instrumentation and computational image analysis, the number of high-resolution structures is steadily increasing. The method cannot only be used to determine high-resolution structures but also to study the dynamic behavior of macromolecular complexes and thus represents a very complementary method to X-ray crystallography. Furthermore, the maximum attainable resolution by cryo-EM has constantly improved in recent years. Most of the high-resolution structures are still in the 3 Angstrom resolution regime but some have even crossed the 2 Angstrom barrier. We have recently installed a new prototype electron microscope which is equipped with a monochromator and a next-generation spherical aberration corrector. This microscope is optically superior to the currently commercially available instruments and can therefore be used to test the resolution limits in cryo-EM. We have used the test specimen apoferritin to determine its structure at 1.25 Angstrom resolution which is sufficient to visualize for the first time individual atoms clearly separated in the density map.

We have used this microscope not only to improve the resolution of the very stable and rigid protein apoferritin. Recently, we also obtained significant improvement in resolution for other more dynamic macromolecular complexes for which one could have expected that the microscope itself may not be a major resolution limiting factor.

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SPEAKER

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Selection of XFEL Single-Particle Diffraction Patterns by Geometric Machine Learning.

Single-particle imaging (SPI) with an X-ray free-electron laser (XFEL) is a novel technique for structural determination, which has the advantages that experiments can be performed at room temperature and do not require sample crystallization. Nevertheless, a central problem in SPI is that high-resolution structures can only be obtained from a sufficiently large number of diffraction patterns of individual molecules, so-called single particles. Here, we present a method that allows for efficient identification of single particles in very large XFEL datasets, operates at low signal levels, and is tolerant to background noise.

Our method relies on Geometric Machine Learning (GML) which first employs Manifold Embedding to convert the high-dimensional space of training diffraction patterns into a representative lower-dimensional space; and then a Nyström Extension protocol to fuse XFEL test datasets into the low-dimensional space of the training dataset. Finally, the test datasets are separated into binary distributions of "single particles" and "non-single particles". As proof of principle, we tested this method with simulated and experimental XFEL datasets of the Coliphage PR772 virus. We compared our method with previously published results and found that GML covers a wide photon-count range, outperforming previous single-particle identification methods in terms of recall. Moreover, a major advantage of GML is its ability to retrieve single particles in presence of structural variability.

Acknowledgements

We acknowledge support by the BioXFEL under NSF Science and Technology Awards No.1231306 (numerical trial models), by the US Department of Energy, Office of Science, Basic Energy Sciences under award DE-SC0002164 (algorithm design and development, and data analysis), and by the UWM Research Growth Initiative.

SPEAKER

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Macromolecular Cryo-EM Structures at Near and True Atomic Resolution.

Cryo-EM has the resolving power to visualize atomic detail of biomolecules. We utilize standard 300 kV transmission electron microscopes (Titan Krios) to image protein complexes in our Stanford-SLAC Cryo-EM Center. We have found to be readily feasible to record sufficient images of vitrified apoferritin in less than ¾ a day for reconstructing its structure at ~1.27-1.34 Å resolution with either a K3 camera and an energy filter or a falcon detector without an energy filter[1]. A quantitative analysis of the maps substantiates the resolvability of all atoms except hydrogen in all the amino acids, water molecules and metal ions. Such capability is not always achieved for all macromolecules because of their compositional and/or conformational heterogeneity. Nevertheless, advanced data processing method can be used to sort out the structure variants from which novel chemical properties of the macromolecules can be derived.

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