AGENDA

MONDAY, FEBRUARY 12TH, 2018

3:00 PM - ONWARDS — **ARRIVAL AND HOTEL CHECK-IN** **CLICK HERE FOR MAP FROM AIRPORT**
MAIN LOBBY

3:00 PM - 5:00 PM — **WORKSHOP AND CONFERENCE REGISTRATION**
MAIN LOBBY

8:30 AM - 4:00 PM — **EXTERNAL ADVISORS MEETING** **CLICK HERE FOR MAP OF MEETING SPACE**
JACKSON

6:00 PM - 7:00 PM — **POSTER SESSION SETUP**
JEFFERSON

5TH BIOXFEL INTERNATIONAL CONFERENCE

TUESDAY, FEBRUARY 13TH, 2018

7:30 AM – 8:30 AM — **LIGHT BREAKFAST - ST. CHARLES**

8:35 AM – 8:45 AM — **CONFERENCE WELCOME - ST. JAMES**
Conference Co-Chairs: James Fraser (UCSF) and Richard Kirian (ASU)

8:45 AM – 11:55 AM — **EXPLOITING THE TIME RESOLUTION OF SFX - ST. JAMES**
Session Chairs: George Phillips (Rice) and Christopher Kupitz (UWM)

8:45 AM – 9:05 AM — Takehiko Tosha (RIKEN)
Time-resolved XFEL crystallography and *in crystallo* spectroscopy for probing reaction dynamics of respiratory metalloenzymes

9:05 AM – 9:15 AM — Discussion

9:15 AM – 9:35 AM — Allen Orville (Diamond Light Source)
Dynamic structural biology at XFELs: Complementary tools applied to etalloenzymes that make or break O_2 bonds

9:35 AM – 9:45 AM — Discussion

9:45 AM – 10:05 AM — Petra Fromme (ASU)
Time-resolved femtosecond crystallography studies of photosystem II and I with X-ray Free Electron Lasers

10:05 AM – 10:15 AM — Discussion

10:15 AM –10:45 AM — **COFFEE BREAK - THE DISTRICT**

10:45 AM – 11:05 AM — Hasan DeMirci (Stanford)
Structure-Based Antibiotic Development Driven by Ambient-Temperature Serial Crystallography of Small and Large Ribosomal Subunits at High Resolution

11:05 AM – 11:15 AM — Discussion

11:15 AM – 11:30 AM	Haiguang Liu (BCSRC) Structure and dynamics of chloride ion pumping rhodopsin
11:30 AM – 11:35 AM	Discussion
11:35 AM – 11:50 AM	Leonie Flueckiger (La Trobe) Rapid mixing and injection for studying molecular dynamics in time-resolved liquid X-ray scattering experiments
11:50 AM – 11:55 AM	Discussion
12:00 PM – 1:45 PM	**LUNCH GENERAL GROUP - ST. CHARLES**
12:00 PM – 1:00 PM	**LUNCH PRESENTATION ON BIOXFEL OPPORTUNITIES FOR UNIVERSITY OF PUERTO RICO STUDENTS - JACKSON**
12:00 PM – 12:45 PM	**LUNCH ASSESSMENT MEETING FOR BIOXFEL POSTDOCS ONLY - MAGAZINE**
1:00 PM – 2:15 PM	**ALTERNATIVE SCIENTIFIC CAREER DISCUSSION PANEL - JACKSON** Chair: Ganesh Subramanian (ASU) Panel: Allison Doerr (Nature Methods), Marc Pusey (iXpressGenes), Stephen Soisson (Merck), Anne Stone (Molecular Dimensions)
2:15 PM – 4:45 PM	**USING XFELS ON NON-CRYSTALLINE TARGETS - ST. JAMES** Session Chair: Abbas Ourmazd (UWM) and Ghoncheh Mashayeki (UWM)
2:15 pm – 2:35 pm	Jochen Hub (Gottingen) Interpretation of (time-resolved) solution X-ray scattering data by explicit-solvent molecular dynamics simulation
2:35 pm – 2:45 pm	Discussion
2:45 pm – 3:05 pm	Michael Brown (U of Arizona) Femtosecond X-ray scattering of membrane proteins with a free electron laser
3:05 pm – 3:15 pm	Discussion
3:15 PM – 3:45 PM	**COFFEE BREAK - THE DISTRICT**
3:45 pm – 4:05 pm	Carolin Seuring (CFEL) X-ray coherent diffraction of amyloid fibrils on graphene: past, present and future
4:05 pm – 4:15 pm	Discussion
4:15 pm – 4:35pm	Brenda Hogue (ASU) Virus conformational landscapes from single-particle X-ray scattering
4:35 pm – 4:45 pm	Discussion
4:45 PM – 5:15 PM	**POSTER BLITZ - ST. JAMES** Session Chair: Richard Kirian (ASU) and James Fraser (UCSF)
6:00 PM - 9:00 PM	**DINNER AT BARCADIA / OHM LOUNGE CLICK HERE FOR MAP** 601 Tchoupitoulas St, New Orleans, LA 70130

Wednesday, February 14th, 2018

8:00 AM – 8:45 AM **Light Breakfast - St. Charles**

8:45 AM – 11:35 AM **Getting Sample to the Beam - St. James**
Session Chairs: Uwe Weierstall (ASU) and George Calvey (Cornell)

8:45 AM – 9:05 AM Andrea Katz (Cornell)
Mixing injector for time-resolved crystallography at X-ray free electron lasers
9:05 AM – 9:15 AM Discussion

9:15 AM – 9:35 AM Marie Gruenbein (MPI-H)
Considerations for SFX data collection at MHz XFELs
9:35 AM – 9:45 AM Discussion

9:45 AM – 10:05 AM Sarah Perry (UMass Amherst)
Graphene microfluidics for room temperature crystallography
10:05 AM – 10:15 AM Discussion

10:15 AM – 10:45 AM **Coffee Break - The District**

10:45 AM – 11:05 AM Alexandra Ros (ASU)
Droplet microfluidics for serial femtosecond crystallography with XFELs
11:05 AM – 11:15 AM Discussion

11:15 am – 11:30 am Peter Berntsen (La Trobe)
Serial crystallography development in partnership with the Australian Synchrotron
11:30 am - 11:35 am Discussion

11:35 PM – 12:30 PM **Lunch General Group - St. Charles**

11:35 PM – 12:30 PM **Lunch Assessment Meeting for all Women Undergrads, Grads, and Postdocs - Magazine**

12:30 PM – 1:30 PM **Keynote Speaker: Krystle McLaughlin (Vassar) - St. James**
Taking a Community Centered Approach to Diversity

1:30 PM – 4:40 PM **New Applications in SFX - St. James**
Session Chairs: Wei Liu (ASU) and Benjamin Stauch (USC)

1:30 PM – 1:50 PM Michael Hennig (LeadXpro)
X-ray Free Electron Laser: Opportunities for drug discovery
1:50 PM – 2:00 PM Discussion

2:00 PM – 2:20 PM Andrea Markelz (UB)
Measuring protein intramolecular dynamics with terahertz light: Functional changes and relevance to biology
2:20 PM – 2:30 PM Discussion

2:30 PM – 2:50 PM Matthias Frank (LLNL)
Progress with solid sample supports for weakly diffracting objects
2:50 PM – 3:00 PM Discussion

3:00 PM – 3:20 PM Adrian Mancuso (EuXFEL)
Recent advances in instrumentation and methodology for XFEL structural studies from the SPB/SFX team at the European XFEL
3:20 PM – 3:30 PM Discussion

3:30 PM – 4:00 PM COFFEE BREAK - THE DISTRICT

4:00 PM – 4:15 PM Matthew Coleman (LLNL)
Using XFELs to characterize nanolipoprotein particles that form HDL and LDL complexes
4:15 PM – 4:20 PM Discussion

4:20 PM – 4:35 PM Gebhard Schertler (ETH-PSI)
Exploring G coupled protein receptors and membrane protein dynamics with X-ray free electron lasers and solution NMR
4:35 PM – 4:40 PM Discussion

4:40 PM – 6:30 PM DINNER ON YOUR OWN

6:30 PM – 7:00 PM MUSICAL PERFORMANCE BY BIOXFEL QUARTET

7:00 PM – 9:00 PM POSTER COMPETITION - JEFFERSON
7:00 - 8:00 EVEN POSTERS
8:00 - 9:00 ODD POSTERS

THURSDAY, FEBRUARY 15TH, 2018

8:00 AM – 8:45 AM LIGHT BREAKFAST - ST. CHARLES

8:00 AM – 12:00 PM POSTER SESSION CLEAN UP - JEFFERSON
*If you do not remove your poster you risk it being thrown away.

8:45 AM – 12:05 PM NEW MEASUREMENT AND ANALYSIS STRATEGIES- ST. JAMES
Session Chair: Nadia Zatsepin (ASU) and Natasha Stander (ASU)

8:45 AM – 9:05 AM Iris Young (LBNL)
XFEL diffraction studies of the oxygen evolving complex of photosystem II
9:05 AM – 9:15 AM Discussion

9:15 AM – 9:35 AM Henry Chapman (DESY)
Incoherent diffractive imaging for structure determination at atomic resolution
9:35 AM – 9:45 AM Discussion

9:45 AM – 10:05 AM David Case (Rutgers)
What can we learn from MD simulations of biomolecular crystals?
10:05 AM – 10:15 AM Discussion

10:15 AM – 10:45 AM **COFFEE BREAK - THE DISTRICT**

10:45 AM – 11:05 AM Helen Ginn (Diamond Light Source Ltd. - Oxford)
The slip-and-slide algorithm: a refinement protocol for detector geometry

11:05 AM – 11:15 AM Discussion

11:15 AM – 11:35 AM Doeke Hekstra (Harvard)
TBD

11:35 AM – 11:45 AM Discussion

11:45 AM – 12:00 AM Chenghua Shao (Rutgers)
XFEL structure deposition and data architecture support in PDB

12:00 AM – 12:05 AM Discussion

12:05 AM – 12:10 PM **STUDENT SPEAKER AWARD AND POSTER PRIZE ANNOUNCEMENTS**

12:10 AM – 12:15 PM **GROUP PHOTO**

12:15 AM – 1:00 PM **LUNCH GENERAL GROUP - ST. CHARLES**

1:00 PM – 4:00 PM **BIOXFEL STC MEETING - FOR CENTER MEMBERS ONLY - ST. JAMES**

1:00 PM – 4:00 PM 10 min (3 powerpoint) talks on current BioXFEL collaborations.
Speakers in alphabetical order.
John Spence

2:45 PM – 3:15 PM **COFFEE BREAK - THE DISTRICT**

KEYNOTE SPEAKER

KRYSTLE MCLAUGHLIN, VASSAR

TAKING A COMMUNITY CENTERED APPROACH TO DIVERSITY

Many studies have demonstrated that increasing levels of diversity in an organization leads to more varied and innovative ideas and outcomes. Efforts towards increasing diversity in STEM fields have increased over the last several years. At almost every level in academia, undergraduate, graduate, postdoctoral, and faculty, there are diversity programs designed to help broaden participation of scholars from underrepresented groups. An important part of the diversity equation, inclusion, is often sidelined. Whereas diversity in a group of people refer to differences in their demographic characteristics, inclusion refers to the creation of an environment that fosters acceptance and involvement of multiple perspectives. Diversity efforts must include a focus on inclusion to see meaningful long-term gains.

Though we can see initial recruitment numbers have improved, the number of scholars from underrepresented backgrounds completing programs or advancing further has not increased as much as would be expected. Experiences in STEM by scholars from underrepresented groups often paint a picture of an unwelcoming culture, prompting recent discussions calling for renewed focus on inclusion. Here I will discuss some strategies towards creating a more inclusive working space for underrepresented scholars in STEM. Improving community and building support systems, thereby facilitating the crucial connections that allow scholars from different backgrounds to foster a sense of belonging and ownership, encourage retention in the field.

BIOGRAPHY

Krystle J. McLaughlin is an Assistant Professor in the Chemistry Department at Vassar College. Research in the McLaughlin Lab involves using x-ray crystallography to characterize proteins that are potential drug targets from diverse microbial systems, such as *Bacteroides ovatus, Staphyloccocus aureus*, and *Mycobacterium* bacteriophages. Dr. McLaughlin is also interested in best practices in science pedagogy and improving chemistry education, with a particular focus on methods to improve retention of women and underrepresented groups. Her most recent publication in *J. Chem Ed.* details an activity to introduce undergraduate students to model building in crystallography using *Coot*. She has created and facilitated several workshops on research preparation for undergraduates, active learning techniques for teachers, and inclusive teaching best practices. As an advocate for diversity in science, Dr. McLaughlin has served since 2013 as the American Crystallographic Association's (ACA) delegate to the AIP Liaison Committee on Underrepresented Minorities, and successfully worked to introduce a Diversity and Inclusion session at ACA meetings, where research and strategies on diversity issues from crystallographers are highlighted. Additionally, Dr. McLaughlin is a member of the Steering Committee for the African Synchrotron Light Source (AfLS), committed towards construction of an advanced light source on the African continent.

Takehiko Tosha, RIKEN

Time-resolved XFEL crystallography and *in crystallo* spectroscopy for probing reaction dynamics of respiratory metalloenzymes

Minoru Kubo, 1.

1. RIKEN SPring-8 Center, 1-1-1 Kouto, Sayo, Hyogo 679-5148, Japan.

Time-resolved (TR) crystallography using X-ray free electron lasers (XFELs), combined with TR optical spectroscopy, is a powerful technique to observe protein dynamics at work in real time. Using SACLA, we have applied this technique to two respiratory metalloenzymes, bovine cytochrome *c* oxidase (CcO) and fungal NO reductase (P450nor).

CcO is the terminal oxidase of cell respiration that catalyzes O_2 reduction to H_2O at the heme-cupper center, coupled with proton pump across the mitochondrial inner membrane. In this study, the structural dynamics of CcO following CO ligand photo-dissociation from the heme was investigated, using serial femtosecond-rotational crystallography (SF-ROX) [1] combined with a pump-probe system [2]. TR-IR *in crystallo* spectroscopy was also preformed to monitor the CO ligand kinetics in the crystalline phase. By the TR crystallographic and spectroscopic analyses, we successfully observed the dynamic process of gate opening in the proton-pump pathway, triggered by CO dissociation from the cupper site in the μs time domain [3].

P450nor is a heme enzyme that catalyzes NO reduction to N_2O in the anaerobic respiration in the fungal mitochondria. To track the enzymatic reaction, a caged substrate (caged NO) was used as a reaction trigger in a pump-probe serial femtosecond crystallography (SFX) system [4]. The kinetics of NO reduction reaction in the crystalline phase was assessed by TR-visible *in crystallo* spectroscopy. Although the crystal packing affects the reaction rate, we captured NO binding to the heme at 20 ms after the caged-NO photolysis [5]. This study demonstrates the utility of caged compounds, which will expand the applications of TR crystallography for dynamic structural analyses of enzymes.

References

[1] Hirata K et al. *Nat. Methods* 11, 734-736 (2014).

[2] Sakaguchi M., Kimura T. et al. *J. Synchrotron Rad.* 23, 334-338 (2016).

[3] Shimada A., Kubo M., Baba S. et al. *Sci. Adv.* 3, e1603042 (2017).

[4] Kubo M. et al. *J. Synchrotron Rad.* 24, 1086-1091 (2017).

[5] Tosha T., Nomura T., Nishida T. et al. *Nat. Commun.* 8, 1585 (2017).

Allen Orville, Diamond Light Source

Dynamic structural biology at XFELs: Complementary tools applied to metalloenzymes that make or break O_2 bonds

Allen M Orville et al

Research Complex at Harwell and

Diamond Light Source, Diamond House, Harewell Science and Innovation Center

Didcot, Oxfordshire, OX11 0DE, United Kingdom

An outstanding frontier challenge in structural biology is to determine time-resolved crystal structures directly from systems engaged in catalysis at physiological pressure and temperature. To fully realize time-resolved/dynamic structural biology, one must understand as much function as possible from the same sample and/or molecular state. However, it is difficult to study oxygen intermediates in metalloenzymes by traditional, synchrotron-based macromolecular crystallography (MX) methods because their reactivity also makes them very sensitive to photoreduction by the X-ray beam.

Many Fe-dependent oxygenases function by activating O_2 to create and then control high-valent intermediates during catalysis of energetically difficult reactions. Such Fe(IV)=O intermediates have been proposed in many systems throughout biology, but typically only detected by spectroscopic methods. In typical crystallographic analyses, it is difficult or impossible to differentiate Fe (II/III)-(OH_2), Fe(II/III)-O_2, Fe(III)-O_2^-, Fe(III)-H_2O_2, Fe(IV)=O species by examining only electron density maps. Moreover, these species are often photoreduced by synchrotron studies conducted at 100 K. Fortunately, the spectroscopic signature of these species are different because they have different electronic structures. Consequently, we have developed on-demand acoustic sample delivery strategies to more efficiently collect fully-correlated, time-resolved serial femtosecond crystallography (tr-SFX) and X-ray emission spectroscopic (tr-XES) data from the same sample and XFEL pulse. The combinations of techniques provides spectroscopic and crystallographic data to support electronic and atomic models of various species throughout the reaction cycles. We have demonstrated with ribonucleotide reductase that it is possible measure X-ray emission spectra and demonstrate the oxidation state of the Mn(IV) Fe(IV) intermediate in microcrystals, which cannot be deduced from typical electron density maps alone.

Acoustic droplet ejection (ADE) is a general, touchless, on-demand method that uses focused sound waves to eject picoliter to nanoliter volume droplets from the surface of one liquid to another location. Our injector delivers discrete sample amounts onto a moving conveyor belt. It is optimized for photochemical and chemical reactions over a wide range of time scales and enables X-ray diffraction in the forward direction simultaneously with XES measurements at 90 degrees. Studies with photosystem II, ribonucleotide reductase R2 and several Fe-dependent oxygenases illustrate the power and versatility of these methods.

Fuller and Gul et al (2017) *Nature Methods* 14, 443–449

Young, Ibrahim, and Chatterjee et al (2016) *Nature* 540, 453–457

Roessler et al (2016) *Structure* 24, 631-640

Support by a Strategic Award from the Wellcome Trust and the Biotechnology and Biological Sciences Research Council (grant 102593).

Petra Fromme, Arizona State University

Time-resolved femtosecond crystallography studies of photosystem II and I with X-ray Free Electron Lasers

Petra Fromme [(see references for co-authors)]

School of Molecular Sciences and Biodesign Center for Applied Structural, Arizona State University, Tempe, Arizona 85287-1604 USA

The study of the dynamics biomolecules is one of the grand challenges of Structural Biology as most structures determined so far only provide a static picture of the molecule. Serial Femtosecond Crystallography (SFX) provides a novel concept for structure determination, where X-ray diffraction "snapshots" are collected from a fully hydrated stream of nanocrystals, using femtosecond pulses from high energy X-ray free-electron lasers (XFELs) [1-4]. The XFEL pulses are so strong that they destroy any solid material, but a femtosecond is so short (1 fs =10^{-15} s) that X-ray damage is diminished and diffraction from the crystals is observed before destruction takes effect [3]. It opens new avenues to determine molecular movies of Photosynthesis "in action" [6-10]. In this talk we will present results from recent experiments to study the dynamic processes in Photosystem II by light-induced time-resolved femtosecond crystallography conducted at LCLS, the X-ray Free Electron Laser in Stanford and also report preliminary results from the first time resolved studies on Photosystem I from the European XFEL in Hamburg, Germany.

The talk will close with a progress report on the development of compact femto and attosecond X-ray Sources at ASU (CXLS and CXFEL) and DESY (AXSIS) [11], which will provide unique new opportunities to study the ultrafast dynamics of reactions of biomolecules with a combination of X-ray diffraction, X-ray spectroscopy and ultrafast optical spectroscopy.

References:

[1] Chapman,HN et al 2011, Nature, 470, 73-77 ;

[2] Fromme P and Spence JC 2011 Curr Opin Struct Biol 2011, 21: 509-516;

[3] Barty,A et al. 2012 Nature Photonics 6, 35–40;

[4] Boutet S et al 2012, Science, 337: 362-364;

[5] Liu W et al 2013, Science 342: 1521-1524;

[6] Aquila,A et al 2012, Optics Express, 20 (3), 2706-16;

[7] Kupitz C et al 2014, Nature 513, 261-5;

[8] Young ID et al. 2016, Nature 543, 131-135 ;

[9], Suga M et al.2017, Nature 543, 131-135 ;

[10] Ayyer, K. et al. Nature 2016, 530, 202-206

[11] Kartner, F.X. et al. 2016, Nuclear Instruments & Methods in Physics Research Section A -Accelerators Spectrometers Detectors and Associated Equipment, 829, 24-29.

This work is supported by the National Science Foundation BIOXFEL STC (NSF-1231306), the Biodesign Institute at Arizona State University, the US National Institutes of Health (NIH), National Institute of General Medical Sciences grants R01 GM095583 and the European Research Council, "Frontiers in Attosecond X-ray Science: Imaging and Spectroscopy (AXSIS)", ERC-2013-SyG 609920

Hasan DeMirci, Stanford PULSE Institute

Structure-based antibiotic development driven by ambient-temperature serial crystallography of small andlarge ribosomal subunits at high resolution

High-resolution ribosome structures determined by cryo X-ray crystallography have provided important insights into the mechanism of translation. Such studies have thus far relied on large ribosome crystals kept at cryogenic temperatures to reduce radiation damage. Here we describe the application of serial femtosecond X-ray crystallography (SFX) using an X-ray free-electron laser (XFEL) to obtain diffraction data from ribosome microcrystals in liquid suspension at ambient temperature. 30S ribosomal subunit microcrystals programmed with decoding complexes and bound to either antibiotic compounds or their next-generation derivatives diffracted to beyond 3.4 Å resolution. Our results demonstrate the feasibility of using SFX to better understand the structural mechanisms underpinning the interactions between ribosomes and other substrates such as antibiotics and decoding complexes. We have also collected full dataset from the dimer of large (50S) ribosomal subunit in 47 minutes of beamtime at the CXI instrument using less than 50 microliter of sample. This structure is the largest one solved to date by any FEL source to near atomic resolution (3 MDa). We expect that these results will enable routine structural studies, at near-physiological temperatures, of the large ribosomal subunit bound to clinically-relevant classes of antibiotics targeting it, e.g. macrolides and ketolides, also with the goal of aiding development of the next generation of these classes of antibiotics. Overall, the ability to collect diffraction data at near-physiological temperatures promises to provide new fundamental insights into the structural dynamics of the ribosome and its functional complexes.

Haiguang Liu, Beijing Computational Science Research Center

Structure and dynamics of chloride ion pumping rhodopsin

Yang Wang[1], Ji-Hye Yun[2], Xuanxuan Li[1], Jae-Hyun Park[2], Chufeng Li[3], Nadia Zatsepin[3], Mark Hunter[4], Jake Koralek[4], Chun Hong Yoon[4], Weontae Lee[2], **Haiguang Liu**[1]

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[3]Physics Department, Arizona State University, Tempe, AZ 85287

[4]LCLS, SLAC national laboratory, 2575 Sand Hill Road, Menlo Park, CA, 94025

The chloride ion pumping rhodopsin was studied with the powerful X-ray lasers and supercomputers, to reveal the molecular mechanism of proteins. The chloride ion pumping rhodopsin (ClR) is a class-A GPCR protein, which utilizes energies from light to actively transport Cl- ions through membranes. In this study, we report, for the first time, an atomic structure determined at room temperature using serial femtosecond X-ray Crystallography (SFX) method using the second chamber with serial sample chamber setup at LCLS. The atomic structure determined using SFX method is very consistent with the structure solved at synchrotrons. Using the SFX structure of ClR as an initial model, extensive molecular dynamics simulations have been carried out to study the pathway of Cl- ions through the rhodopsin channel. By using all-atom molecular dynamics simulation method, the plausible pathways of Cl- ion were observed (Figure 1). The residues that undergo substantial conformational changes during the ion transportation have been identified. We will carry out time-resolved experiments using pump-probe method at LCLS to validate the results from simulations. Structures at three time delays will be determined to exam the conformational changes during the ion transportation process in December 2017.

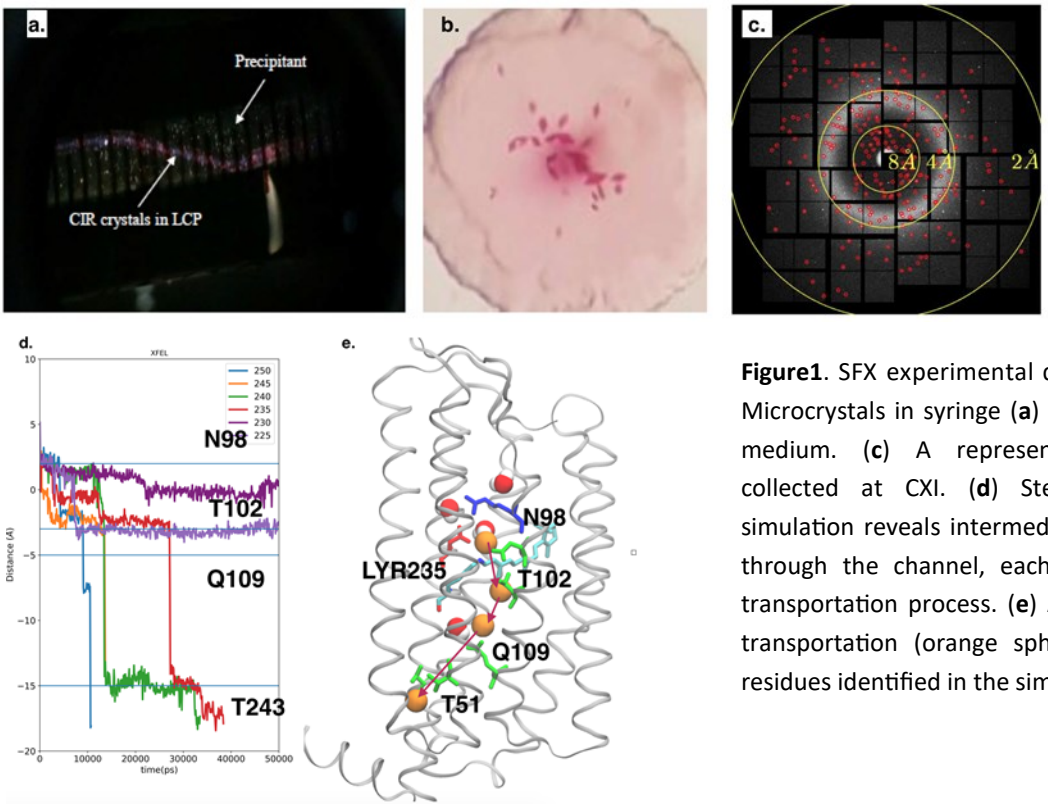


Figure1. SFX experimental data and simulation results. Microcrystals in syringe (**a**) and in plate (**b**) both in LCP medium. (**c**) A representative diffraction pattern collected at CXI. (**d**) Steered molecular dynamics simulation reveals intermediate states as Cl- ion moves through the channel, each curve shows a simulated transportation process. (**e**) A plausible pathway for ion transportation (orange sphere, Cl- ion) and the key residues identified in the simulations.

Acknowledgement: The National Natural Science Foundation of China award to H. Liu (#11575021, U1530401, and U1430237) and Korean National Research Foundation (2016R1A5A1004694 to W. Lee, NRF-2016R1A6A3A04010213n to J. H. Yun) support the project. Data were collected at LCLS, supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. DE-AC02-76SF0051.

Leonie Flueckiger, La Trobe University

Rapid mixing and injection for studying molecular dynamics in time-resolved liquid X -ray scattering experiments

Leonie Flückiger[1], Lilian Hor[1,2], Daniel Langley[1], Saumitra Saha[4], Connie Darmanin[1], Peter Berntsen[1], Nigel Kirby[5], Tim Ryan[5], Lakshmi Wijeyewickrema[2], Robert Pike[2] and Brian Abbey[1]

[1]ARC Centre of Excellence in Advanced Molecular Imaging, Department of Chemistry and Physics, La Trobe University, Melbourne, VIC 3086, Australia; [2]Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, VIC 3086, Australia; [4]ARC Centre of Excellence for Advanced Molecular Imaging, Theoretical Condensed Matter Physics Group, School of Physics, The University of Melbourne, Parkville, VIC 3010, Australia; [5]Australian Synchrotron, 800 Blackburn Road, Clayton, Victoria 3168, Australia

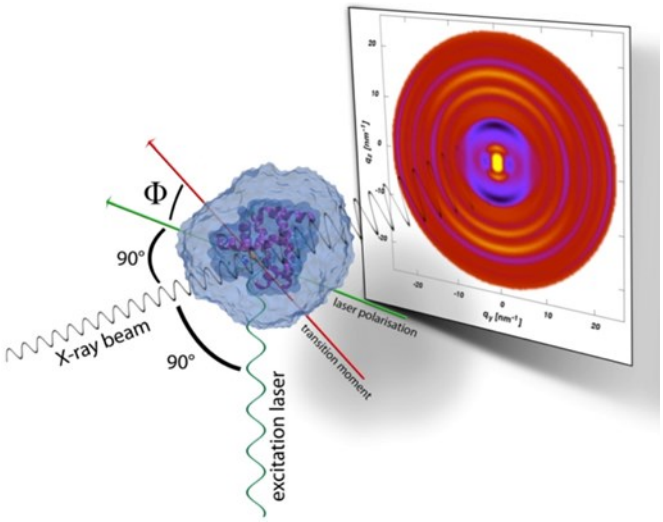
The dynamics of biomolecules studied under physiologically relevant conditions provides the best possible insight into their functional behaviour within their native environment. Whilst the atomic resolution structure of complex biomolecules in solution is still a challenge which remains to be solved, important clues can be gained by studying their real-time conformational dynamics. This information can be obtained via analysis of solution X-ray scattering data, which can be analysed to track changes in the molecular envelope during chemical reactions. The development of XFEL based solution scattering experiments to probe molecular dynamics offers significant advantages to the experimenter. These include, the ability to minimise radiation damage which both limits resolution and complicates the data analysis, an improved signal-to-noise and the ability to much more finely sample biomolecular dynamics. Whilst photo-activated pump-probe experiments at XFELs are now established, chemically-induces kinetic studies at XFELs, particularly in the context of solution scattering, are still in their infancy. Here we discuss the design and development of rapid mix-and-inject devices (MID) for time-resolved small angle X-ray scattering (SAXS) experiments performed on biomolecules in a free-standing liquid jet. Our current device designs are able to achieve mixing times on the order of µs, providing an accurate trigger for chemically-driven molecular dynamics. The mixer is coupled directly to a gas dynamic virtual nozzle (GDVN) in order to focus the mixed solution into a free-standing jet using hydrodynamic forces. Simulations and preliminary experimental data of stable jets with diameters ranging from 1 to 20 µm are presented which have an associated flow-rate of around 5 µl/min. The devices we present are fabricated from SU-8 and PDMS using photolithography and will find immediate applications in the study of the large-scale conformational changes that occur during protein complex formation.

Jochen Hub, Gottingen University

Interpretation of (time-resolved) solution X-ray scattering data by explicit-solvent molecular dynamics simulation

Jochen S. Hub

Georg-August-University Göttingen, Institute for Microbiology and Genetics, Göttingen, Germany



Small and wide-angle X-ray scattering (SWAXS) provides a view on structures, ensembles, and dynamics of proteins in solution. However, the interpretation of the signals has remained challenging due to the low information content of SWAXS data, scattering contributions from the solvent (hydration layer and excluded solvent), and due to unclear confidence intervals of structural models that were refined against the data. We aim to overcome such problems by combining SWAXS data with explicit-solvent molecular dynamics (MD) simulation. Specifically, we have developed methods for SWAXS curve prediction and for structure refinement against SWAXS data based on explicit-solvent MD and Bayesian inference [1,2]. The calculations fully account for scattering contributions from the hydration layer and excluded solvent, thereby avoiding any solvent-related fitting parameters.

Recently, we used such methods to interpret time-resolved SWAXS (TR-SWAXS) data of myoglobin (Mb), following the ultrafast "quake-like" dissipation of energy after CO-photodissociation. The simulations revealed that the protein quake is characterized by single pressure peak that propagates in a highly anisotropic and asynchronous manner across the protein and further into the solvent. By computing TR-SWAXS patterns from the simulations, we could interpret features in the reciprocal-space SWAXS signals as specific real-space dynamics, such as CO displacement and quake propagation.

A Guinier analysis applied to recent experimental TR-SAXS data of Mb revealed underdamped oscillations of the radius of gyration and of protein volume, which were interpreted as underdamped oscillations of the entire protein, thus challenging the view of overdamped global protein dynamics [4]. Our simulations offer an alternative interpretation of the data. The calculations suggest that the small-angle signals reflect mainly solvent and not protein dynamics, thereby reconciling the TR-SAXS data the idea of overdamped protein dynamics. Moreover, the analysis demonstrates that an accurate modeling of solvent contributions is required to interpret the data.

[1] Chen and Hub, *Biophys. J.* **107**, 435-447 (2014); Chen and Hub, *Biophys. J.* **108**, 2573–2584 (2015); Chen and Hub, *J. Phys. Chem. Lett.*, **6**, 5116–5121 (2015); Shevchuk and Hub, *PLoS Comp Biol* **13**, e1005800 (2017); Cordeiro *et al.*, *Nucleic Acids Res.* 45, 1501-2015 (2017)

[2] http://waxsis.uni-goettingen.de; Knight and Hub, *Nucleic Acids Res.*, **43**, W225-W230 (2015)

[3] Brinkmann and Hub, *PNAS* **113**, 10565–10570 (2016); Brinkmann and Hub, *J. Chem. Phys.,* **143**, 104108 (2015)

[4] Levantino et al., *Nat. Commun.*, **6**, 6772 (2015)

Michael Brown, University of Arizona

Femtosecond X-ray scattering of membrane proteins with a free electron laser

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We describe the use of X-ray free electron laser (XFEL) technology in a pump-probe, time-resolved wide-angle X-ray scattering experiment to structurally interrogate the conformational changes of membrane proteins, with an emphasis on G-protein-coupled receptors. The XFEL beam of the Linac Coherent Light Source (LCLS) has a very high peak X-ray brightness, which is key for probing elusive ultra-fast structural changes during the biological function of protein molecules. With XFEL detection, femtosecond time-resolved small- and wide-angle X-ray scattering (TR-SWAXS) experiments afford unprecedented opportunities in membrane biophysics. For the pump, either an optical parametric amplifier (OPA) or a Nd:YLF laser is used at the Coherent X-ray Imaging (CXI) instrument of LCLS. The protein in detergent micelles is delivered to the X-ray beam using micro-jet technology involving a gas-dynamic virtual nozzle (GDVN) of 50-mm diameter. The 2D images of the scattered X-rays are recorded with an ultra-fast Cornell-SLAC Pixel Array Detector (CSPAD) with pump laser on-versus-off frequency of 60 Hz (X-ray pulse frequency 120 Hz) generating light minus dark-state data for specified time points. The 2D-scattered X-ray intensities are radially integrated to generate 1D-scattering profiles (scattered X-ray intensity versus momentum transfer vector *Q*). On-the-fly data analysis using the OnDA software package reveals the light-triggered ‘‘protein quake’’ during the very early stages of the activation. Data reduction involves singular value decomposition (SVD) together with all-atom molecular mechanics simulations. We combine MD and QM/MM simulations of rhodopsin in ground and excited states to provide a mechanistic interpretation of the changes observed in the radius of gyration. Establishing whether there is a single activation pathway or an energy landscape mechanism will be a significant step to understanding the functional GPCR activation mechanisms.

CAROLIN SEURING, CFEL

X-RAY COHERENT DIFFRACTION OF AMYLOID FIBRILS ON GRAPHENE

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There is a conspicuous gap in our ability to determine the structures of biological filaments such as amyloid fibrils present in Alzheimer's and Parkinson's disease patients. Such filaments are periodic along the fibril axis, therefore cannot be crystallized for structure determination by conventional X-ray crystallography, instead requiring the use of complementary structural methods. We developed a new approach to X-ray fiber diffraction using a free-standing graphene support and single nanofocused X-ray pulses of femtosecond duration from an X-ray free electron laser. We measured low-background diffraction from a limited number of amyloid fibrils aligned on the graphene substrate, providing superior measurements than obtainable at synchrotron radiation sources and exhibiting noticeable asymmetry. Improvements of this low-background method, that have been realized so far, and potential applications for imaging weakly scattering objects at XFELs and electron microscopes will be discussed.

Brenda Hogue, Arizona State University

Virus conformational landscapes from single-particle X-ray scattering

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X-ray free-electron lasers (XFELs) provide a novel approach to single-particle (SP) virus studies. We report coherent diffraction imaging experiments conducted at the Linac Coherent Light Source (LCLS) at SLAC National Laboratory as part of the SP imaging initiative (SPI). SPI consists of an international team of collaborators working to develop XFEL-based high-resolution imaging of non -crystalline targets, such as viruses, cells, and proteins. Bacteriophage PR772, one of several model viruses, was delivered to the LCLS Atomic Molecular Optics (AMO) XFEL femtosecond soft X-ray pulsed beam. Single virus particle diffraction snapshots from the experiment were analyzed to determine the 3D structure and conformational landscape of PR772 to a detector-limited resolution of 9 nm (1). Data analysis identified a single dominant conformational coordinate controlling an expansion of the virus reminiscent of virus "swelling", coupled with emptying of the virus internal contents (2). PR772 is similar to bacteriophage PRD1. Recent studies indicate that PRD1 uses its internal lipid membrane to form a nanotube that facilitates viral genome delivery (3). The conformational changes revealed by analysis of PR772 diffraction at LCLS show changes similar to those observed in the PRD1 studies. The results provide important new information about the biology of PR772. Viruses undergo conformational changes critical for their life cycles. Our analysis suggests that SP XFEL scattering has the potential to shed new light on changes that viruses undergo during their life cycles. Complementary studies with cryoEM advances opens new opportunities to reveal the conformational landscape of a virus population which will help provide insight into their dynamic nature relevant to biological processes during infection. Ongoing cryoEM studies and future XFEL/cryoEM experiments capitalizing on the complementary single-particle approaches will be discussed.

Acknowledgements

[1]Research at UWM supported by the US Department of Energy, Office of Science, Basic Energy Sciences award DE-SC0002164 (algorithm design & development); US National Science Foundation awards STC 1231306 (numerical trial models & data analysis) and 1551489 (underlying analytical models); UWM Research Growth Initiative.

[2]Research at SLAC National Accelerator Laboratory LCLS supported by US Department of Energy, Office of Science, Office of Science, Office of Basic Energy Sciences under contract DE-AC02-76SF00515.

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Andrea Katz, Cornell University

Mixing injector for time-resolved crystallography at X-ray free electron lasers

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Mix-and-inject serial crystallography at X-ray free electron laser sources provides a window for observing structural changes of biomolecules as they function. This exciting new technique can capture atomically detailed structures of intermediate states to provide more complete understanding of biomolecular machines. I will discuss recent developments in mixing injector technology that enable measurements of chemically initiated reactions in crystals on time scales ranging from milliseconds to seconds [1]. A new interface between the mixer and sample chamber reduces the time required to assemble an injector and increases the ease of operation. These robust mixing devices have been used to probe enzyme structural dynamics over a broad range of time points [2]. They have been successfully implemented at serial femtosecond crystallography beamlines at LCLS and European XFEL.

This project is supported by BioXFEL, an NSF Science and Technology Center, grant 1231306.

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Marie Gruenbein, Max Planck Institute

Condisderations for SFX data collection at MHz XFELs

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The new megahertz (MHz) X-ray free-electron lasers (XFEL) promise data collection in an extremely rapid and sample-efficient manner. However, this can only hold true under the stringent condition that pristine sample is provided at a rate commensurate to the XFEL pulse rate which imposes severe constraints on sample delivery. Liquid jet injection is ideally suited for this task and the method of choice for biological samples. However, the high intensity of the XFEL pulse not only destroys the exposed sample in the jet but the energy deposited by the XFEL beam results in an explosion, generating a gap in the liquid jet transporting the sample [1]. Moreover, a shock wave is launched, propagating along the jet and producing ns-long pressure jumps on the order of 1-10 kbar within the jet medium, possibly affecting the sample [1]. Recent findings and implications from serial femtosecond crystallography experiments studying the described effects will be discussed.

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Sarah Perry, UMass Amherst

Graphene microfluidics for room temperature crystallography

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Coupling microfluidic technology with advanced protein crystallography techniques for *in situ* analysis is an area of research where significant advances can be made. Microfluidic platforms have the benefit of not only enabling experiments at small volumes, but also of creating an environment free of inertial or convective effects while providing exquisite control over local conditions and gradients. A significant problem in microfluidic-based crystallography is the background scattering resulting from the interaction of X-rays with the device materials, which may reduce the signal-to-noise ratio obtained from small or weakly diffracting crystals. These challenges can be overcome by decreasing the overall thickness of the microfluidic device. In particular, we utilize graphene as an ultra-thin X-ray compatible window material. Furthermore, the mechanical strength, gas impermeability, and conductivity of graphene have the potential to enable a tremendous range of structural biology experiments including room temperature serial crystallography, the analysis of oxygen-sensitive proteins, high-throughput ligand screening, and electric-field stimulated crystallography experiments.

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Alexandra Ros, Arizona State University

Droplet microfluidics for serial femtosecond crystallography with XFELs

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Sample waste resulting from the low pulse frequency of current X-ray free electron lasers (XFELs) remains an unsolved issue for serial femtosecond crystallography (SFX). All crystals injected continuously between the fs laser pulses run to waste and do not contribute to the analysis. The majority of protein crystals are time consuming and costly to prepare as well as difficult to obtain in suspensions of adequate concentration and quantities required for full dataset recording. Current XFEL pulse frequencies are in the range of 10 Hz to 120 Hz resulting in waste of the majority of crystals. Approaches to reducing sample volume include the development of jets which slowly extrude crystals suspended in a viscous medium, such as for example lipidic cubic phase (LCP). However, not all proteins can be crystallized or maintained in the viscous media and the viscous media might increase the background of the diffraction signal. Furthermore, viscous jets are too slow for refreshing sample matching the fast pulse repetition within a pulse train of the European XFEL. Other approaches including gas dynamic virtual nozzle (GDVN) switching, acoustic injection or fixed-target methods have special requirements and caveats such as repetition rate, increased background or incompatibility with time-resolved methods.

Here, we propose utilizing microfluidic liquid-in-liquid droplets coupled to a GDVN to reduce the volume of sample required to collect a full data set for an SFX experiment. By generating small water-in-oil droplets at a frequency synchronized with the XFEL pulses, an order of magnitude less sample volume may be required for a full SFX data set compared to traditional GDVN injections. This method thus overcomes limitations for proteins which are extremely difficult to crystalize, expensive to prepare or are not compatible with the high viscosity media required for LCP injectors. We developed 3D-printed water-in-oil droplet generators and a droplet frequency detector that can be employed in a typical SFX experiment. Through tuning of the flow rate ratios of the intersecting oil and water phases, droplets in a frequency range from 10Hz up to 120Hz can be generated. We tested this approach at the Macromolecular Femtosecond Crystallography (MFX) instrument at the SLAC National Accelerator Laboratory and at the EuXFEL.

Peter Berntsen, La Trobe University

Serial crystallography development in partnership with the Australian Synchrotron

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Jun Aishima[2], Tom Caradoc-Davies[2] and Brian Abbey[1].

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A novel set-up to perform serial millisecond crystallography (SMX) has been established at the Australian Synchrotron. The project is based around the rapid readout capacity of the Eiger 16Ṁ detector installed at the MX2 microcrystallography beamline and utilizes a syringe pump injection system for continuously streaming protein crystals, suspended in a highly viscous matrix, into the X-ray beam. This project incorporates a number of design elements that allows the beamline to be changed from cryo-crystallography to room temperature SMX within 15 min to facilitate routine use of the method. Proof of concept experiments were carried out and here we will describe the design and performance of this new high-viscosity injector which is named ”Lipidico”.

Michael Hennig, leadXpro

X-ray free electron laser: Opportunities for drug discovery

Robert Cheng, Rafael Abela and Michael Hennig

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Past decades have shown the impact of structural information derived from complexes of drug candidates with their protein targets to facilitate the discovery of safe and effective medicines. Despite recent developments in single particle cryo-electron microscopy, X-ray crystallography has been the main method to derive structural information. The unique properties of X-ray Free Electron Laser (XFEL) with unmet peak brilliance and beam focus allow X-ray diffraction data recording and successful structure determination from smaller and weaker diffracting crystals shortening timelines in crystal optimization. To further capitalize on the XFEL advantage, innovations in crystal sample delivery for the X-ray experiment, data collection and processing methods are required. This development was a key contributor to serial crystallography allowing structure determination at room temperature yielding physiologically more relevant structures. Adding the time resolution provided by the femtosecond X-ray pulse will enable monitoring and capturing of dynamic processes of ligand binding and associated conformational changes with great impact to the design of candidate drug compounds.

Figure: Sample delivery systems for serial crystallography. The most frequently used system at the moment are injector-based. All can be used for room temperature data collection, but the fixed target approach provides the option for cryo-crystallography as well.

Recent Publications

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Andrea Markelz, University at Buffalo

Measuring protein intramolecular dynamics with terahertz light: Functional changes and relevance to biology

Yanting Deng, Mengyang Xu, Deepu Koshy George, Catherine Luck, Akansha Sharma and Andrea Markelz

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As Austin and coworkers showed over 45 years ago [1], thermally activated motions are critical to protein function, however the characterization of these motions has been challenging. In the last 20 years new optical instrumentation in the critical terahertz (THz) frequency range has enabled unprecedented characterization of these dynamics revealing changes in the collectivity and orientation of motions with functional state for enzymes and photoactive proteins [2-5]. In this talk I will review measurements of protein intramolecular vibrations, their directionality and their impact on steering function. The various shortcomings of standard simulation methods to analyze the optical results will be discussed as well as possible strategies to overcome these. This work was supported by National Science Foundation MRI^2 grant DBI2959989, IDBR grant DBI1556359, and MCB grant MCB1616529, and the Department of Energy BES grant DE-SC0016317.

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DOEKE HEKSTRA, HARVARD UNIVERSITY

TITLE TBD

Adrian Mancuso, European XFEL

Recent advances in instrumentation and methodology for XFEL structural studies from the SPB/SFX team at the European XFEL

This presentation will outline the basic properties of the newly operational European XFEL [1], in particular focusing on the capabilities of the SPB/SFX Instrument [2] which is the European XFEL's only purpose-designed instrument to support biological structure studies. The suite of instrumentation at SPB/SFX will be presented—including focusing optics, sample delivery technology and the capabilities of its 2D detector, along with highlights from its commissioning and first experiments that took place from September 2017.

If time permits, some research highlights from the SPB/SFX team will also be presented, which predominantly include developments in methods to determine the three-dimensional structure of non-crystalline specimens in both theoretical and experimental aspects [3-5].

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Matthew Coleman, Lawrence Livermore National Laboratory

Using XFELs to characterize nanolipoprotein particles that form HDL and LCL complexes

Coleman, M.A.,[1,2] Shelby, M.,[1] Gilbile, D.,[2] Grant, T.,[3] Seuring, C.,[4] Segelke, B.,[1] He W.,[1] Evans A.C.,[1] Cheng, H.,[2] Pakendorf, T.,[4] Fischer, P.,[4] Hunter, M.S.,[5] Batyuk, A.,[5] Hogue, B.,[6] Zatsepin, N.,[6] Kuhl, T.,[2] Barthelmess M.,[4] Wagner, A.,[7] Meents, A.[4] and Frank M.[1,2]

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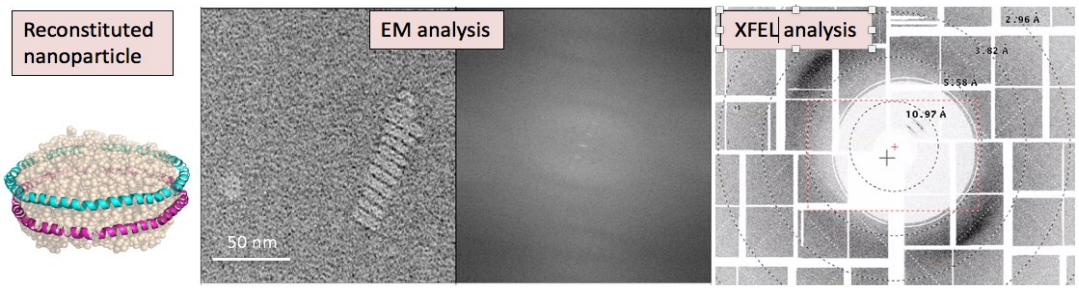
The overall goal of this project is to develop methods for high-throughput and high-resolution biological imaging that will enable temporal and spatial studies for formation of High Density Lipid (HDL) and Low Density Lipid (LDL) particles. HDL and LDL particles are involved in lipid and cholesterol scavenging and transport. These nanolipoprotein particles (NLPs; aka nanodiscs), form 10-25 nm discs and can be reconstituted using cell-free expression systems. The resulting discs consist of a 5 nm lipid bi-layer surrounded by a "belt" of apolipoproteins and represent a stable intermediate state in HDL and LDL formation. Cell-free expressed, assembled, and purified particles were submitted for high-throughput crystallization trials through the High-Throughput Crystallization Screening Center at the Hauptman-Woodward Medical Research Institute. A total of 65 different conditions resulted in crystals that ranged in size from sub-5 um to ~100 um. Optimized crystals showed similar rod-like morphologies for both types of nanoparticles. Optimized crystals were pipetted (40 - 60 uL per) on to a Roadrunner compatible Si support with 8 um pores at moderate crystal densities (10^4 cm^{-2}) in a hydrated 100% Rh environmental chamber. Wafers with crystals were mounted in the high-speed Roadrunner goniometer for sample delivery at the LCLS MFX- end station and X-ray diffraction data was collected using a 3 um focused beam. Diffraction images revealed similar 2D fiber-like diffraction (up to ~11.5 A) for both forms of the HDL and LDL particles. We achieved a maximum hit rate of approximately 3% for ApoA1 (HDL) and 2% for ApoE4 (LDL) crystals with an average acquisition rate of ~4.2X10^4 shots in under 30 mins. The ApoA1 (HDL) showed distinct Bragg peaks along the meridian whereas more diffuse spots were observed for the ApoE4 (LDL) samples. Diffuse arcs observed at ~4.5 Å d-spacing centered around the perpendicular to the meridian are from the lipid bilayer. Layer spacing of diffraction along the meridian translates to ~5.5 nm in real space for both ApoA1 and ApoE4 containing samples. These patterns are consistent with Rouleau structure previously reported from EM studies. These diffraction patterns (diffuse Bragg peaks and layer lines) could also be replicated by 2D Fourier transformation of the EM images of the Rouleau structures. This represents the first comparative study of HDL and LDL particles using XFELs. Overall, cell-free expression represents a unique solution to address multiple bottlenecks in the production, purification, and characterization of lipid-binding proteins that were previously difficult to obtain. On-going studies will further refine the resolution and therefore structure of these proteins involved in membrane and cholesterol transport.



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Gebhard Schertler, ETH Zurich-PSI

Exploring G protein coupled receptors and membrane protein dynamics with X-ray free electron lasers and solution NMR

Gebhard F.X. Schertler

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Free-electron lasers are X-ray sources with unprecedented peak brilliance and time structure. We are applying them to explore serial crystallography for membrane proteins using bacteriorhodopsin as a model system. Our collaborative international team was able to trigger its photo cycle in lipidic cubic phase, and we have observed reaction intermediates at room temperature. From a series of snap shots taken after different delay times we were able to construct an informative movie of bacteriorhodopsin. The order changes of water molecules close to the retinal could be observed, and we can now explain important changes in the proton affinity to the Schiff base nitrogen which is central to the proton pump mechanism of bacteriorhodopsin. We can observe already after 16 ns disordering of a water cluster that is directly hydrogen bonded to the Schiff base. The difference densities are further increasing over time. This change is an important part of the mechanism that modulates the affinity of the proton to the Schiff base nitrogen. Later structural changes raise the pKa of Asp85 to the point where it spontaneously accepts a proton from the Schiff base. With another collaborative Swiss team we have successfully introduced N15-labeled valine in a conformationally stabilized beta 1 adrenergic receptor and used the labeled positions for evaluating backbone dynamics of this GPCR. We were able to see heterogeneous responses across the receptor after binding a number of agonists and antagonists. But, remarkably, we were able to pick up a homogenous response reflecting the signaling transmission within the receptor in a region distant from the ligand binding site. In addition, we were able to observe changes of loop dynamics in the ligand entrance channel of the adrenergic receptor.

New G-protein-coupled receptor crystal structures: insights and limitations
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Backbone NMR reveals allosteric signal transduction networks in the β1-adrenergic receptor
S Isogai, X Deupi, C Opitz, FM Heydenreich, CJ Tsai, F Brueckner, ...
Nature 530 (7589), 237-241 (2016)

Iris Young, Lawrence Berkeley National laboratory

XFEL Diffraction studies of the oxygen evolving complex of photosystem II

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X-ray free electron lasers (XFELs) provide a unique opportunity for time resolved, damage-free studies of dynamic systems. We have applied this technique to the study of the oxygen-evolving complex (OEC) in photosystem II (PSII), the membrane-bound protein responsible for water splitting and oxygen evolution in plants and photosynthetic cyanobacteria. In order to probe the structure of the OEC in multiple illuminated states and evaluate the possible oxygen-evolving mechanisms consistent with these structures, we conducted simultaneous X-ray emission spectroscopy/X-ray diffraction experiments at the XFEL facility at LCLS. We recently published the first high-resolution room-temperature diffraction data for the dark and twice-illuminated (2F) states of PSII. Differences between these structures and cryogenic structures with similar packing and unit cells evidence the importance of room temperature data collection of crystals in near-native conditions. Several key advancements in data processing capabilities have contributed to significantly improved data quality over the course of recent experiments, including development of the *cctbx.xfel* graphical user interface for rapid feedback and changes to the handling of XFEL still images in the *dials.stills_process* pipeline. We will discuss the above developments in relation to the advancement of the PSII data analysis.

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Henry Chapman, DESY

Incoherent diffractive imaging for structure determination at atomic resolution

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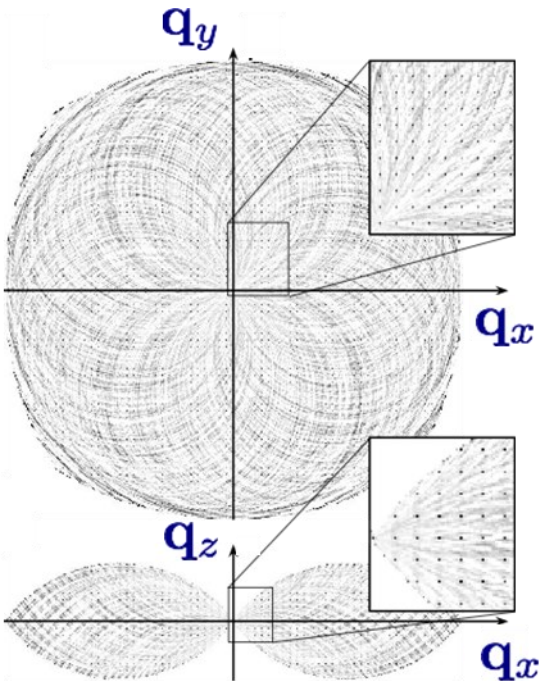

A map of 3D correlations at locations $\mathbf{q} = \mathbf{k}_1 - \mathbf{k}_2$ of simulated fluorescence intensities from a Fe-bearing protein crystal exposed to a single X-ray FEL pulse shorter than the Fe core-hole lifetime. From the 4.6×10^{12} correlations, one already observes periodicity of the Fourier components, indicating the (crystalline) structure.

We proposed a method of utilising incoherent and fluorescence photons to obtain structures of molecules [1], based on Hanbury-Brown Twiss intensity interferometry [2]. The information of the atomic arrangements of the emitting atoms is determined from correlations of the angularly-resolved emission intensities, detected within the coherence time of the emission process. This can be achieved by using the natural time-gating of X-ray free-electron laser pulses which can be made as short as the 2 fs coherence time set by the lifetime of the inner-shell X-ray fluorescence radiation.

We plan to explore this method on metal-bearing proteins, by detecting XFEL-induced fluorescence using large-area pixellated detectors. A detector has about three million pixels, giving 5×10^{12} intensity correlations in a single shot. The correlation between a pair of pixels located at scattering wave-vector directions (relative to the sample) of $\mathbf{k}_1$ and $\mathbf{k}_2$ encode the strength of the Fourier component of the object given by $\mathbf{q} = \mathbf{k}_1 - \mathbf{k}_2$. Remarkably, the trillion observations of $I(\mathbf{k}_1)$ $I(\mathbf{k}_2)$ map out a *volume* $\mathbf{q} = \mathbf{k}_1 - \mathbf{k}_2$ of Fourier space (see Figure) rather than the usual two-dimensional manifold accessible in a coherent diffraction experiment. This means that true three-dimensional information can be obtained from measurements in a single orientation.

This 3D imaging will be discussed, as well as several more advantages over the conventional crystallographic techniques that have been in use for the last 100 years. The fluorescence emission for an atom such as Fe is about 200 times the probability for scattering from a carbon atom, allowing measurements on single molecules, in solution. The chemical specificity of the emission means systems can be examined in natural environments, and chemical states (such as oxidation) can be discriminated. Analogues can be made of all diffraction experiments (powder diffraction, solution scattering, time-resolved). We expect atomic resolution without suffering from atomic form factors that limit high-resolution coherent scattering measurements. The method can be easily combined with diffraction, and thus may have great impact for understanding the structure, dynamics, and energetics of proteins and other materials.

A. Classen, et al. *PRL* **119**, 053401 (2017)
R. Schneider *et al. Nature Physics* 2017 on-line; doi:10.1038/nphys4301

David Case, Rutgers University

What can we learn from MD simulations of biomolecular crystals?

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The adaption of graphical processing units (GPUs) to biomolecular simulations has made microsecond-scale simulations of biomolecular crystals available on a nearly-routine basis [1-5]. Typically, a super-cell consisting of several crystallographic unit cells becomes the periodically-repeating unit in the simulation; in this talk, I will consider super-cells with up to 125 unit cells. Here are some ways this data might be used:

- Straightforward comparisons between computed and experimental average structures and atomic displacement parameters can be used to identify problems in biomolecular force fields [1-4]. The high accuracy and precision crystallography (compared to NMR or other solution methods) makes such comparisons uniquely informative, and the statistics of such comparisons benefit from having many copies of chains in equivalent environments in the simulation.
- Structural fluctuations in the simulations can be used to estimate diffuse scattering intensities, which can be compared to recent measurements using modern detectors [5,6]. I will show examples of results for lysozyme in three crystal forms, using data collected at CHESS by Steve Mesiburger and Nozomi Ando as a reference. These provide important insights into the contributions to diffuse scatter from water and from lattice vibrations of the protein.
- Simulations provide a model for density fluctuations in regions of "disordered" or "bulk" solvent (mainly water). Such models appear to account for solvent contributions to Bragg intensities in ways that are a systematic improvement over the procedures used in most protein structure refinement protocols.
- Simulations provide a plausible, if imperfect, model for conformational heterogeneity in biomolecular crystals. Having both Bragg intensities computed from the average electron density (as a refinement target) and hints from the trajectory itself as to the nature of the underlying conformational transitions, James Holton has created and refined atomic models with many more than the traditional number of "alternate locations"; these can closely reproduce the synthetic Bragg intensities. We hope that such models may provide clues about how to construct better models to refine against real data.
- TeraHertz spectroscopy in biomolecular crystals, and its orientation dependence [7], can be directly compared to predictions from MD simulations.

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Helen Ginn, Diamond Light Source-Oxford University

The slip-and-slide algorithm: a refinement protocol for detector geometry

Geometry correction is traditionally plagued by mis-fitting of correlated parameters, leading to all sorts of 'decoy' local minima. This makes it a messy, difficult job, requiring significant skilled human intervention to create a meaningful definition of detector geometry. Segmented detectors pose an enhanced risk of mis-fitting: even a minor confusion of detector distance and panel separation can prevent improvement in data quality. The slip-and-slide algorithm breaks down effects of the correlated parameters and their associated target functions by shifting from parameterisation in Cartesian coordinates to a spherical coordinate system. This change allows an incredibly simplified mechanism for geometry refinement with a significant reduction in human judgement required. The target function used is like that of other geometry refinement protocols (reducing the offsets between observed and predicted reflection positions). However, the spherical coordinate system is now divided into two types of parameters, which are sensitive to different aspects of the usual target function. Due to the refinement of decoupled parameters, this geometry method also reaches convergence and is more insensitive to noise. This technique can quite powerfully bring out tilts in detectors not completely perpendicular to the beam, and is supported by improvement in indexing rates and more accurate estimation of anomalous differences.

Reference:

Ginn & Stuart, J. Synchrotron Rad. (2017). 24, 1152–1162

Matthias Frank, Lawrence Livermore National Laboratory

Progress with solid sample supports for weakly diffracting objects

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Solid sample supports (aka “fixed targets”) offer some potential advantages over aerosol or liquid jet based sample introduction for biological imaging with XFELs. With solid sample supports, the sample density and sample properties can be characterized and optimized before an X-ray imaging experiment and samples can be archived afterwards. Solid sample supports can help orient and align samples (e.g., fibrils), keep 2D protein crystals flat, and allow tilting the sample in a controlled fashion with respect to the incident X-rays during the X-ray measurement. Solid sample supports may also facilitate new types of pump-probe experiments that involve integrating microfluidics for controlled sample mixing or applying an electrical field to a sample. At the same time, this sample introduction approach comes with practical challenges, including undesired X-ray scatter background from the support, potential distortion of a sample due to interaction with the support surface, and the need to protect the sample from dehydration in vacuum. Moreover, most fixed target measurements at LCLS and other XFELs, so far, have been relatively slow due to limited scanning speed and precision (typically ~1 shot/second). Here we describe recent progress in addressing these challenges with solid sample supports for weakly diffracting objects, such as 2D protein crystals, protein fibrils, and nanoliprotein particles (NLPs) and discuss the new opportunities provided by fast scanning stages, such as the Roadrunner system (developed by CFEL) that allows data acquisition at the full 120 Hz repetition rate of LCLS and, potentially, beyond.

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CHENGHUA SHAO, RUTGERS UNIVERSITY

XFEL STRUCTURE DEPOSITION AND DATA ARCHITECTURE SUPPORT IN PDB

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Hard X-ray Free Electron Lasers (XFEL) are enabling new breakthroughs in structural biology research, as evidenced by recent structure depositions to the Protein Data Bank (PDB, pdb.org). Annual XFEL structure deposition rates have grown rapidly over the last 3-years from 6 structures in 2013 to 67 in 2016, illustrating the rapid pace of technical developments in this area. The high brightness of the coherent photon pulse from the XFEL enables data collection on microcrystals maintained at near-physiological conditions, while avoiding the detrimental effects of radiation damage. As the femtosecond duration of XFEL photon pulses match the time scales of certain molecular motions, direct observation of previously inaccessible biological processes are now possible. Recent Serial Femtosecond Crystallography (SFX) studies at XFEL facilities include enzyme intermediate and transition state structures, time-resolved structural changes, and even molecular movies.

In order to meet the myriad challenges of archiving XFEL data and metadata in a manner that allows PDB users to fully understand the import of these experiments and reproduce important results, the Worldwide Protein Data Bank (wwPDB, wwpdb.org) partnership has worked with the wwPDB PDBx/mmCIF Working Group and experts from the XFEL community to develop new PDBx/mmCIF metadata extensions that faithfully represent XFEL experiments (https://github.com/pdbxmmcifwg/xfel-extension). These data items help to support deposition of structures determined by XFEL methods. Herein, we review the current state of XFEL structures in the PDB archive, providing information relating to growth in number of depositions, data collection and experimental protocols, structure types, and metrics pertaining to both molecular and structural complexity. With selected high-profile examples, we also describe how to access XFEL/SFX structural data and metadata using the RCSB.org website of the RCSB Protein Data Bank (RCSB PDB), the US Regional Data Center for the wwPDB organization.

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