BioXFEL

AGENDA

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

	MONDAY, FEBRUARY 7TH, 2022
11:00 am – 4:00 pm	**External Advisors Meeting**
2:00 pm – 6:00 pm	**Professional Development Workshop: Inclusive Mentoring**

9TH BIOXFEL INTERNATIONAL CONFERENCE

	TUESDAY, FEBRUARY 8TH, 2022
11:00 am – 11:10 am	**Conference Welcome** **Conference Chairs: Tonya Kuhl, UC-Davis & Thomas Grant, UB**
	SESSION I: John Spence Memorial Session Chair: Henry Chapman, CFEL
11:10 am – 12:00 pm	**Henry Chapman (CFEL)** Going through a phase: John Spence and his interests in coherent imaging
12:00 pm – 12:30pm	**Coffee Break**
12:30 pm – 1:00 pm	**Nadia Zatsepin (LaTrobe University)** On a different note: some colourful and musical memories of John Spence
1:00 pm – 1:30 pm	**Jian-Min Zuo (University of Illinois-Champagne)** Electron Coherent Diffractive Imaging Inspired by John Spence
1:30 pm – 2:00 pm	**Rick Kirian (ASU)** Nanodrop Fluctuation X-Ray Scattering
2:00 pm – 3:00 pm	**Lunch Break**
	SESSION II: Bioapplications Session Chairs: Gebhard Schertler, PSI & Marina Galchenkova, CFEL
3:00 pm – 3:30 pm	**Jason Stagno (NIH)** Studying ligand-inducible phase changes in macromolecular crystals
3:30 pm – 3:50 pm	**Stephanie Wankowitz (UCSF)** Ligand binding remodels protein side chain conformational heterogeneity
3:50 pm – 4:10 pm	**Maximillian Wratnik (PSI)** Molecular snapshots of drug release over eleven orders of magnitude in time
5:30 pm – 7:00 pm	**Poster Session - Even Number Presentations**

BioXFEL

AGENDA, continued

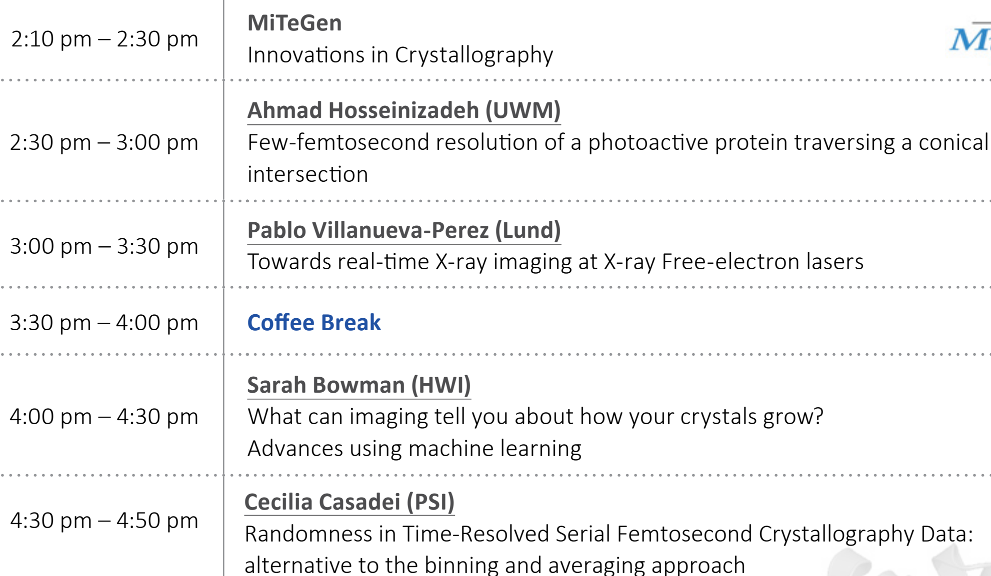
All Times are given in Eastern Standard Time (EST)

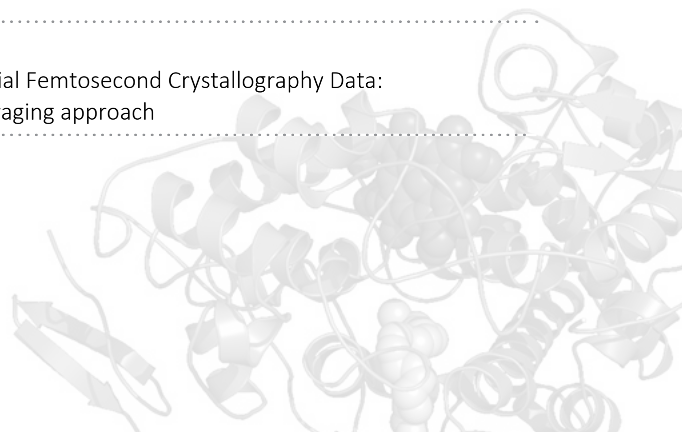
	WEDNESDAY, FEBRUARY 9TH, 2022
	SESSION III: Non-XTAL Experiments Session Chairs: Richard Kirian, ASU & Elijah Gonzalez, Rice
11:00 am – 11:30 am	**Hyotcherl Ihee (KAIST)** Exploring Ultrafast Protein Structural Dynamics with Time-Resolved X-ray Liquidography
11:30 am – 12:00 pm	**Foivos Perakis (Stockholm University)** Resolving protein dynamics with X-ray photon correlation spectroscopy at the European XFEL
12:00 pm – 12:30 pm	**Coffee Break**
12:30 pm – 12:50 pm	**Edwin Peña-Martínez (UPR)** Characterizing the Effects of Non-coding Mutations in Cardiac Transcription Factor-DNA Interactions
1:00 pm – 2:00 pm	**Keynote Speaker: Sarah Holder (SLAC), Diversity and Inclusion in Science** Chairs: Bill Bauer (HWI) and Jorvani Cruz-Villarreal (ASU)
2:00 pm – 3:00 pm	**Lunch Break**
	SESSION IV: Methods & Instrumentation Session Chairs: Lois Pollack, Cornell & Iris Chang, SLAC
3:00 pm – 3:30 pm	**Andrea Markelz (UB)** Simplifying Protein Collective Vibrational Assignment Through Symmetry
3:30 pm – 4:00 pm	**Mark Hunter (LCLS)** Biology and Sample Delivery at LCLS
4:00 pm – 4:20 pm	**Sarthak Saha (Umass Amherst)** A Microfluidic Device for Room Temperature Crystallography
4:20 pm – 4:40 pm	**Coffee Break**
4:40 pm – 5:10 pm	**Robert Bücker (CSSB-Hamburg)** Serial Electron Diffraction for Macromolecules – Chances and Challenges.
5:10 pm – 5:40 pm	**J. Mia Lahey-Rudolph (Luebeck)** Diffracting intracellular protein crystals - optimizing delivery of insect cells.
5:45 pm – 7:00 pm	**Poster Session - Odd Number Presentations**

AGENDA, continued

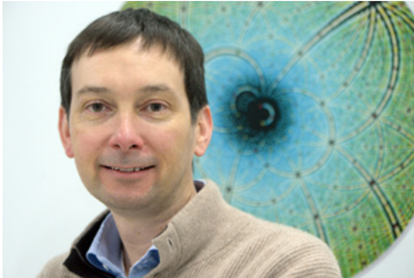
All Times are given in Eastern Standard Time (EST)

	THURSDAY, FEBRUARY 10TH, 2022
	SESSION V: Bottlenecks Session Chairs: Tonya Kuhl, UC-Davis & Diandra Doppler, ASU
11:00 am – 11:30 pm	**Kara Zeilinksi (Cornell)** The Importance of Pre-Screening Ligand Binding In Crystallo for Mix-and-Inject Serial Crystallography
11:30 am – 12:00 pm	**Valerio Mariani (SLAC)** Remote real-time experiment monitoring for fast decision making: challenges and opportunities
12:00 pm – 12:30 pm	**Coffee Break**
12:30 pm – 1:00 pm	**Filipe Maia (Uppsala University)** Overcoming the limits of ultrafast X-ray single-particle imaging
1:00 pm – 1:30 pm	**Megan Shelby (LLNL)** A fixed-target approach to overcoming crystallization, sample environment, and sample delivery obstacles for room temperature serial crystallography.
1:30 pm – 2:10 pm	**Lunch Break**
	SESSION VI: Machine Learning Session Chairs: Abbas Ourmazd, UWM & Roshanak Etemadpour, UWM
2:10 pm – 2:30 pm	**MiTeGen** Innovations in Crystallography
2:30 pm – 3:00 pm	**Ahmad Hosseinizadeh (UWM)** Few-femtosecond resolution of a photoactive protein traversing a conical intersection
3:00 pm – 3:30 pm	**Pablo Villanueva-Perez (Lund)** Towards real-time X-ray imaging at X-ray Free-electron lasers
3:30 pm – 4:00 pm	**Coffee Break**
4:00 pm – 4:30 pm	**Sarah Bowman (HWI)** What can imaging tell you about how your crystals grow? Advances using machine learning
4:30 pm – 4:50 pm	**Cecilia Casadei (PSI)** Randomness in Time-Resolved Serial Femtosecond Crystallography Data: alternative to the binning and averaging approach





KEYNOTE SPEAKER



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HENRY CHAPMAN

Center for Free-Electron Laser Science CFEL, DESY, 22607 Hamburg, Germany
The Hamburg Centre for Ultrafast Imaging, 22761 Hamburg, Germany
Department of Physics, Universität Hamburg, 22761 Hamburg, Germany

Going through a phase: John Spence and his interests in coherent imaging

Many of us knew John Spence as the remarkable, generous, and prolific Director of Science who played the leading role in launching the "Biology with X-ray Free Electron Lasers (BioXFEL)" NSF Science and Technology Center and steering it to achieve a cohesive, united, and successful collaborative center that nurtured methods such as serial crystallography and diffractive imaging through their formative years. He undertook all that with characteristic skill, insight, assurance, and equanimity, as a leading expert on the science of X-ray free-electron lasers when X-ray free-electron lasers had only just started up. Not many people were willing to take the risk on these new sources, but John did so wholeheartedly, based on his knowledge and discussions and debates he undertook with the best experts to be found on potential issues and pitfalls. These facilities also offered John an irresistible fresh blank slate and so many opportunities to supply new ideas and inventions.

But how was it that John was poised for XFEL research? He began his career in electron microscopy and literally wrote the book on that subject[1]. His work on electron microdiffraction (and some more books[2,3]) introduced ways to obtain atomic-resolution coherent images free of aberrations via holography, projection imaging, and scanning imaging. With the advent of high-brightness third-generation synchrotron sources there was a need for similar developments of coherent imaging with X-rays. John founded the series of Coherence workshops to bring these fields together after the first demonstration by John Miao and David Sayre of iterative phasing of the diffraction pattern of a non-periodic object[4]. I was fortunate to team up with John on diffractive imaging experiments at the Advanced Light Source[5]. Together we received support from the NSF Center for Biophotonics Science and Technology, which was led by Dennis Matthews of LLNL and who had created the first lab-based soft X-ray laser in 1984[6]. This took us to the FLASH FEL in Hamburg[7] and finally to first experiments at LCLS[8]. I will discuss some of John's early experiments and ideas, and leave plenty of time for members of the audience to add their reminisces or to correct mine.

References:

1 J. C. H. Spence, *Experimental High Resolution Electron Microscopy* (Oxford UP, 2013)
2 J. C. H. Spence and J. M. Zuo, *Microelectron Diffraction* (Springer, 1992)
3 J. M. Zuo and J. C. H. Spence, *Advanced Transmission Electron Microscopy* (Springer, 2017)
4 J. Miao, P. Charalambous, J. Kirz, and D. Sayre, Nature 400, 342 (1999).
5 S. Marchesini, H. He, H. N. Chapman, S. P. Hau-Riege, A. Noy, M. R. Howells, U. Weierstall, and J. C. H. Spence, Phys. Rev. B 68, 140101 (2003)
6 D. Matthews et al. Phys. Rev. Lett. 54, 110 (1985)
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8 H. N. Chapman et al., Nature 470, 73 (2011)

KEYNOTE SPEAKER

SARAH HOLDER

BIO:

Sarah Holder is the Diversity, Equity and Inclusion Program Manager for SLAC National Accelerator Laboratory in Menlo Park, CA. In this role they work with scientific and support teams across the laboratory to better understand the barriers to creating inclusion, so that they can work together to address them. Sarah is also currently working on writing their dissertation to finish up a Ph.D. in Education at the University of Virginia. Their research looks at the role of academic peer support systems in STEM higher education programs, using a critical and intersectional framework.

Science has always been about innovation and discovery, and the scientific challenges we face today require thoughtful, diverse, and inclusive teams that can work together to move us forward. However, deeply entrenched beliefs and systemic hurdles keep us from attracting and retaining the diversity of talent we need in our organizations. In this interactive keynote discussion, we will address the ways these beliefs and systems impact early career scientists in particular and share some promising practices for addressing these challenges.

SPEAKER

JIAN-MIN ZUO

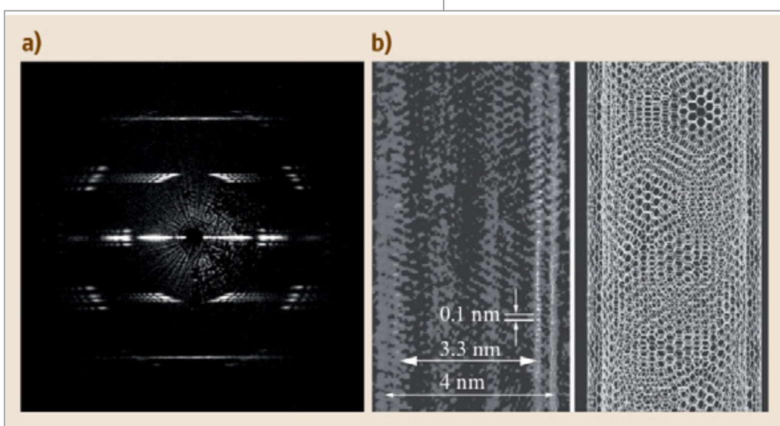
Dept of Materials Science and Engineering, University of Illinois, Urbana-Champaign

Electron Coherent Diffractive Imaging Inspired by John Spence.

Electron Coherent Diffractive Imaging (CDI), which John Spence started his journey into diffractive imaging and non-crystallographic phase problem, has been continually developed from single particle CDI to today's atomic resolution ptychography. As John Spence succinctly summarized in his Chapter in Springer Handbook of Microscopy, "The demand for higher-resolution 3-D noninvasive imaging with old and new radiation sources continues unabated in both materials science and biology. New convex constraints are steadily being discovered, leading to steady incremental advances. Looking back, it seems clear now that diffractive imaging has made a decisive contribution to imaging science, with exciting possibilities for further development".

This talk reviews the development of electron CDI and discusses latest development in electron diffraction and future possibilities. The background is John long-time dedication to electron diffraction and crystallographic problems. Compared to X-rays and neutrons, electrons have a small wavelength (of the order of a few picometers), and the large scattering cross-section. Inside a transmission electron microscope (TEM), the electron beam can be focused down to < 1 Å in diameter with the current reaching hundreds of picoamps (1 pA = 6.3x106 e/s), which gives a large scattering power for the electron beam. Since electron diffraction was discovered by Davisson and Germer, and Thomson and Reid, in 1927, transmission electron diffraction and the related electron imaging have developed into powerful tools for the analysis of materials, such as proteins and transistor devices. However, dynamical diffraction makes electron scattering very much different. At ASU, the collaboration between the author and John Spence led to the development of quantitative convergent beam electron diffraction (QCBED) and the observation of chemical bonds, while John has continually worked on the dynamical inversion problem with his breakthrough published in a recent Physical Review Letter paper.

This talk will also show how the ideal of diffractive imaging and computer processing can be generalized with the recent development of fast pixelated electron detectors. The concept of cepstral STEM will be introduced based on coherent speckles formed by interference of electron diffuse scattering. It will be demonstrated how cepstral STEM can be applied to determine crystal disorder, which is very much of current interest in the materials community.



Electron CDI of Double-Walled Carbon Nanotube

SPEAKER

RICHARD A. KIRIAN

Arizona State University Department of Physics, Tempe, Arizona, 85287

Nanodrop Fluctuation X-Ray Scattering

The extreme intensities of XFELs raised the possibility of time-resolved imaging of non-crystalline biomolecules in solution via the method of Fluctuation X-Ray Scattering (FXS). The idea of FXS originated in the late 1970s[1], decades before the necessary diffract-and-destroy paradigm became a reality. FXS is based on the fact that two-point intensity correlations obtained from millions of snapshot diffraction patterns yields far more information than a normal scattering profile does. Proof-of-principle experiments along with many algorithm developments have demonstrated FXS imaging on large biomolecular assemblies (viruses) at low resolution[2,3]. Despite exciting progress, it is still not clear if FXS will ever yield biomolecular images with molecular resolution. Unfortunately, the giant boost in information that FXS analysis yields comes with a seemingly insurmountable drop in the signal-to-noise ratio[4]. Here I discuss the challenges that we face in the development of FXS, and how the use of nanodrops or nanosheets might bring about a much-needed boost in the signal-to-noise ratio of FXS measurements.

References:

1 Kam, Z. Determination of Macromolecular Structure in Solution by Spatial Correlation of Scattering Fluctuations. *Macromolecules* 10, 927–934 (1977).

2 Kurta, R. P. *et al.* Correlations in Scattered X-Ray Laser Pulses Reveal Nanoscale Structural Features of Viruses. *Physical Review Letters* 119, (2017).

3 Pande, K. *et al.* Ab initio structure determination from experimental fluctuation X-ray scattering data. *Proceedings of the National Academy of Sciences* 115, 11772–11777 (2018).

4 Kirian, R. A., Schmidt, K. E., Wang, X., Doak, R. B. & Spence, J. C. H. Signal, noise, and resolution in correlated fluctuations from snapshot small-angle x-ray scattering. *Physical Review E* 84, (2011).

Acknowledgement:

This work is supported by NSF awards 1231306, 1943448, and 1817862.

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SPEAKER

JASON STAGNO

Stagno, J.R.[1], Ramakrishnan, S.[1], Conrad, C.E.[1,12], Ding, J.[1], Yu,P.[1], Bhandari, Y.R.[1], Lee, Y.-T.[1], Pauly, G.[2], Yefanov, O.[3], Wiedorn, M.O.[3], Knoska, J.[3,4], Oberthür, D.[3], White, T.A.[3], Barty, A.[3], Mariani, V.[3], Li, C.[3,5], Brehm, W.[3], Heinz, W.F.[6], Magidson, V.[6], Lockett, S.[6], Hunter, M.S.[7], Boutet, S.[7], Zatsepin, N.A.[5,13], Zuo, X.[8], Grant, T.D.[9], Pandey, S.[10], Schmidt, M.[10], Spence, J.C.H.[5], Chapman, H.N.[3,4,11], & Wang, Y.-X.[1]

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4. Department of Physics, Universität Hamburg, Hamburg, Germany;
5. Department of Physics, Arizona State University, Tempe, AZ, USA;
6. Optical Microscopy and Analysis Laboratory, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Frederick, MD, USA;
7. Linac Coherent Light Source, SLAC National Accelerator Laboratory, Menlo Park, CA, USA;
8. X-ray Science Division, Argonne National Laboratory, Lemont, IL, USA. Department of Structural Biology;
9. Jacobs School of Medicine and Biomedical Sciences, SUNY University at Buffalo, Buffalo, NY, USA;
10. Kenwood Interdisciplinary Research, Complex Physics Department, University of Wisconsin-Milwaukee, Milwaukee, WI, USA;
11. Centre for Ultrafast Imaging, Universität Hamburg, Hamburg, Germany;
12. Present address: Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA;
13. Department of Chemistry and Physics, ARC Centre of Excellence in Advanced Molecular Imaging, La Trobe Institute for Molecular Science, La Trobe University, Melbourne 3086 Victoria, Australia.

Studying ligand-inducible phase changes in macromolecular crystals

The growing field of time-resolved structure determination faces significant challenges. Although much progress has been made in mapping out local changes in photocycles of light-activated proteins, the vast majority of macromolecules function through interactions with ligands or other biomolecules, many of which can involve large and more complex structural changes. Such changes in a crystal are often energetically and mechanically impeded by lattice restraints, and when forced by mass action, can disrupt lattice order to the extent of uninterpretable or total loss in diffraction. To circumvent these challenges, we have performed extensive characterization studies on ligand-inducible phase changes in crystals of adenine riboswitch aptamer RNA. The addition of ligand to the crystals triggers a sequence of reversible lattice changes that are directly associated with conformational switching. Using a combination of time-resolved methods, including polarized video microscopy, atomic force microscopy, and X-ray diffraction, we have identified a minimum of four distinct lattices and the atomic structures of each. Moreover, the three methods collectively demonstrate that molecular switching occurs cooperatively over fractional crystal volumes, and that smaller crystals yield more rapid and more uniform phase transitions. Currently, we are exploring other methods to improve molecular synchrony such as the use of a photocaged ligand and crystals grown under microgravity.

SPEAKER

STEPHANIE A. WANKOWICZ

Stephanie A. Wankowicz, Saulo H.P. de Oliveira, Daniel W. Hogan, Henry van den Bedem, James S. Fraser

Ligand binding remodels protein side chain conformational heterogeneity

While protein conformational heterogeneity plays an important role in many aspects of biological function, including ligand binding, its impact has been difficult to quantify. Macromolecular X-ray diffraction is commonly interpreted with a static structure, but it can provide information on both the anharmonic and harmonic contributions to conformational heterogeneity. Here, through multiconformer modeling of time- and space-averaged electron density, we measure conformational heterogeneity of 743 stringently matched pairs of crystallographic datasets that reflect unbound/apo and ligand-bound/holo states. When comparing the conformational heterogeneity of side chains, we observe that when binding site residues become more rigid upon ligand binding, distant residues tend to become more flexible, especially in non-solvent exposed regions. Among ligand properties, we observe increased protein flexibility as the number of hydrogen bonds decrease and relative hydrophobicity increases. Across a series of 13 inhibitor bound structures of CDK2, we find that conformational heterogeneity is correlated with inhibitor features and identify how conformational changes propagate differences in conformational heterogeneity away from the binding site. Collectively, our findings agree with models emerging from NMR studies suggesting that residual side chain entropy can modulate affinity and point to the need to integrate both static conformational changes and conformational heterogeneity in models of ligand binding.

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SPEAKER

MAXIMILLIAN WRANIK

Maximillian Wranik[1], Tobias Weinert[1], Chavdar Slavov[2], Tiziana Masini[3], Antonia Furrer[1], Natacha Gaillard[1], Dario Gioia[3], Marco Ferrarotti[3], Daniel James[1], Hannah Glover[1], Melissa Carrillo[1], Demet Kekilli[1], Florian Dworkowski[4], Robin Stipp[1], Petr Skopintsev[1], Steffen Brünle[1], Tobias Mühlethaler[1], John Beale[1], Dardan Gashi[4], Karol Nass[4], Dmitry Ozerov[5], Philip Johnson[4], Claudio Cirelli[4], Camila Bacellar[4], Markus Braun[2], Chris Milne[3], Andrea Cavalli[3], Joseph Wachtveitl[2], Michel O. Steinmetz[1,6], Jörg Standfuss[1]

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2 Institute of Physical and Theoretical Chemistry, Goethe University, Frankfurt am Main, Germany.
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6 Biozentrum, University of Basel, 4056 Basel, Switzerland.

Molecular snapshots of drug release over eleven orders of magnitude in time

The dynamic interplay between proteins and their ligands is central to molecular biology, pharmacology, and drug development but is difficult to resolve experimentally. Using time-resolved serial crystallography, we studied the release of the photochemical affinity switch azo-combretastatin A4 from the colchicine site of the anti-cancer target tubulin. Thirteen logarithmically spaced temporal snapshots at near-atomic resolution, complemented by time-resolved spectroscopy and molecular dynamics simulations show how the cis to trans isomerization of the azobenzene bond stretches the ligand within its binding pocket in the picoseconds to nanoseconds range, followed by stepwise opening of a gating loop within microseconds, and completion of the unbinding reaction through col-lapse of the binding pocket within milliseconds. Ligand changes and unbinding are accompanied by global tubulin-backbone rearrangements. Our results have implications for the mechanism of action of anti-tubulin drugs and provide a general experimental framework to study protein-ligand interaction dynamics.

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SPEAKER

HYOTCHERL IHEE

Department of Chemistry and KI for the BioCentury, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Republic of Korea; Center for Advanced Reaction Dynamics, Institute for Basic Science (IBS), Daejeon 34141, Republic of Korea

Exploring Ultrafast Protein Structural Dynamics with Time-Resolved X-ray Liquidography.

Time-resolved x-ray liquidography (TRXL) has been established as a powerful experimental tool for investigating molecular structural dynamics in solution. We have applied this method to various small molecules including diatomic molecules, haloalkanes, organometallic complexes [1-3] to reveal structural dynamics over timescales from femtoseconds to milliseconds. Moreover, the technique has been applied to protein molecules to unveil structural dynamics of proteins in solution [4-9]. One of the representative experimental methods to study protein structural dynamics is time-resolved x-ray crystallography. Time-resolved x-ray crystallography can determine three-dimensional structure of proteins with an atomic resolution, but the stringent prerequisite requiring highly-ordered and radiation-resistant single crystals have limited its applicability toward more various proteins. The problem can be overcome by applying time-resolved x-ray diffraction directly to protein solutions rather than protein single crystals. This time-resolved x-ray scattering method applied on the solution-phase samples is named time-resolved x-ray liquidography (TRXL), which is analogous to time-resolved x-ray crystallography applied to crystalline samples. We will present our recent results including the achievement of femtosecond TRXL using an x-ray free-electron laser. Using femtosecond TRXL, we investigated the ultrafast structural dynamics of homodimeric hemoglobin (HbI), which is a model system for studying allosteric structural transition and cooperativity of proteins. From the kinetic analysis and structure refinement performed using the TRXL data of HbI, we identified a reaction intermediate (I0) that is formed within a picosecond and unveiled the ultrafast structural response of HbI upon photoexcitation including the protein quake and coherent motion. In addition to the structural change of protein itself, we captured the change of the electron density of the hydration shell by analyzing the small-angle x-ray scattering (SAXS) region which is dominantly affected by the change of the hydration shell.

References:

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SPEAKER

FOIVOS PERAKIS

Department of Physics, AlbaNova University Center, Stockholm University, S-106 91 Stockholm, Sweden

Resolving protein dynamics with X-ray photon correlation spectroscopy at the European XFEL

In this presentation I will highlight research opportunities and challenges in probing structural dynamics of biomolecular systems using X-ray Photon Correlation Spectroscopy (XPCS) [1]. The development of new X-ray sources, such as 4th generation storage rings and X-ray free-electron lasers (XFELs), provides promising new insights into molecular motion. Employing XPCS at these sources allows to capture a very broad range of timescales and length scales, spanning from femtoseconds to minutes and atomic scales to the mesoscale. I will discuss the scientific questions that can be addressed with these novel tools for two prominent examples: protein dynamics in crowded environments and aggregation phenomena, which take place on micro and sub-microsecond timescales.

Investigating proteins in solutions with extremely high dose rates–as generated by XFEL pulses–is accompanied by many challenges like beam driven dynamics and structural changes due to radiation damage. We explore these effects with a combined megahertz small angle X-ray scattering (MHz-SAXS) and megahertz X-ray photon correlation spectroscopy (MHz XPCS) experiment at the MID instrument at European XFEL [2]. We probe the dynamics in a concentrated gamma-globuline (Ig) solution-PEG (>80% IgG), which is a prototypical antibody protein. The MHz-SAXS signal reveals threshold values for a maximum tolerable dose of 10 kGy independent of dose rate. However, the MHz-XPCS correlation functions reveal microsecond diffusion dynamics of the Ig molecules within their first coordination shell, with a pronounced dependence both on dose rate and absolute dose.

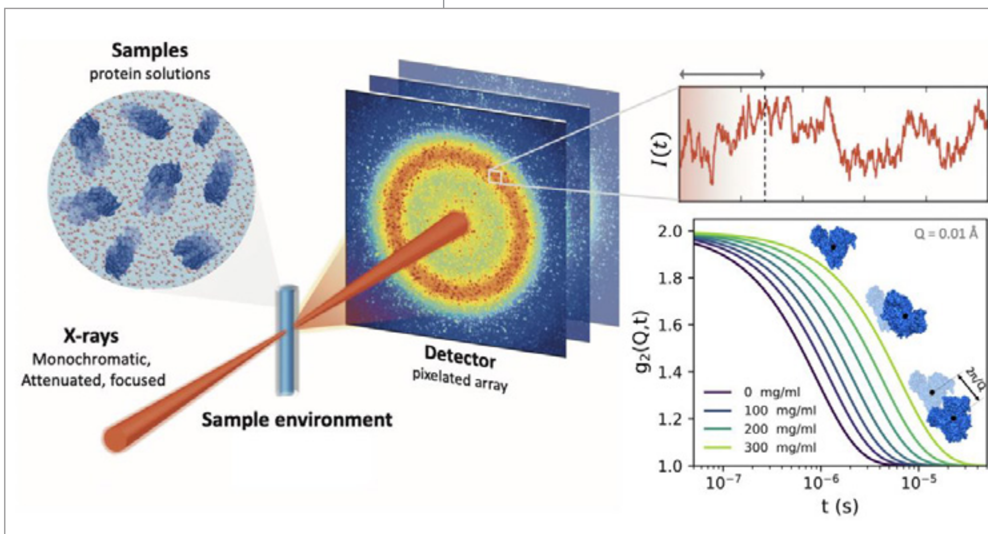

FIG. 1 A schematic of protein X-ray Photon Correlation Spectroscopy (XPCS). The coherent scattering intensity is recorded as a function of time, which fluctuates due the changes of the speckle pattern. By calculating the intensity correlation function g2 one can obtain information about the molecular-level dynamics.

References:

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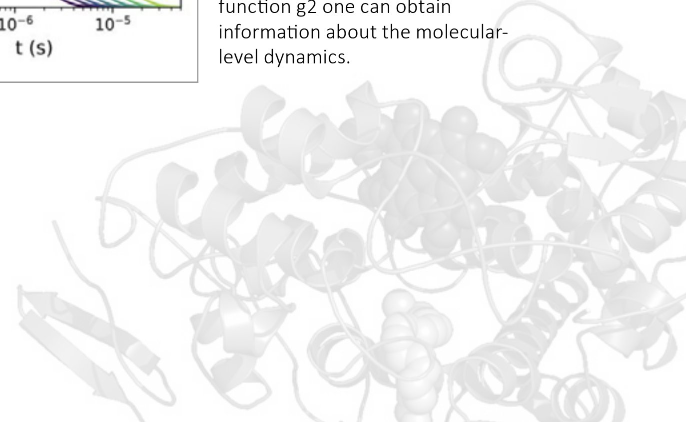

SPEAKER

EDWIN G. PEÑA-MARTÍNEZ

Edwin G. Peña-Martínez, José A. Rodríguez-Martínez

University of Puerto Rico-Río Piedras Campus, San Juan PR, Edwin.pena1@upr.edu

Characterizing the Effects of Non-coding Mutations in Cardiac Transcription Factor-DNA Interactions

Congenital heart disease (CHD) is the most common birth defect that results in structural abnormalities in the heart. Mutations in cardiac transcription factors (TFs), such as NKX2-5, have been shown to cause CHD. However, mutations within the non-coding genome and their associations to diseases, like CHD, have remained underexplored. Non-coding genomic variation affects how TFs recognize DNA and may disrupt TF-DNA binding. Our goal is to understand how non-coding variants affect TF-DNA interactions involved in heart development. First, using the ~84 millions Single Nucleotide Polymorphisms (SNPs) identified in the 1000 Genomes Project, I identified 8,475 that are predicted to affect NKX2-5 binding. To prioritize variants, we furthered filtered our binding predictions to include variants that localize within previously identified cardiac enhancers or that have been previously identified in Genome Wide Association Studies. In summary, we identified 901 SNPs found in cardiac enhancers, and 30 disease-associated SNPs from the GWAS catalog predicted to affect NKX2-5 binding sites. NKX2-5 binding to a selection of the identified variants will be tested by Electrophoretic Mobility Shift Assay (EMSA) and Biolayer Interferometry (BLI). Successful completion of this project will provide insight to the molecular mechanism of non-coding mutations on heart development. Additionally, these findings will aid in developing genetic tests to diagnose these SNP-associated diseases.

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SPEAKER

ANDREA MARKELZ

Jeffrey Mckinney[1], Deepu George[1], Yanting Deng[1], Alex Mcnulty-Romaguera[1], Xiaotong Zhang[2], Jason B. Benedict[2], Tod Romo[3], A. M. Grossfield[3], and A. G. Markelz[1]

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Simplifying Protein Collective Vibrational Assignment Through Symmetry

Anisotropic terahertz microspectroscopy (ATM) isolates protein collective vibrations from a highly congested spectrum based on the direction of the vibrational transition dipole[1,2]. ATM changes with inhibitor binding and photo excitation reveal that the vibrational bath evolves with functional state. By comparing the measurements to calculations, specific structural motions can be assigned to spectral bands. The complexity of the anisotropic spectral structure is dependent on the level of alignment of the molecules. This alignment is achieved using protein crystals, which are routinely used for structure determination and typically have 30-70% water by volume. One expects that the lowest symmetry crystals will have the most spectral structure, whereas higher symmetry crystals will have decreasing structure due to the dipole cancellation. Here we examine crystal symmetry effects for hen egg white lysozyme crystals (HEWL). ATM measurements of triclinic, monoclinic and tetragonal HEWL crystals find that indeed the spectral structure decreases with increasing symmetry. We compare the measurements to normal mode ensemble analysis calculations and find good agreement with the resulting symmetry in the anisotropic spectra. By comparing the spectrum from different crystal symmetry groups of the same protein CEWL, we identity the conserved vs unique spectral features across molecular arrangements in lattices. Peak near 40 cm^{-1} and 55 cm^{-1} were common among three lattice systems while a peak near 20 cm^{-1} was observed only in triclinic CEWL. This serves as a guide in determining the direction of the modes with respect to the molecule and identifying whether crystal contact forces influence the spectrum. The experimental spectrum is compared and contrasted to an ensemble averaged calculated anisotropic spectrum.

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SPEAKER

MARK HUNTER

Biological Sciences and Sample Environment and Delivery Departments, LCLS

Sample preparation, characterization and delivery at LCLS

Sample handling, preparation, characterization, and delivery at LCLS are handled by the Sample Environment and Delivery (SED) department. As LCLS is progressing to the LCLS-II era and beyond, many R&D activities are being pursued by SED to improve current user operations as well as prepare for future possibilities at current and planned instruments, such as the Tender X-ray Instrument (TXI). An overview of existing hardware and injection methods that are supported at LCLS as well as the current state of the wetlab spaces at LCLS and future space and equipment expansion possibilities will be brought up for discussion and feedback. Current research areas including automated Droplet on Demand (DoD) and sheet jets of varying thicknesses for use at all hutches of LCLS, including recent results of in house and user beamtimes, will be covered. A critical component to the effort is the integration of SED activities with the NIH Center for Structural Dynamics in Biology (SDB) at LCLS. The integrated work includes automation and work towards streamlined LCLS experiments for the general bioscience user. How these current efforts are improving outcomes during experiments and how we envision them enhancing engagement in experiments, *e.g.* user-assisted commissioning and complete user operation of the DoD system and MFX beamline, will be detailed.

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SPEAKER

SARTHAK SAHA

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A Microfluidic Device for Room Temperature Crystallography

X-ray crystallography is the primary technique for determining the 3D structure of proteins and facilitates understanding the effects of protein structure on function. One of the key bottlenecks of X-ray crystallography remains the need to loop out protein crystals from the crystallization buffer and cryo-cool them for X-ray data collection. The process of crystal manipulation is very sophisticated and often ends up damaging the protein crystals. Although data collection at cryogenic temperature stabilizes the crystal, it also freezes out functional motions. Collecting room temperature data allows for the observation of conformational ensembles which can help in understanding protein function. We have developed a microfluidic device that allows on chip crystallization and room temperature data collection. We have employed UV curable polymer to fabricate the device with a high level of X-ray transparency. This enables data collection without the need to harvest the crystals. We have demonstrated the room temperature data collection of the hub domain of human calcium/calmodulin-dependent protein kinase II (CaMKII). The crystals were grown inside the device and RT data collection yielded 2.5Å resolution data. We also observed serval extra residues not visible in previous cryogenic data. This device not only eliminates the need for manual manipulation of crystals for room temperature data collection, but it also facilitates serial crystallography at the synchrotron.

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SPEAKER

ROBERT BÜCKER

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Serial Electron Diffraction for Macromolecules – Chances and Challenges.

Serial electron diffraction[1] is an emerging three-dimensional electron diffraction[2] method performed in a scanning transmission electron microscope (STEM), where, in analogy to fixed-target serial X-ray crystallography at XFELs or synchrotrons, data from a large ensemble of nano-crystals is merged into a high-resolution structure solution. Despite the comparable collection strategy, the scattering properties of electrons lead to some specificities, such as a lower inelastic energy deposition, higher sensitivity to hydrogens and charge states, and incompatibility with crystals of size above 1 µm.

Like its X-ray counterpart, SerialED is typically performed in the limit of rapidly acquired single or dosefractionated diffraction snapshots, minimizing deleterious effects of radiation damage. However, the flexibility of a STEM enables a much broader range of data collection strategies, blurring the lines between serial crystallography (SX), momentum-resolved STEM (4D-STEM), and massively automated rotation electron diffraction[3].

I will present results from a range of highly radiation-sensitive biological and organic samples, discuss approaches for collection, data processing[4] and sample preparation, and give an outlook on ongoing projects towards time-resolved SerialED and new sample classes.

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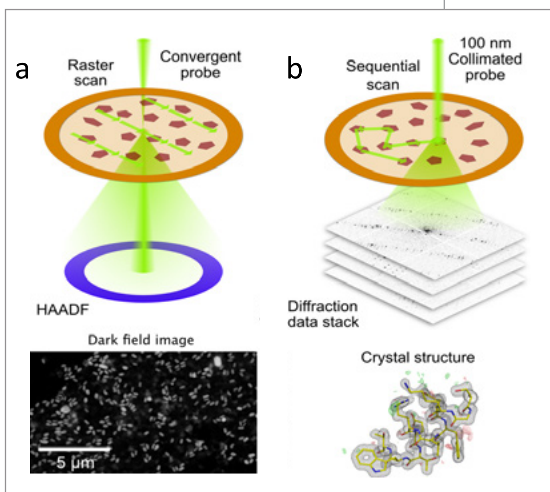

Figure 1: Schematic of a SerialED data collection. (a) A sample grid region is screened in STEM dark-field mode for crystals. Crystal positions are stored. (b) Directly after the screening, crystals are illuminated sequentially with a collimated nano-beam, and a stack of serial diffraction data is collected.

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Diffracting intracellular protein crystals - optimizing delivery of insect cells.

Protein crystals in living cells have been observed surprisingly often in all domains of life. With this crystallization approach, it is possible to grow a huge number of micron-sized high quality protein crystals in the crowded environment of the cell in a short time.[1] We are establishing a streamlined process towards a systematic use of *in cellulo* crystallization in insect cells for structural biology[2-4].

The gene of interest is cloned into baculovirus transfer vectors. The associated recombinant baculoviruses are generated to infect insect cells. Crystal formation can be detected a few days after infection[5]. If intracellular crystallization was successful, protein crystals can be extracted from the cell and diffraction data of the *in cellulo*-grown crystals are collected using serial crystallography approaches. Either XFELs or highly brilliant synchrotron sources are employed, depending on the average crystal diffraction volume.[3,4,6,7] The arsenal of potentially binding cofactors within the cell is large, and at near physiological conditions of a respective host, therefore one can identify high affinity binding ligands in the electron density[6], which can be exploited as an alternative method to elaborate soaking experiments. In our hands, more than 20 different proteins in living insect cells have been successfully crystallized.

Today, *in cellulo* crystallization is still a niche method. This is due to major bottlenecks: The fraction of crystal containing cells in a batch varies between more than 80 % and less than 1 %, depending mainly on the recombinant protein sequence. In addition, cell lysis and crystal purification out of the cellular debris, accompanied by necessary changes of environmental conditions, can reduce crystal integrity[1,2].

As presented in this talk, one solution to these limitations is optimization of data collection strategies for this special kind of sample, and so we have established and tested techniques for serial diffraction data collection from *in cellulo* grown crystals directly within the living insect cells. These innovative approaches avoid crystal purification and transfer of the living, crystal-containing cells, and furthermore allow direct screening of cell cultures for successful *in cellulo* protein crystallization using the X-ray beam[5].

In the future, the current *in cellulo* crystallization pipeline has to be tuned to enable structural studies also on targets with a low intracellular crystallization efficiency, e.g. by integrating fluorescence-based cell sorting to enrich the proportion of crystal containing cells. Much of the underlying molecular biology of this fascinating phenomenon intracellular protein crystallization remains to be discovered in this relatively young research field. Our results pave the way to a more efficient use of crystal containing insect cells as suitable targets for serial diffraction data collection at synchrotrons and XFELs.

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Acknowledgement:

MLR acknowledges a PhD scholarship from the Joachim Herz Foundation. Partly supported by the German Federal Ministry for Education and Research (BMBF; grant 05K18FLA).

SPEAKER

KARA ZIELINSKI

Kara Zielinski and Lois Pollack

Cornell University

The Importance of Pre-Screening Ligand Binding In Crystallo for Mix-and-Inject Serial Crystallography

Serial Femtosecond Crystallography (SFX) at X-ray Free Electron Lasers (XFELs) is used to determine room temperature, damage-free, protein structures from micron-sized crystals.[1] With its demonstrated success, attention has now turned to Mix-and-Inject Serial Crystallography (MISC), which exploits the small dimensions of the microcrystals to enable rapid mixing for diffusion initiated reactions. This rapid diffusion is achieved by mixing injectors, which precisely position microfluidic mixers just upstream of a Gas Dynamic Virtual Nozzle (GDVN) to carry the freshly mixed crystals into the X-ray interaction region.[2,3] These mixing injectors are robust and have been used successfully at multiple XFEL facilities for many biological systems, including for remote experiments.[4,5]

Most of the effort for sample preparation for MISC goes into producing a high yield of microcrystals with a high diffractive quality. This no easy feat and all these qualities are essential, but there is another important characteristic that is often forgotten: confirmation of ligand binding *in crystallo*. If the ligand cannot bind inside the crystal, the MISC experiment will not yield useful data. Many features of the crystal system itself, such as protein packing within the crystal, the accessibility of the active site, and the size solvent channels that allow for the transport of ligands into the crystal, can impede the ability of the ligand to bind. Crystals that have only been optimized for diffractive quality can be very misleading, as many experimenters will put in the work to collect days' worth of promising diffraction data, just to eventually find no ligand is present, likely due to issues with the crystal system. Although a lot of progress has been made with real-time data analysis, it is still not trivial, especially for MISC experiments. Thus, it is very difficult to assess the presence of a ligand fast enough to make adjustments during a beamtime. Additionally, it can take a lot of time and effort to find a new crystal form that is amenable to MISC and still has a high diffractive quality.

The only way to avoid the pitfall of conducting a MISC experiment with crystals that are not well suited for the method is to assess ligand binding *in crystallo* well in advance of the beamtime. Orthogonal techniques, such as various spectroscopic or fluorescence methods, are well suited to evaluate if the ligand can bind inside the crystal. We have recently demonstrated the use of Electron Paramagnetic Resonance Spectroscopy to confirm ligand binding *in crystallo* with the model system of myoglobin and azide.[6] We present the results of a recent experiment with a high-value target that showed no ligand binding due to the active site being blocked inside the crystal and demonstrate a strategy for sample preparation and pre-screening of new biological target to better ensure the success of these information-rich MISC experiments.

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SPEAKER

VALERIO MARIANI

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Remote real-time experiment monitoring for fast decision making: challenges and opportunities.

Traditionally, experiments at XFELs are carefully planned by the research groups, who then travel to the facilities, collect data on the instrument, and retrieve the desired physical information. The sophistication of the workflow and the volume of data to analyze often lead to gaps of months or even years between the experiment itself and the achievement of the desired outcome. Several elements are now coming together to disrupt this established model.

The commissioning of next generation light sources like LCLS-II, capable of collecting data at an unprecedented scale, is emphasizing the challenges of not only processing, but even just storing such a large amount of data for prolonged periods of time. Researchers are required to optimize their data collection strategies, maximizing the information density of the recorded data, and avoiding unproductive use of instrument time, rapidly depleting sample and data storage space. Researchers now need to assess the quality of the data while the data is still being collected. A rapid interpretation of the scientific content of the data is also a requirement. This information can then be used to steer the next steps of the experiments in a favorable direction.

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Recently, software development has focused on the development of tools for real-time data analysis. These software packages must usually interact with the data acquisition system of the facility to capture data as it is being collected. They are often difficult to install and configure: detailed knowledge of the technical details of the facility's operations, like data formats and network topologies, is frequently required. Considerable programming skills are also often necessary to operate these programs and to adapt them to the wide range of experiments that take place at XFELs. The scientific interpretation of the processed data also poses its own set of challenges, demanding complex interactions between software developers and scientists, to bridge the divide between programming expertise and scientific domain knowledge.

The ongoing COVID pandemic has also disrupted the traditional approach to scientific experiments in a second important way. Movement restrictions and safety requirements have often made it impossible for researchers to physically travel to the facility to conduct experiments. Scientists now work from their home institutions and on-site presence is minimal. Unfortunately, most of the available real-time monitor software is designed to be run on the beamline computing resources, frequently on a small subset of the available machines. Specific versions of software libraries or operating systems are often required to install the programs. Additionally, the graphical user interfaces that present the data processing to the researchers are in most cases designed to be used on a desktop or laptop computer rather than remotely over the internet. All these limitations severely hinder the usage of real-time monitoring software in remote experiments.

This presentation will focus on these challenges, and on the opportunities that they introduce, with particular focus on the OM (OnDA Monitor) real-time experiment monitor software, of which the authors are core developers.

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SPEAKER

FILIPE MAIA

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Overcoming the limits of ultrafast X-ray single-particle imaging.

The dream of imaging single molecules was instrumental to the construction of X-ray free-electron lasers (XFELs). The extremely bright and short pulses provided by XFELs make it possible to collect the diffraction pattern of a particle before its destruction (Neutze et al. 2000) which was successfully proved at FLASH more than a decade ago (Chapman et al. 2006). Since then, the method of flash X-ray imaging (FXI) has been used to image live cells (van der Schot et al. 2015), cell organelles (Hantke et al. 2014) and in particular the giant Mimivirus in both two dimensions (Seibert et al. 2011) and three dimensions (Ekeberg et al. 2015). The inauguration of the European XFEL marked the beginning of the high-intensity, high-repetition-rate and high data-rate era of XFELs, and it has been shown that FXI can take full advantage of those rates (Sobolev et al. 2020).

Yet, FXI has not yet fulfilled its promise of high-resolution sub-nanometer imaging. In this talk I will present the evolution of FXI, and in particular highlight our latest results from the European XFEL. I will also discuss what prevents FXI from achieving the aforementioned high-resolution as well as some possible solutions to overcome the current limits of ultrafast X-ray single-particle imaging.

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SPEAKER

MEGAN SHELBY

Shelby, M.L.,[1] Gilbile, D.,[2] Liu, Z.,[2] Lyubimov, A.,[3] Grant, T.D.,[4,5] Bauer, W.J.,[5] Crespo, N., [4,5] Fischer, P.,[6] Pakendorf, T.,[6] Hennicke, V.,[6] Wierman, J.L.,[3] Russi, S.,[3] Cohen, A.E.,[3] Hunter, M.S.,[7] Batyuk, A.,[7] Meents, A.,[6] Coleman, M.A.,[1,2] Kuhl, T.L.,[2] and Frank, M.[1,2]

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A fixed-target approach to overcoming crystallization, sample environment, and sample delivery obstacles for room temperature serial crystallography.

Serial femtosecond crystallography (SFX) at X-ray free electron lasers (XFELs) has proven a powerful tool for room temperature characterization of protein structure and dynamics, especially for micro- and nanocrystals of samples intractable to crystallization, for instance membrane proteins with solubility and stability limitations. However, delivery of the microcrystalline sample into the X-ray beam path while maintaining a controlled environment with low X-ray scatter background, and with minimal sample consumption, remain technical challenges, especially where this methodology is applied to samples that are difficult to purify and crystallize in large quantities or prove unstable under conventional jetting conditions.

A fixed target sample delivery approach provides key solutions by lowering sample consumption and maintaining a hydrated environment, while allowing greater flexibility in sample distribution and handling. We have demonstrated the use of polymer thin films, graphene, and all-polymer microfluidic chips for on-chip sample crystallization, delivery, encapsulation, and support as a generally applicable method for high-throughput and high-resolution biological imaging at room temperature, providing a low-volume, low-background approach to protein SFX. A fixed target geometry will also enable novel sample manipulation and triggering applications through thin film functionalization and modification of the substrate design. Implementation of a polymer thin-film encapsulation approach has been successful for both synchrotron and XFEL serial diffraction data collection, delivering a robust new tool for fixed target delivery while minimizing sample handling and consumption.

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SPEAKER

AHMAD HOSSEINIZADEH

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Few-femtosecond resolution of a photoactive protein traversing a conical intersection.

The structural dynamics of a molecule are determined by the underlying potential energy landscape. Conical intersections are funnels connecting otherwise separate potential energy surfaces. Posited almost a century ago, conical intersections remain the subject of intense scientific interest. In biology, they have a pivotal role in vision, photosynthesis and DNA stability. Accurate theoretical methods for examining conical intersections are at present limited to small molecules. Experimental investigations are challenged by the required time resolution and sensitivity. Current structure-dynamical understanding of conical intersections is thus limited to simple molecules with around ten atoms, on timescales of about 100 fs or longer. Spectroscopy can achieve better time resolutions but provides indirect structural information. Here we present few-femtosecond, atomic-resolution videos of photoactive yellow protein, a 2,000-atom protein, passing through a conical intersection. These videos, extracted from experimental data by machine learning, reveal the dynamical trajectories of de-excitation via a conical intersection, yield the key parameters of the conical intersection controlling the de-excitation process and elucidate the topography of the electronic potential energy surfaces involved.

Acknowledgments:

This research was supported by the US Department of Energy, Office of Science, Basic Energy Sciences under award DE-SC0002164, and by the US National Science Foundation under award STC 1231306 and DBI-2029533. N. Breckwoldt and R. Santra were supported by the Cluster of Excellence 'CUI: Advanced Imaging of Matter' of Deutsche Forschungsgemeinschaft (DFG)–EXC 2056–project no. 390715994.

BioXFEL

SPEAKER

PABLO VILLANEUVA-PEREZ

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Towards real-time X-ray imaging at X-ray Free-electron lasers

The unique high-brilliance or coherent flux provided by X-ray free-electron lasers (XFELs)[1] has opened new opportunities to explore nature with coherent X-ray imaging techniques, such as in-line holography. When implemented in XFELs, such methods can expand the achievable spatial and temporal resolutions compared to storage rings. Together with other XFEL properties, this unique capability has brought the scientific community to build such facilities worldwide[2,3]. Despite their potential and significant interest in the scientific community, XFELs are scarce, and their availability for users is limited. Thus, approaches to increasing availability and reducing the entry barrier to new groups will help optimize the usage and exploit the unique capabilities of XFELs.

This talk will discuss how machine learning combined with simple analytical algorithms can lead to online or real-time analysis tools for coherent X-ray imaging, such as in-line holography. These real-time processing algorithms will provide experimental feedback to researchers at XFELs. Through this feedback mechanism, we hope that the success of experimental campaigns can be increased by enabling the researchers to evaluate the performance and the impact of changing their experimental configuration in real time. Specifically, this talk presents three lines of work towards this goal.

First, we will discuss a machine learning approach to enable advanced phase-retrieval reconstructions via flat-field corrections. Flat-field correction (FFC) is a technique that mitigates illumination and experimental effects that hinder the applicability of phase-retrieval algorithms, especially in the near-field domain. Given the stochastic nature of the self-amplified spontaneous emission responsible for XFELs' high brilliance, conventional FFC approaches do not provide a satisfactory solution to perform imaging from single XFEL pulses. Thus, we study the applicability of analytical[4] and machine-learning algorithms capable of performing FFC from single-shot experiments. Second, we will discuss real-time phase-retrieval reconstructions in the near-field regime. For that purpose, we will present an unsupervised Deep-learning framework that can robustly obtain phase reconstructions by adding the image formation's physics for a specific setup and configuration. This framework, christened PhaseGAN[5], will not only enable real-time reconstructions but also reconstructions when only simulations or data from other experiments are available. Finally, we will present X-ray multI-projection imaging[6], an approach capable of retrieving 3D information from single pulses, and how machine-learning algorithms can enhance the 3D capabilities of this approach when only a sparse number of radiographs are collected instead of a complete tomographic acquisition.

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SPEAKER

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What can imaging tell you about how your crystals grow? Advances using machine learning

One of the primary bottlenecks in biomolecular crystallography is identifying conditions in which a biomolecule will crystallize; imaging methods are vital to the process of screening for successful crystallization hits. When crystallization screening is carried out in a high-throughput manner, the enormous volume of images needing processing requires a large commitment of time and effort to locate successful crystallization conditions. To address this obstacle, machine learning approaches are used to empower better detection of crystals. This includes the MAchine Recognition of Crystallization Outcomes (MARCO) scoring algorithm, which was trained and tested on brightfield crystallization images in a collaborative effort between HWI, major pharmaceutical companies, and other crystallization facilities worldwide[1]. To further enhance crystal detection, we can integrate standard brightfield imaging with additional imaging modalities, including Ultraviolet Two-Photon Excited Fluorescence (UV-TPEF) and Second Harmonic Generation (SHG), which enables detection of submicron crystals and crystals under precipitate, as well as distinguishing between protein and salt crystals. We have recently developed a Graphical User Interface called MARCO-Polo, which uses the MARCO algorithm on brightfield images coupled to a viewing platform for multiple imaging modalities, such as UV-TPEF and SHG[2]. Different imaging modes are subject to different complications, including optical aberrations, resolution limitations, and behavior with respect to obstruction. Our current efforts are focused on ways to improve image processing and analysis for crystallization screening, with specific emphasis on machine learning approaches to combine the information from brightfield images and alterative imaging modalities to facilitate steps towards automated imaging to streamline crystal detection and acquisition.

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Acknowledgement:

Support is gratefully acknowledged from NIH NIGMS grant R24GM141256.

BioXFEL

SPEAKER

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Randomness in time-resolved serial crystallography data: alternatives to the binning and averaging approach.

Time binning approaches have proved successful in dealing with the issues of sparsity and partiality in time-resolved serial crystallography and produced molecular movies that offer unprecedented insight into the function of biomolecules in action. Yet the averaging procedure that underlies such approaches normally requires large numbers of frames: this often means binning over large time windows, which implies a loss of timing information.

While the data manifold of the time-evolving system is intrinsically one-dimensional - and its trajectory likely explores only a low-dimensional subspace of the high-dimensional data space, the physical specificities of the diffraction experiment introduce sparsity and partiality, artificially increasing the apparent dimension of the subspace in which the data points lie. Time-lagged embedding mitigates such issues and allows - at least in favourable situations - to reconstruct the underlying system dynamics by singular value decomposition in the supervector space (Singular Spectrum Analysis). Nonetheless this procedure is impractical in the case of large data sets, and in some circumstances may not be able to provide a low-rank approximation of the system dynamics[1]. Time-lagged embedding can be combined with data filtering in supervector space to ease these issues. This can be done - for instance, by using a set of orthonormal trigonometric functions as subspace basis, or alternatively a data-driven set of basis functions. The latter approach is called the nonlinear Laplacian spectral analysis (NLSA)[1], which was first applied to serial crystallography in [2].

These concepts are exemplified using synthetic models and preliminary serial crystallography results from the membrane protein bacteriorhodopsin in the first ps after photoactivation.

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