AGENDA

Monday, February 11th, 2019

3:00 PM - onwards — **Arrival and Hotel Check-in** [Click Here for Map from Airport]
Hyatt Regency Mission Bay
1441 Quivira Rd
San Diego, CA 92109

3:00 PM - 5:00 PM — **Conference Registration & Poster Session Setup**

8:30 AM - 4:30 PM — **External Advisors Meeting - Mission ballroom**

6th BioXFEL International Conference

Tuesday, February 12th, 2019

7:30 AM – 8:30 AM — **Light Breakfast - Regatta Pavilion**

8:35 AM – 8:45 AM — **Conference Welcome - Mission Ballroom**
Conference Co-Chairs: Brenda Hogue (ASU) and Nadia Zatsepin (ASU)

8:45 AM – 11:45 AM — **Structure from Single Particles, Fibrils, and Nanocrystals - Mission Ballroom**
Session Chairs: Peter Schwander (UWM), Ganesh Subramanian (ASU)
Filipe Maia (Uppsala), Joe Chen (ASU)

8:45 AM – 9:05 AM — Filipe Maia (Uppsala)
High repetition rate single-particle imaging at the European XFEL
9:05 AM – 9:15 AM — Discussion

9:15 AM – 9:35 AM — David Wojtas (University of Canterbury)
Analysis of XFEL serial diffraction data from individual fibrous assemblies
9:35 AM – 9:45 AM — Discussion

9:45 AM – 10:05 AM — Brent Nannenga (ASU)
MicroED: cryo-electron diffraction of 3D microcrystals
10:05 AM – 10:15 AM — Discussion

10:15 AM –10:45 AM — **Coffee Break - Regatta Pavilion**

10:45 AM – 11:05 AM — Peter Zwart (LBL)
Recent developments in fluctuation X-ray scattering
11:05 AM – 11:15 AM — Discussion

11:15 AM – 11:35 AM — Ivan Vartaniants (DESY)
Single-particle imaging without symmetry constraints at an X-ray free-electron laser
11:35 AM – 11:45 AM — Discussion

11:50 AM – 1:00 PM **LUNCH GENERAL GROUP - REGATTA PAVILION**

12:00 AM - 12:50 PM **LUNCH ASSESSMENT MEETING FOR 1ST AND 2ND YEAR GRADUATE STUDENTS - PALM I**

1:00 PM - 2:30 PM **KEYNOTE SPEAKER: PATRICIA RAUN (VIRGINIA TECH) - MISSION BALLROOM**
Communicating Science
Session Chair: Petra Fromme (ASU)

2:30 PM - 3:00 PM **POSTDOCTORAL AWARD RECIPIENT TALK: BENJAMIN STAUCH (USC) - MISSION BALLROOM**
Session Chairs: George Phillips (Rice)

3:00 PM – 5:20 PM **NEW VISIONARY METHODS IN SPI AND NANOCRYSTAL IMAGING - MISSION BALLROOM**
Session Chairs: George Phillips (Rice), Andrew Shevchuk (ASU)
Marc Messerschmidt (ASU), Sabine Botha (ASU)

3:00 PM – 3:20 PM Ghoncheh Mashayekhi (UWM)
Machine learning delivers more bang for the same data
3:20 PM – 3:30 PM Discussion

3:30 PM– 3:50 PM Kartik Ayyer (CFEL)
How small can we go? An analysis of the limits of single particle imaging using experi mental data
3:50 PM – 4:00 PM Discussion

4:00 PM – 4:30 PM **COFFEE BREAK - REGATTA PAVILLION**

4:30 PM – 4:50 PM Saša Bajt (DESY)
Extreme focusing with high numerical aperture X-ray lenses
4:50 PM – 5:00 PM Discussion

5:00 PM – 5:15 PM Abhishek Singharoy (ASU)
Hybrid methods for structure-determination at the dawn of exascale supercomputers
5:15 PM – 5:20 PM Discussion

6:00 PM - 9:00 PM **DINNER AT STONE BREWING-LIBERTY STATION CLICK HERE FOR MAP**
Site is accessible by cab, UBER, or Lyft. Please tell your driver to take you to "Breakfast Republic Point Loma" which will drop you next to the Sims Road Entrance of Stone Brewing.

Wednesday, February 13th, 2019

8:00 AM – 8:45 AM **Light Breakfast - Regatta Pavilion**

8:45 AM – 12:10 PM **Time-Resolved Serial Femtosecond Crystallography - Mission Ballroom**

Session Chairs: Keith Moffat (U of Chicago), Ishwor Poudyal (UWM)
Syun-ru Yeh (Albert Einstein), Lan Zhu (ASU)

8:45 AM – 9:05 AM Martin Weik (IBS)
Excited-state dynamics of photoswitchable fluorescent proteins as measured by time-resolved serial femtosecond crystallography

9:05 AM – 9:15 AM Discussion

9:15 AM – 9:30 AM Nadia Opara (University of Basel)
Demonstration of femtosecond X-ray pump X-ray probe diffraction on protein crystals

9:30 AM – 9:35 AM Discussion

9:35 AM – 9:50 AM Ji-Hye Lee (Korea University)
Time-resolved pH jump studies using serial femtosecond X-ray crystallography

9:50 AM – 9:55 AM Discussion

10:00 AM – 10:20 AM **Coffee Break - Regatta Pavilion**

10:20 AM – 10:40 AM Joerg Standfuss (PSI)
Dynamics of bacteriorhodopsin activation studied using synchrotrons and X-ray lasers

10:40 AM – 10:50 AM Discussion

10:50 AM– 11:10 AM Emina Stojkovic (Northeastern Illinois University)
Myxobacterial phytochromes as light-regulated enzymes suitable for XFEL studies

11:10 AM 11:20 AM Discussion

11:20 AM – 11:40 AM Jose Olmos (Rice University)
Enzyme Intermediates Captured by Mix-and-Inject Serial Crystallography with an X-ray Free Electron Laser

11:40 AM – 11:50 PM Discussion

11:50 AM – 12:05 AM Thomas Szyperski / Surya Pulavarti (University at Buffalo)
NMR meets XFEL

12:05 AM – 12:10 PM Discussion

12:10 PM – 1:00 PM **LUNCH GENERAL GROUP - REGATTA PAVILION**

12:10 PM – 1:00 PM **LUNCH PRESENTATION ON BIOXFEL OPPORTUNITIES FOR UNIVERSITY OF PUERTO RICO STUDENTS - PALM II**

1:00 PM – 3:45 PM **SERIAL SYNCHROTRON CRYSTALLOGRAPHY AND CRYO-EM - MISSION BALLROOM**

Session Chairs: Martin Fuchs (Brookhaven), Darya Marchany (UPR)
Marius Schmidt (UWM), Shatabdi Roychowdhury (ASU)

1:00 PM – 1:20 PM Shibom Basu (EMBL)
Serial crystallographic data analysis at the Swiss Light Source

1:20 PM – 1:30 PM Discussion

1:30 PM – 1:50 PM Jose Martin- Garcia (ASU)
Monochromatic and Poly-chromatic Serial Crystallography at Synchrotron Sources

1:50 PM – 2:00 PM Discussion

2:00 PM – 2:20 PM Cornelius Gati (Stanford University)
Structural Biology of G Protein-coupled Receptors: New Opportunities from XFELs and CryoEM

2:20 PM – 2:30 PM Discussion

2:30 PM – 2:45 PM **COFFEE BREAK - REGATTA PAVILION**

2:45 PM – 3:05 PM Vignesh Kasinath (Berkeley)
Epigenetic silencing by PRC2: Visualizing Cofactor Mediated Regulation by Cryo-EM

3:05 PM – 3:15 PM Discussion

3:15 PM – 3:35 PM Dominik Oberthuer (CFEL)
MHz Serial Crystallography

3:35 PM – 3:45 PM Discussion

3:45 PM – 4:00 PM **POSTER BLITZ - MISSION BALLROOM**

Session Chair: Thomas Grant (UB)

4:00 PM – 6:30 PM **DINNER ON YOUR OWN**

6:30 PM – 7:00 PM **MUSICAL PERFORMANCE BY BIOXFEL QUARTET - REGATTA PAVILION**

7:00 PM – 9:00 PM **POSTER COMPETITION - REGATTA PAVILION**

7:00 - 8:00 EVEN POSTERS

8:00 - 9:00 ODD POSTERS

Thursday, February 14th, 2019

8:00 AM – 8:45 AM **Light Breakfast - Regatta Pavilion**

8:00 AM – 8:45 AM **Poster Session Clean Up - Regatta Pavilion**
*If you do not remove your poster you risk it being thrown away. For those participating in the workshop, please LEAVE your posters and we will have them moved for you.

8:45 AM – 10:05 AM **Serial Crystallography Data Analysis Developments - Mission Ballroom**
Session Chair: Rick Millane (U of Canterbury), Chufeng Li (ASU)

8:45 AM – 9:05 AM Thomas White (DESY)
Horror stories from serial crystallography data processing, and how CrystFEL can help
9:05 AM – 9:15 AM Discussion

9:15 AM – 9:35 AM Ti-Yen Lan (Princeton)
Analysis of Sparse Serial Microcrystal Diffraction Data Collected at a Storage-ring Source
9:35 AM – 9:45 AM Discussion

9:45 AM – 10:00 AM Joe Chen (ASU)
Shape transform phasing
10:00 AM – 10:05 AM Discussion

10:05 AM – 10:30 AM **Coffee Break - Regatta Pavilion**

10:30 AM – 12:00 PM **Serial Crystallography Instrumental Developments - Mission Ballroom**
Session Chairs: Martin Trebbin (UB), Megan Shelby (LLNL)

10:30 AM – 10:50 AM Sebastian Guenther (DESY)
Roadrunner III & IV: High-speed fixed-target sample delivery
10:50 AM – 11:00 AM Discussion

11:00 AM – 11:20 AM Martin Trebbin (UB)
Microfluidic reaction control for time-resolved structure determination at XFELs and synchrotrons
11:20 AM – 11:30 AM Discussion

11:30 PM – 11:50 PM Juraj Knoska (CFEL)
Novel 3D printed mixers and injectors for time-resolved structural biology
11:50 PM – 12:00 PM Discussion

12:00 PM - 2:00 PM	**BIOXFEL SAMPLE DELIVERY INTERACTIVE SESSION & LUNCH - REGATTA PAVILION** Session Chairs: Nadia Zatsepin (ASU), Brenda Hogue (ASU)
12:00 PM - 12:15 PM	Biology Workshop Discussion - **Mission Ballroom**
12:15 PM - 1:15 PM	Lunch Workshop Session - **Regatta Pavilion**
1:15 PM - 2:00 PM	Summary of Sample Delivery Needs for Biology - **Mission Ballroom**
2:00 PM - 2:15 PM	**STUDENT SPEAKER AWARD ANNOUNCEMENT - MISSION BALLROOM**
2:15 PM - 2:30 PM	**GROUP PHOTO**
2:30 PM – 5:00 PM	**BIOXFEL STC MEETING - FOR CENTER MEMBERS ONLY - MISSION BALLROOM**
3:00 PM – 5:00 PM	Review of 2019 BioXFEL Assessment Data (20 minutes) 10 min (3 powerpoint) talks on current BioXFEL collaborations. Speakers in alphabetical order. John Spence
4:00 PM – 4:15 PM	**COFFEE BREAK - REGATTA PAVILION**

KEYNOTE SPEAKER

Patty Raun, Virgina Tech

Communicating Science

Effective public engagement is key to solving wicked problems. An engaged, educated, and scientifically literate public is the only path to sound public decision-making. But the gap between research conducted at universities sand the public understanding of that research is enormous. Americans hold fairly positive views about research and researchers, but they have a limited understanding of what researchers do--and of research findings. Although Americans respect scientists and engineers, their information about science and technology comes primarily from the Internet, television, and newspapers (NSF, 2016). Most of our country's population has very little formal training in science or research (U.S. Census Bureau, 2016), making effective communication of research findings both essential and challenging. In addition, with ever-increasing specialization, nearly as large a gap of understanding can exist between researchers in different fields.

The communication skills gained through this approach transfer to collaborative innovation and creative problem-solving, with many "side benefits" along the way. For example, researchers who are effective at connecting across differences can help build diversity in the STEM workforce. Building collaborative skills in undergraduate and graduate students results in cohorts of graduates who are able to work with others effectively across a variety of types of "difference": research specialty, gender, race, ethnicity, age, and more.

Biography

Patricia Raun is Director of the Center for Communicating Science at Virginia Tech. During her years as a professional actor and professor of theatre she was inspired to share the powerful tools of her work to support skills of connection and communication in scientists, technology professionals, and scholars. As a theatre voice specialist, her mission is to promote positive transformation by developing healthy and varied voices—both literal and figurative—in individuals, institutions, and communities. Inspired by her work with the Alan Alda Center for Communicating Science at Stony Brook University, her particular interests include empathy development, serious games, and collaboration.

Filipe Maia, Uppsala

High repetition rate single-particle imaging at the European XFEL

Filipe R.N.C. Maia[1,2]

[1] Laboratory of Molecular Biophysics, Dept. of Cell and Molecular Biology, Uppsala University, 75124 Uppsala, Sweden.

[2] NERSC, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

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, last year, of the European XFEL marks the beginning of the high-intensity, high-repetition-rate and high data-rate era of XFELs, bringing the dream closer to reality.

This last decade has also witnessed a spectacular rise in the data volumes acquired in X-ray diffraction experiments. When I started my PhD we were collecting images at FLASH at around one hertz. Nowadays we're collecting data at the European XFEL at a peak rate into the megahertz. This has enabled the development of new techniques that exploit this richness and were not possible before. In this talk I will present the evolution of X-ray diffraction imaging, and in particular highlight the latest results from the European XFEL. I will also demonstrate the importance of developing robust structure validation procedures for the long-term success and wider adoption of the method as well as discuss what new techniques might be over the horizon and how to best make use of this wealth of data to extract as much new knowledge as possible.

Chapman, H. N. *et al.* Femtosecond diffractive imaging with a soft-X-ray free-electron laser. *Nat. Phys.* **2,** 839–843 (2006).

Ekeberg, T. *et al.* Three-Dimensional Reconstruction of the Giant Mimivirus Particle with an X-Ray Free-Electron Laser. *Phys. Rev. Lett.* **114,** 98102 (2015).

Hantke, M. F. *et al.* High-throughput imaging of heterogeneous cell organelles with an X-ray laser. *Nat. Photonics* **8,** 943–949 (2014).

Neutze, R., Wouts, R., Van Der Spoel, D., Weckert, E. & Hajdu, J. Potential for biomolecular imaging with femtosecond X-ray pulses. *Nature* **406,** 752–7 (2000).

Seibert, M. M. *et al.* Single mimivirus particles intercepted and imaged with an X-ray laser. *Nature* **470,** 78–81 (2011).

van der Schot, G. *et al.* Imaging single cells in a beam of live cyanobacteria with an X-ray laser. *Nat. Commun.* **6,** 5704 (2015)

David Wojtas, University of Canterbury

Analysis of XFEL serial diffraction data from individual fibrous assemblies

Wojtas, D. H.,[1] Seuring, C.,[2] Ayyer, K.,[2] Liang, M.,[3] Mossou, E.,[4,5] Forsyth, V. T.,[4,5] Chapman, H. N.,[2,6,7] and Millane, R. P.[1]

1. Computational Imaging Group, Department of Electrical and Computer Engineering University of Canterbury, Christchurch, New Zealand; 2. Center for Free-Electron Laser Science, Deutsches Elektronen-Synchrotron (DESY), Notkestrasse 85, 22607 Hamburg, Germany; 3. Linac Coherent Light Source, SLAC National Accelerator Laboratory, Menlo Park, California, USA; 4. Institut Laue-Langevin, Grenoble, France; 5. Faculty of Natural Sciences, Keele University, UK; 6. Department of Physics, University of Hamburg, Hamburg, Germany; 7. Centre for Ultrafast Imaging, University of Hamburg, Hamburg, Germany

Application of X-ray free-electron lasers (XFELs) through serial femtosecond x-ray crystallography (SFX) [1] opens up new opportunities for imaging single fibrous biomolecular assemblies. Fibrous assemblies are long, slender systems that have periodicity along one axis, but with little or no crystallinity in the lateral plane [2]. They are therefore intermediate between 3D crystals and single particles, and their diffracted intensity is weak in the absence of the coherent amplification provided by a crystal. The potential to target individual assemblies circumvents cylindrically averaging of the diffracted intensity that occurs with rotationally disordered, mm-sized samples used in conventional x-ray fiber diffraction with synchrotron sources. Fibrous molecules and assemblies are ubiquitous in biology, with their fibrous nature often being key to their biological function, making them interesting targets for XFEL studies.

We have conducted experiments with fibrous systems at the CXI end-station at the LCLS, using both liquid-jet and fixed-target delivery techniques [3, 4]. Fiber diffraction theory that is relevant to the experimental design of each experiment will be introduced. Technical differences between each kind of experiment will be described, including some unforeseen complications that were encountered. Image processing techniques for processing the large volumes of data and to identify and extract well-oriented diffraction and identify patterns due to single fibrils. The two sample delivery techniques are compared by characterizing their ability to produce well-oriented diffraction data and their relative levels of background diffuse scattering.

Efforts to obtain structural information from the useful data are then described. This first step involves determining the orientation of the assembly for each frame from the diffraction features. In the case of a crystalline amyloid-forming oligopeptide of unknown structure, analysis of the diffraction patterns allows determination of orientations in 3D [3]. The data can then be merged into 3D reciprocal space, allowing the individual structure amplitudes to be measured. Structure determination using this data is currently in process. This approach offers a new route to the study of fibrous systems in biology.

The experiments described here were conducted as part of a large collaboration between investigators at UC, CFEL, LCLS and ILL. The work is supported by the NZ Marsden Fund and the Human Frontiers Science Program (RGP0010/2017).

1. H. N. Chapman et al., *Nature* **470**, 73-77 (2011).
2. R. P. Millane, in *International Tables for Crystallography Vol. B,* edited by U. Shmueli, IUCr, 568-583 (2010).
3. D. H. Wojtas et al., IUCrJ **4,** 794-811 (2017).
4. C. Seuring et al., Nature Comm. **1**(6), doi:10.1038/s41467–018–04116–9 (2018**)**.

Brent Nannenga, Arizona State University

MicroED: Cryo-electron diffraction of 3D microcrystals

One of the critical bottlenecks in protein crystallography is the growth of large, well-ordered crystals for conventional X-ray experiments. For difficult targets, such as membrane proteins or protein complexes, the process of optimizing conditions for large, high-quality crystals can be extremely difficult. Micro electron-diffraction, or MicroED, is a structure determination method that uses a cryo-transmission electron microscope (cryo-TEM) to collect electron diffraction data from microcrystals. This technique has been successfully used to determine structures of many targets from crystals orders of magnitude smaller than what is needed for X-ray diffraction experiments. Here, we will describe the MicroED method and continued improvements to facilitate MicroED structure determination for more difficult crystalline samples.

Peter Zwart, Lawrence Berkeley National Laboratory

Recent developments in fluctuation X-ray scattering

Zwart, P.H. [*1,2], Donatelli J.J.[1,3] & Pande, K.[1,2]

1. Center for Advanced Mathematics in Energy Research Applications, Lawrence Berkeley National Laboratory, Berkeley, CA 94720 USA; 2. Molecular Biophysics and integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720 USA; 3. Department of Mathematics, Computational Research Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720 USA.; * e-mail: PHZwart@lbl.gov

Fluctuation X-ray scattering (FXS) is a biophysical structural characterization technique that overcomes low data-to-parameter ratios encountered in traditional X-ray scattering methods used for studying non-crystalline samples (1-3). By collecting a series of ultrashort X-ray exposures on an ensemble of particles at a free-electron laser, information-dense experimental data can be extracted that ultimately result in structures with a greater level of detail than can be obtained using traditional X-ray solution scattering methods, ultimately allowing one to visualize details of structural dynamics that may be inaccessible through traditional methods.

After a successful application of FXS on single particle data (4), we have now demonstrated the practical feasibility of FXS from experimental data from multiple particle solution of the PBCV-1 virus (5, 6), Fig 1. One of our findings is that the required number images needed to obtain decent correlation data is far lower than originally expected by use of information-theoretic approaches in noise reduction. As a result, data collection times can be reduced to less than a few minutes, reducing the overall consumption of biological sample and beamtime. In addition to these results, we will present an assessment of the effect of various sources of noise on the experimental data, and how to account for these effects in the data processing.

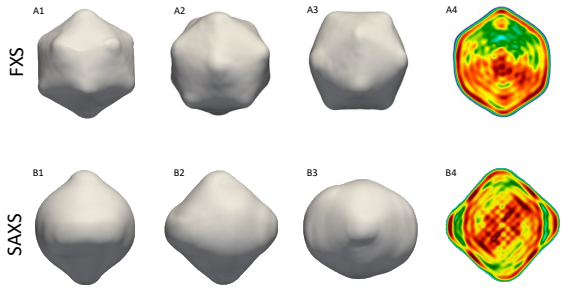

Figure 1. The structure of PBCV-1 as determined from FXS data (A1-4) is of higher quality and contains contains far more details at the structure determined from the associ-

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4. Kurta RP, *et al*. Correlations in Scattered X-Ray Laser Pulses Reveal Nanoscale Structural Features of Viruses. Phys Rev Lett. 2017;119 (15):158102.

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6. Pande K, *et al*. Free-electron laser data for multiple-particle fluctuation scattering analysis. Sci Data. 2018;5:180201.

IVAN VARTANIANTS, DESY

SINGLE-PARTICLE IMAGING WITHOUT SYMMETRY CONSTRAINTS AT AN X-RAY FREE ELECTRON-LASER

M. Rose[1], S. Bobkov[2], K. Ayyer[3], R. P. Kurta[4], D. Dzhigaev[1], Y.-Y. Kim[1], A. Morgan[3], C.H. Yoon[5], D. Westphal[6], J. Bielecki[6,4], J.A. Sellberg[7,6], G. Williams[8], F.R.N.C. Maia[6], O.M. Yefanov[3], V. Ilyin[2], A. P. Mancuso[4], H. N. Chapman[3], B.G. Hogue[8], A. Aquila[5], A. Barty[3] and I.A. Vartanyants [1,9,*]

[1] DESY, Hamburg, Germany; [2] NRC 'Kurchatov Inst.', Moscow, Russia; [3] CFEL, Hamburg, Germany, [4] Eu. XFEL Schenefeld, Germany; [5] LCLS, SLAC, Menlo Park, CA, USA; [6] Lab. Mol. Bioph., Uppsala Univ., Sweden; [7] Biomed. X-Ray Phys., KTH Royal Inst. Techn., Stockholm, Sweden; [8] Brookhaven Nat. Lab., Shirley, NY, USA; [9] Biodesign Cent. Immunother., Arizona St. Univ., Tempe, USA;[9] NRNU MEPhI, Moscow, Russia

*e-mail: (Ivan.Vartaniants@desy.de)

The analysis of a single particle imaging (SPI) experiment performed at the AMO beamline at LCLS [1] as part of the SPI initiative [2] will be presented. A workflow for the three-dimensional (3D) virus reconstruction of PR772 bacteriophage from measured single particle data will be discussed [3]. It consists of several well defined steps (see Fig. 1) including: single hit diffraction data classification, refined filtering of the classified data, reconstruction of 3D scattered intensity from the experimental diffraction patterns by orientation determination, and final 3D reconstruction of the virus electron density without symmetry constraints. Single particle diffraction patterns from X-ray free electron laser (XFEL) pulses encode rich information of their structure. Here, we employ a selection routine driven by principal component analysis (PCA) to improve the data quality used for orientation determination and three-dimensional (3D) structure recovery of nano-scale viruses measured at the Linac Coherent Light Source (LCLS) XFEL. Our results highlight the structural fingerprint of a 3D virus that can be obtained from diffraction space and *ab initio* structure recovery *via* iterative phase retrieval. The analysis developed here revealed and quantified nano-scale features of the PR772 virus with resolution below 10 nm and with a clear indication that the structure was compressed in one direction and, as such, deviates from ideal icosahedral symmetry.

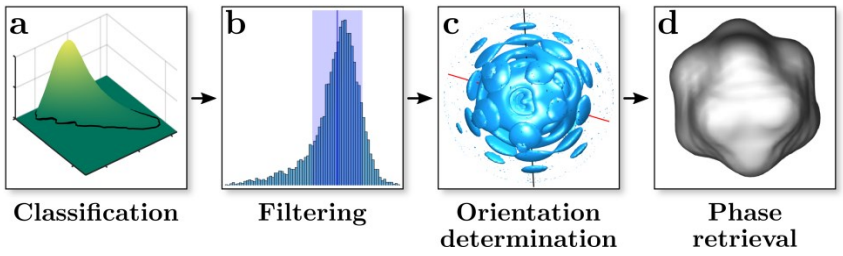

Fig. 1 Workflow of the SPI experiment towards single particle reconstruction. a) Single hit classification of the initial data. b) Refined filtering of the classified data. c) Orientation determination. d) Particle reconstruction.

REFERENCES:

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[2] A. Aquila *et al.*, *The linac coherent light source single particle imaging road map* , Struct. Dynam. **2**, 041701 (2015).

[3] M. Rose *et al.*, *Single-particle imaging without symmetry constraints at an X-ray free-electron laser*, IUCrJ **5**, 727-736 (2018).

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Benjamin Stauch, University of Southern California

Designing GPCR photoswitches for time-resolved crystallography

Benjamin Stauch, Andrii Ishchenko, Anna Shiriaeva, and Vadim Cherezov

Bridge Institute, University of Southern California, Los Angeles

G protein coupled receptors are a privileged class of drug targets, accounting for more than 30% of FDA approved drugs[1]. They undergo large-scale conformational changes between inactive and active states that span internal, local, and global helical motions, and the nano- to millisecond timescale, allowing them to process external stimuli such as binding of endogenous ligands or drugs, and relaying those to intracellular effectors such as G proteins. However, the exact sequence and dynamics of this conformational coupling mechanism is unknown, requiring crude interpolation between known start and end states[2,3], with gaps being filled by computer simulation, or indirect experimental readouts[4]. X-ray free electron lasers (XFELs) facilitate direct observation of dynamic events with temporal resolution only limited by their (femtosecond) pulse length[5], and are ideally suited for time-resolved structural studies of challenging systems such as membrane proteins. Recently, studies of the structural dynamics of light-sensitive bacterial transmembrane proteins[6] have demonstrated the feasibility of this approach, and bring investigations of pharmaceutically more relevant targets such as GPCRs into immediate reach. Using light to trigger conformational change, and to synchronize this conformational change across a crystal, is prerequisite for studies of fast to intermediate dynamics. The vast majority of proteins is not light-sensitive, and the design of a photoswitch for generic GPCRs is a formidable task. We aim to develop photoswitchable adenosine receptor ligands to study activation of the A2A receptor by bioisosteric replacement of known agonists by photoswitchable substructures. A key challenge is to obtain well-diffracting crystals of the receptor-ligand complex while functionalization of ligand tends to decrease its receptor affinity. To overcome this obstacle, we develop a *ligand exchange* method that allows us to obtain crystals of GPCRs in complex with relatively weak binders, and multiplex crystallization trials and structure determination of many candidate compounds in parallel. We demonstrate the power of this method by obtaining several new ligand co-crystal structures of known GPCRs, and by rapidly expanding structural coverage of the pharmacology of a novel GPCR starting from a single crystal hit. We discuss limitations of our method and outline the steps towards a molecular movie of GPCR activation dynamics.

This work is supported by the NSF BioXFEL STC Postdoctoral Research Award.

1 Stauch, B. & Cherezov, V. Serial Femtosecond Crystallography of G Protein-Coupled Receptors. *Annual review of biophysics*, doi:10.1146/annurev-biophys-070317-033239 (2018).

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Ghoncheh Mashayekhi, University of Wisconsin-Milwaukee

Machine learning delivers more bang for the same data

G. Mashayekhi, A. Dashti, A. Hosseinzadeh, R. Fung, P. Schwander, A. Ourmazd

Department of Physics, University of Wisconsin-Milwaukee, Milwaukee, WI 53211, USA

We apply geometric machine learning to experimental data in order to determine the functionally relevant dynamical properties of biological nanomachines. As pointed out by Frauenfelder long ago [1], function plays out on energy landscapes. Using particles in equilibrium and without any time-resolution, it is now possible to map energy landscapes, and determine the continuous conformational changes associated with biological function.

On occasion, one wishes to learn not only the sequence of events, but also their exact timing, e.g., in processes involving conical intersections traversed after photon absorption. In such cases, the available timing information is often highly inaccurate. We have developed and validated a mathematically rigorous data-analytical approach capable of extracting accurate dynamical information from noisy experimental data recorded with extreme timing uncertainty. This capability can be applied whenever dynamical information is compromised by noise and timing uncertainty. Do XFEL-based pump-probe experiments ring a bell?

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KARTIK AYYER, CFEL

HOW SMALL CAN WE GO? AN ANALYSIS OF THE LIMITS OF SINGLE PARTICLE IMAGINING USING EXPERIMENTAL DATA

Kartik Ayyer[1], Andrew J. Morgan[2], Anton Barty[3], Henry N. Chapman[3,4,5]

[1]Max Planck Institute for the Structure and Dynamics of Matter, Luruper Chaussee 149, 22761 Hamburg, Germany

[2]ARC Centre of Excellence for Advanced Molecular Imaging, School of Physics, The University of Melbourne, Parkville, VIC 3010, Australia

[3]Center for Free-Electron Laser Science, Deutsches Elektronen Synchrotron DESY, Notkestraße 85, 22607 Hamburg, Germany

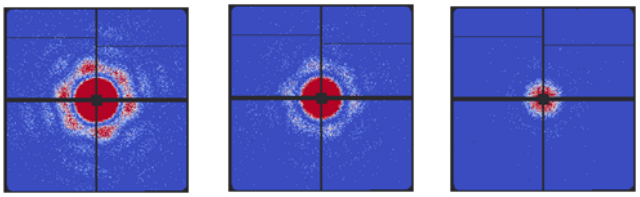
[4]Department of Physics, University of Hamburg, Luruper Chaussee 149, Hamburg, Germany

[5]The Center for Ultrafast Imaging, University of Hamburg, Luruper Chaussee 149, Hamburg, Germany

X-ray single particle imaging (SPI) has till now been performed to relatively low resolution on relatively large particles which scatter strongly. This has left open the question of whether we can reliably determine structures when the signal levels are much lower. Although simulations and proof-of-principle experiments have been performed with very sparse photon counts, they haven't definitively answered whether this is possible under realistic conditions, such as non-isotropic background, incident fluence fluctuations and contamination by non-single particle patterns.

We approach this problem by considering a publicly available experimental dataset from 60-nm PR772 viruses collected at LCLS and reduce the signal by throwing away a random fraction of photons in every frame. This simulates the effect of a weaker X-ray beam, or of a smaller particle with proportionately lower background. We reconstruct the electron densities and examine various metrics as a function of the signal level. Encouragingly, we find that the reconstruction quality remains consistent even when 1/256th the photons are used, corresponding to a sub-10 nm particle size.

The second part of the talk will be about the other big challenge in SPI analysis, namely single hit classification. While a plethora of machine learning methods exist to classify the patterns at higher signal levels, they often break down as the scattered signal becomes weak. We discuss a possible way forward using a combination of a multi-model 2D EMC and more conventional manifold embedding.



Saša Bajt, DESY

Extreme focusing with high numerical aperture X-ray lenses

Saša Bajt

DESY, Notkestrasse 85, 22607 Hamburg, Germany

Novel X-ray optics combined with new x-ray sources offer new opportunities to focus x-rays to unprecedented intensities. In this presentation we will present x-ray diffractive optics based on multilayers, also called multilayer Laue lenses (MLLs), which are similar to x-ray zone plates but fabricated by layer deposition. The resolution of any such diffractive optic is governed by the width of the smallest feature in such a structure—the zone width or layer width. As a first requirement for the multilayers from which MLLs are made is that the layers are smooth and abrupt. Improvements in multilayer technology and an improved understanding of material properties enabled us to deposit layers thinner than 1 nm. As long as these layers are also precisely and correctly placed in the structure, a resolution of this order is considered achievable, at least theoretically. Unlike in lithographically prepared zone plates in MLLs, the aspect ratio between the smallest zone width (layer thickness) and the zone plate optical thickness can be extremely high. This is critical when using these lenses with hard X-rays (> 5 keV) and it results in very high efficiencies of MLLs. Additionally, each layer in the MLL has to be correctly tilted to satisfy the Bragg condition [1,2]. Only then will every layer contribute to the focus to ensure high efficiency and high numerical aperture.

Using these kinds of MLLs we successfully demonstrated sub-10 nanometer focusing in one dimension [3] and in two dimensions [4]. Layer placement errors produce aberrations in the lens. To correct such aberrations in the lenses it is critical to perform precise measurements on the lenses and implement corrections directly into the next fabrication process. Ideally these measurements should be done at a synchrotron beamline. However, such beamlines are very rare and highly subscribed. The access to them is unpredictable and not unreliable. Therefore, we are developing methods and solutions based on a lab-based x-ray source.

Highly intense beams can also be achieved by compressing x-ray pulses from XFELs in time. This can be done with highly efficient volume structures such as a pair of volume transmission gratings [1]. All these developments require the preparation of multilayer structures of over 100 microns thick (or ideally even larger), consisting of more than 50 000 layers, which is extremely challenging. This talk with give an overview, current status and the future outlook on the development and performance of such state-of-the-art X-ray optics.

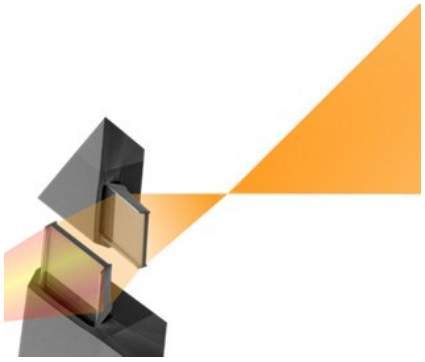


Figure 1: A multilayer Laue lens (MLL) acts as cylindrical mirror (focuses light in one direction) and therefore we need two of them placed orthogonal to each other to achieve a 2D focus.

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Abhishek Singharoy, Arizona State University

Hybrid methods for structure-determination at the dawn of exascale supercomputers

Abhishek Singharoy,[1] Letty SalasEstrada,[2] Thomas Grant,[3] Derek Mendez,[4] Alan Grossfiled,[2] Nadia Zatsepin,[1 1] Richard Kirian, [1] Natasha Stander,[1] Petra Fromme, [1] Michael Brown,[4] Joachim Frank,[5] Ali Vashti,[6] Peter Schwander, [6] Abbas Ourmazd[6]

Accurate structure determination from medium to low-resolution experimental data necessitates a balance between extensive global and local sampling of atomistic models, yet with stereochemical correctness of backbone and sidechain geometries. Molecular Dynamics simulations, particularly through the application of enhanced sampling and Baysean inferencing schemes provide today a robust way of achieving this balance for hybrid structure determination. Engendering a high-throughput real space refinement approach, called molecular dynamics flexible fitting (MDFF), the protocol performs at scale unto 10000 nodes on national supercomputers. In this presentation, the capabilities of MDFF will be showcased with three distinct applications of imminent relevance to the BioXFEL community: **(a)** refinement of disordered residues within crystal contacts of Photosystem II, **(b)** determination of protein quakes from scattering profiles in bovine-rhodopsin, and **(c)** elucidation of time-resolved minimum-free energy pathways from single particle images of calcium channels. The common theme that underlies these three applications is the role of large-scale conformational transitions within the functional cycle of the proteins. Information on the dynamics remain embedded within the diffraction, scattering, or single-particle data. Taking advantage of the highly efficient conformational search capabilities provided by MDFF, this dynamical information is revealed, uncertainty in the data is quantified, and inferences are drawn on the biological functions of the proteins. To this end, MDFF refinements deliver **(a)** geometry of the oxygen evolving complex in the dark-state of PSII, **(b)** light-induced activation step of rhodopsin, and **(c)** mechanism of ion binding to the Calcium ion channel.

[1] Arizona State University

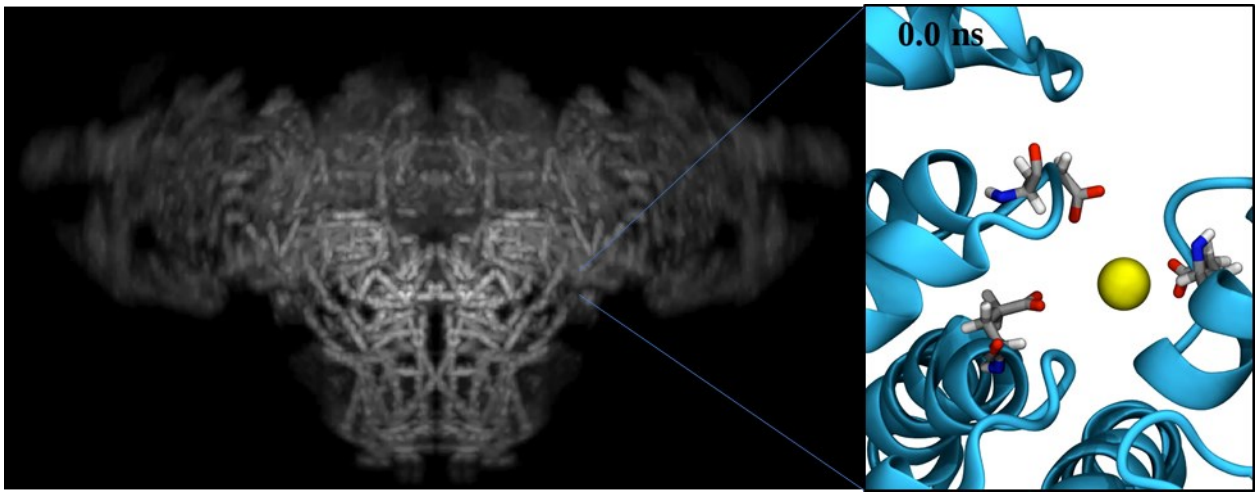
[2] University of Rochester

[3] Sunny University of Buffalo

[4] University of Arizona

[5] Columbia University

[6] University of Wisconsin Milwaukee



From data to model using molecular dynamics simulations

Martin Weik, Institut de Biologie Structurale

Title TBD

Weik, M.

Institut de Biologie Structurale, Grenoble, France

Reversibly photoswitchable fluorescent proteins (RSFP) are essential tools in advanced fluorescence nanoscopy of live cells (such as PALM and RESOLFT). They can be repeatedly toggled back and forth between a fluorescent (*on*) and a non-fluorescent (*off*) state by irradiation with light at two different wavelengths.

Our consortium combines time-resolved serial femtosecond crystallography (TR-SFX), ultrafast absorption spectroscopy in solution and simulation methods to study *off*-to-*on* [1] and *on*-to-*off* photoswitching (unpublished) intermediates in the RSFP rsEGFP2 on the ultra-fast photochemical time scale. *Off*-to-*on* photoswitching involves an excited state intermediate in which the chromophore adopts a twisted conformation, midway between the stable configurations of the *on* and *off* states. This observation has been confirmed by simulations and has allowed us to rationally design a mutant with a two-fold increased photoswitching quantum yield [1].

The presented research has been conducted by the following scientists: Adam, Aquila, Barends, Bourgeois, Boutet, Byrdin, Cammarata, Carbajo, Colletier, Coquelle, de la Mora, Demachy, Doak, Feliks, Fieschi, Field, Foucar, Grünbein, Guillon, Hilpert, Hunter, Jakobs, Joti, Kloos, Koglin, Lane, Lévy, Liang, Macheboeuf, Mizohata, Motomura, Nango, Nass, Nass-Kovacs, Owada, Ridard, Robinson, Roome, Roux, Ruckebusch, Seaberg, Schiro, Schlichting, Shoeman, Sliwa, Stricker, Sugahara, Thepaut, Togashi, Tono, Uriarte, Weik, Woodhouse, Yabashi.

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Nadia Opara

Demonstration of femtosecond X-ray pump X-ray probe diffraction on protein crystals

Nadia Linda Opara[1,4,5,*], Istvan Mohacsi[1,2], Mikako Makita[1], Daniel Castano-Diez[4], Ana Diaz[1], Pavle Juranić[1], May Marsh[1], Alke Meents[2], Christopher J Milne[1], Aldo Mozzanica[1], Celestino Padeste[1], Valérie Panneels[1], Marcin Sikorski[3], Sanghoon Song[3], Henning Stahlberg[4,5], Ismo Vartiainen[1], Laura Vera[1], Meitian Wang[1], Philip R Willmott[1] & Christian David[1]

[1]*Paul Scherrer Institut, Villigen PSI, Switzerland*
[2]*Deutsches Elektronen-Synchrotron DESY, Germany*
[3]*LCLS, SLAC National Accelerator Laboratory, USA*
[4]*C-CINA, Biozentrum, University of Basel, Basel, Switzerland*
[5]*Swiss Nanoscience Institut, Basel, Switzerland*
*Correspondence by e-mail: nadia.opara@gmail.com

The development of X-ray free-electron lasers (XFELs) has opened the possibility to investigate the ultrafast dynamics of biomacromolecules using X-ray diffraction [1]. Whereas an increasing number of structures solved by means of serial femtosecond crystallography at XFELs is available [2-10], the effect of radiation damage on protein crystals during ultrafast exposures has remained an open question [11-12]. We used a split-and-delay line based on diffractive X-ray optics [13] at the LCLS XFEL to investigate the time dependence of X-ray radiation damage to lysozyme crystals. For these tests, crystals were delivered to the X-ray beam using a fixed target approach. The presented experiments provide probe signals at eight different delay times between 19 and 213 femtoseconds after a single pump event, thereby covering the time scales relevant for femtosecond serial crystallography [14].

Even though significant impact on the crystals was observed at long time scales after exposure with a single X-ray pulse, the collected diffraction data did not show significant signal reduction that could be assigned to beam damage on the crystals in the sampled time window and resolution range. This observation is in agreement with estimations of the applied radiation dose, which in our experiment was clearly below the values expected to cause damage on the femtosecond time scale. The experiments presented here demonstrate the feasibility of time resolved pump-multi-probe X-ray diffraction experiments on protein crystals.

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Ji-hye Lee, Korea University

Time-resolved pH jump studies using serial femtosecond X-ray crystallography

Lee, J.-H., Lee, D. B., Nam, J. W., An, J. S., Yang, T., Seok, J. H., and Kim, K. H.

Department of Biotechnology & Bioinformatics, Korea University, Sejong 30019, Korea.

A green-emitting fluorescent protein variant, NowGFP, has a anionic tryptophan-based chromophore (Thr65-Trp66-Gly67) and is stable with respect to pH and temperature, unlike its predecessor, WasGFP. NowGFP shows characteristic photoswitching from a green-emitting form to a cyan-emitting form, induced by acidic pH. We have noticed that the pH jump-induced conformational change of NowGFP is limited to local active site rearrangement without disrupting the crystal packing, which can be a suitable target protein for developing a pH jump platform for time-resolved serial femtosecond X-ray crystallography (TR-SFX). The crystal structures of NowGFP were determined at pH 4.0, 6.5, and 9.0 at both cryogenic and room temperature using synchrotron and XFEL sources. In a typical β-barrel structure, major conformational changes occur at the chromophore and Lys61, a key residue in chromophore ionization, and hydrogen-bond networks comprising Lys61, Glu222, Thr203 and Ser205, which was connected to the indole ring of the chromophore at the active site. In addition to structural differences at cryo and room temperature, the results of our recent TR-SFX studies using cage molecule-driven pH jump are described.

This work was supported by grants from the National Research Foundation of Korea (NRF) funded by the Korean government (MSIP) (NRF-2016R1E1A1A01942558, K.H.K.; NRF-2018R1D1A1A02049225, J.-H.L.) and the BK21 Plus program through the NRF of the ME.

Joerg Standfuss, Paul Scherrer Institute

Dynamics of bacteriorhodopsin activation studied using synchrotrons and X-ray lasers

Jörg Standfuss

Laboratory of Biomolecular Research, Department of Biology and Chemistry, Paul Scherrer Institute, Villigen PSI

Representing a collaboration of scientists from Uni. Gothenburg, Uni. Tokyo, Uni. Kyoto, PSI, ASU, SACLA, LCLS and SwissFEL.

X-ray free electron lasers provide exciting new opportunities to study the structural dynamics of proteins. By integrating sample efficient high viscosity injectors into pump probe setups, it is now possible to determine whole series of structural snapshots by time-resolved serial femtosecond crystallography and assemble them to molecular movies of proteins in action.

Based on our recent studies of bacteriorhodopsin, I will outline the possibilities but also the challenges that have to be overcome before we can routinely study structural rearrangements at ambient temperature and in real time. One of the current bottlenecks is that access to XFEL facilities will likely remain scarce for the foreseeable future. To allow experiments at synchrotron sources, we have adapted high viscosity injector systems to carry out routine room-temperature serial millisecond crystallography (SMX) at the Swiss Light Source (1). Such SMX experiments allow to improve density and homogeneity of crystal preparations for efficient time-resolved data collection.

Time-resolved serial crystallography has now allowed to study the structural reorganizations and proton transfer steps in bR with astounding detail and over many orders of magnitude in time. Mechanistically bR can be divided into an extracellular half and a cytoplasmic half with the retinal chromophore positioned roughly in the middle of the membrane. The first principal step in the pumping mechanism is the light induced isomerization of retinal in the femtosecond range, which provides the energy for the reaction (2). In the second step, the energy is used to change the protein conformation within microseconds to allow proton release from the retinal Schiff base towards the extracellular release group via a water mediated hydrogen-bonding network (3). In the third principal step, the protein changes again after several milliseconds to allow uptake of a proton from the intracellular side of the membrane (4). These sequential rearrangements throughout the bR photocycle follow the basic predictions of an alternate access model and may provide a template to understand the principal transport steps in other membrane pumps.

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Emina Stojkovic, Northeastern Illinois University

Myxobacterial phytochromes as light-regulated enzymes suitable for XFEL studies

Stojković, E.A.[1] and Schmidt, M.[2]

1. Department of Biology, Northeastern Illinois University, Chicago, IL, 60625, USA and 2. Department of Physics, University of Wisconsin-Milwaukee, Milwaukee, WI, 53211, USA

Phytochromes (PHYs) are photoreceptor proteins first discovered in plants, where they control a variety of photomorphogenesis events. PHYs as photochromic proteins can reversibly switch between two distinct states: a red light absorbing (Pr) and a far-red light absorbing (Pfr) form. The discovery of Bacteriophytochromes (BphPs) in non-photosynthetic bacteria has opened new frontiers in our understanding of the mechanisms by which these natural photoswitches can control single cell development, although the role of BphPs *in vivo* remains largely unknown. BphPs are dimeric proteins that consist of a photo-sensory core module (PCM) and enzymatic domain, often histidine kinase. The PCM is composed of three domains (PAS, GAF, PHY). It holds a covalently bound linear tetrapyrrole (biliverdin, BV) chromophore. Upon absorption of light, the double bond between BV rings C and D isomerizes and switches the protein between Pr and Pfr states. The two BphPs from the non-photosynthetic myxobacterium *S. aurantiaca, Sa*BphP1 and *Sa*BphP2, have distinct photochemistry, although they bind the same bilin chromophore and share a large sequence identity. Unlike classical BphPs, the wild-type *Sa*BphP1 lacks a conserved histidine that stabilizes the bilin chromophore in the Pr and Pfr states. On the other hand, this His is present in the wild-type *Sa*BphP2. Hence, the two distinct phytochromes will provide a unique platform to explore the function of this important His. We solved the structures of both *Sa*BphP1 and *Sa*BphP2 in wild-type and mutant forms involving substitutions of the conserved His. The wild-type *Sa*BphP2 crystals diffract to a resolution better than 1.9 Å at the synchrotron, and microcrystals to about 2.3 Å at the Japanese X-ray Free Electron Laser (XFEL) SACLA, the highest resolution for PCMs so far. Although structures of Pr and Pfr states have been determined from other phytochromes, direct structural information of various intermediates is lacking. To investigate the structural changes that occur in the phytochrome chromophore, its binding-pocket and the adjacent PHY domain with Time-Resolved Serial Femtosecond X-ray crystallography (TR-SFX), we plan to study *Sa*BphP2 using the ultrashort X-ray pulses available at XFELs. Analysis of the diffraction patterns will yield detailed structural information of the signal generation and transduction at near atomic resolution. Our experiments will not only show structural changes near the chromophore, but also describe signal transduction into the PHY domain which moves several tens of Angstroms after the light stimulus in real time. Our goal is to provide a comprehensive view of the transition from early events in signal transduction to later protein relaxations, revealing how enzymatic activity in the intact protein is controlled/regulated by the light stimulus generated over 100 Angstrom distance.

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Jose Olmos, Rice University

Enzyme intermediates captured by mix-and-inject serial crystallography with an X-ray Free Electron Laser

Olmos, Jr., J.L.,[1] Pandey, S.,[2] Martin-Garcia, J.M.,[3] Calvey, G.,[4] Katz, A.,[4] Knoska, J.,[5] Kupitz, C.,[2] Hunter, M.S.,[6] Liang, M.,[6] Oberthuer, D.,[5] Yefanov, O.,[5] Wiedorn, M.,[5] Heymann, M.,[5] Holl, M.,[3] Pande, K.,[5] Barty, A.,[5] Miller, M.D.,[1] Stern, S.,[5] Roy -Chowdhury, S.,[3] Code, J.D.,[3] Nagaratnam, N.,[3] Zook, J.,[3] Norwood, T.,[2] Poudyal, I.,[2] Xu., D.,[1] Koglin, J.,[6] Seaberg, M.,[6] Zhao, Y.,[3] Bajt, S.,[5] Grant, T.,[7] Mariani, V.,[5] Nelson, G.,[8] Subramanian, G.,[3] Bae, E.,[9] Fromme, R.,[3] Fung, R.,[2] Schwander, P.,[2] Frank, M.,[10] White, T.,[5] Weierstall, U.,[3] Zatsepin, N.,[3] Spence, J.,[3] Fromme, P.,[3] Chapman, H.N.,[3] Pollack, L.,[4] Tremblay, L.,[8] Ourmazd, A.,[2] Phillips, Jr., G.N.,[1] Schmidt, M.[2]

1. Department of BioSciences, Rice University, Houston, Texas 77005, USA; 2. Physics Department, University of Wisconsin, Milwaukee, Milwaukee, Wisconsin 53206, USA; 3. Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287, USA 4. Department of Applied and Engineering Physics, Cornell University, Ithaca, New York 14853, USA 5. Center for Free-Electron Laser Science, DESY, Hamburg, Germany 6. SLAC National Accelerator Laboratory, Menlo Park, California 94025, USA 7. Hauptman-Woodward Institute, Buffalo, New York 14203, USA 8. BioXFEL National Science Foundation Science and Technology Center, Buffalo, New York 14203, USA 9. Seoul National University, Seoul, South Korea 10. Lawrence Livermore National Laboratory, Livermore, California 94551, USA

The visualization of protein motions is one of the many exciting, cutting-edge uses of an X-ray free electron laser. Ever since the first structure of an enzyme was solved, the discovery of the mechanism and dynamics of reactions catalyzed by biomolecules has been the key goal for the understanding of the molecular processes that drive life on earth at the atomic scale. Despite a large number of successful methods for trapping reaction intermediates, the direct observation of an ongoing reaction at runtime has been possible only in rare and exceptional cases. Here, we demonstrate a general method for capturing enzyme catalysis "in action" by "mix-and-inject serial crystallography". Specifically, we follow the catalytic reaction of the Mycobacterium tuberculosis beta-lactamase with the 3rd generation antibiotic ceftriaxone by time-resolved serial femtosecond crystallography. The results reveal, in near atomic detail, antibiotic cleavage and inactivation on the millisecond to second time scales including the crossover from transition state kinetics to steady-state kinetics. In a widely collaborative effort involving the BioXFEL science and technology center, these results show promise towards structure-based enzymology using an XFEL. We hope that our work will enable mix-and-inject serial crystallography for time-resolved studies of a broad variety of interesting enzymes.

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Enzyme intermediates captured “on the fly” by mix-and-inject serial crystallography. *BMC Biology* 2018 **16**:59 https://doi.org/10.1186/s12915-018-0524-5

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Thomas Szyperski & Surya Pulavarti, SUNY Buffalo

NMR meets XFEL

Shibom Basu, European Molecular Biology Laboratory

Serial crystallography data analysis at the Swiss Lightsource

Shibom Basu[1,2], Jakub W. Kaminski[1], Ezequiel Panepucci[1], Chia-Ying Huang[1], Rangana Warshamanage[1], Meitian Wang[1] and Justyna Aleksandra Wojdyla[1]

[1]Swiss Light Source, Paul Scherrer Institute, 5232 Villigen PSI, Switzerland
[2]European Molecular Biology Laboratory (EMBL), 71 Avenue des Martyrs, 38000 Grenoble, France

At the Swiss Light Source (SLS) macromolecular crystallography (MX) beamlines, the collection of serial crystallography (SSX) diffraction data includes micro-crystals (≥ 10-µm) being delivered via solid supports (e.g., mesh-loop or chip) as well as injectors (Weinert et al., 2017). When the crystals are mounted on solid supports, small wedges of rotation data (i.e., so-called minisets) of typically 10-20° are collected. This advanced data collection strategy from fixed target microcrystals is generally known as serial synchrotron crystallography (SSX) (Diederichs & Wang, 2017). A recently developed *in meso in situ* serial crystallography (IMISX) plate is an alternative to the standard fixed target technique, in which membrane proteins are crystallized in a glass sandwich (Huang *et al.*, 2015, 2016). The IMISX plate allows crystallization of membrane proteins in the lipidic cubic phase (LCP) and collection of SSX data from crystals in native condition without the need for crystal harvesting.

Our SSX data collection is facilitated by the recent *DA+* data acquisition and analysis software developments. The *SSX suite* allows easy, efficient and high throughput measurements on a large number of crystals. The fast continuous diffraction-based two dimensional grid scan method allows initial location of microcrystals (Wojdyla et al., 2016). The *CY+ GUI* utility enables efficient assessment of a grid scan's analysis output and subsequent collection of multiple wedges of data (so-called minisets) from automatically selected positions in a serial and automated way. The automated data processing (*adp*) routines adapted to the SSX data collection mode provide near real time analysis for data in both CBF and HDF5 formats. The automatic data merging (*adm*) is the latest extension of the *DA+* data analysis software routines. It utilizes the *sxdm* (SSX data merging) package (Basu et al., accepted), which provides automatic online scaling and merging of minisets and allows identification of minisets subset resulting in the best quality of the final merged data. The results of both *adp* and *adm* are sent to the MX MongoDB database and displayed in the web-based tracker, which provides user with on-the-fly feedback about the experiment. In the talk, automatic SSX data collection, real-time analysis, and user-friendly data visualization will be discussed and opportunities for various types of SX data collection at the SLS will be highlighted.

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JOSE MARTIN-GARCIA, ARIZONA STATE UNIVERSITY

MONOCHROMATIC AND POLY-CHROMATIC SERIAL CRYSTALLOGRAPHY AT SYNCHROTRON SOURCES

J. Martin-Garcia 1,2, M-Y. Lee 1,2, D. Mendez 1,3, C. Li 1,3, L. Zhu 1,2, E. Chun 1,2, N. Stander 1,3, G. Subramanian 1,3, G. Nelson 1,3, H. Hu 3, J. Coe 1,2, N. Nagaratnam 1,2, S. Roy-Chowdhury 1,2, D. Kissick 5, A. Ishchenko 4, C. Conrad 1,2, G. Ketawala 1,2, D. James 6, Z. Dobson 1,2, S. Zhang 1,2, J. Zook 1,2, C. Ogata 5, N. Venugopalan 5, S. Xu 5, V. Srajer 8, R. Henning 8, J. Spence 1,3, U. Weierstall 1,3, P. Fromme 1,2, N. Zatsepin 1,3, R. Fischetti 5, V. Cherezov 4, W. Liu 1,2

1. Center for Applied Structural Discovery, Biodesign Institute, Arizona State University, Tempe, AZ, USA; 2. School of Molecular Sciences, Arizona State University, Tempe, AZ, USA; 3. Department of Physics, Arizona State University, Tempe, AZ, USA; 4. Department of Chemistry, University of Southern California, Los Angeles, CA, USA; 5. Advanced Photon Source, Argonne National Laboratory, Chicago, IL, USA; 6. Paul Scherrer Institute, Villigen, Switzerland; 7. Centre for Free-Electron Laser Science, Deutsches Elektronen-Synchrotron, Hamburg, Germany; 8. BioCARS, University of Chicago, IL, USA

Since the first successful serial crystallography (SX) experiment at a synchrotron radiation source, the popularity of this approach has continued to grow, showing that 3rd generation synchrotrons can be viable alternatives to scarce X-ray free electron laser sources. Here, we present injector-based SMX experiments carried out at the Advanced Photon Source (APS). Monochromatic beam experiments were conducted at the GM/CA 23-ID-D beamline. Micro-crystals of lysozyme, phycocyanin, human adenosine A_{2A} receptor (A_{2A}AR), and proteinase K were delivered to the X-ray beam suspended in lipidic cubic phase, or a high molecular weight PEO (MW=8,000,000), using the LCP injector. The structures of the A_{2A}AR, phycocyanin, proteinase K and lysozyme were determined to 3.1 Å, 3.1 Å, 2.65 Å, and 2.05 Å resolution, respectively. Pink beam experiments were conducted at the BioCARS beamline (14-ID-B) on micro-crystals of proteinase K and A_{2A}AR. The broad bandwidth (5%) yielded full reflections and thus only a few thousand diffraction patterns were required to build a complete dataset. The structures of proteinase K and A_{2A}AR were determined to 4.2 Å and 1.8 Å resolution using 24 and 4 consecutive 100 ps X-ray pulse exposures, respectively. Strong PK data were processed using existing Laue approaches, while weaker A_{2A}AR required an alternative data processing strategy. Monochromatic and pink beam experiments demonstrate the feasibility of serial data collection at the APS using micro-crystals. Upcoming developments in beamline optics will increase intensity by a factor of ten. In addition, the intensity will also be increased by another factor of ten from the planned APS-U upgrade enabled by the two orders of magnitude increase in Brightness. All these developments will enable the use of smaller micro-crystals as well as the SMX of larger macromolecules.

Grant Acknowledgement:

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Cornelius Gati, Stanford University

Structural Biology of G protein-coupled receptors: New opportunities from XFELs and Cryo-EM

Cornelius Gati

G protein-coupled receptors (GPCRs) are ubiquitous cellular gatekeepers and are involved in the regulation of virtually every physiological process in the human body. Due to their biomedical relevance, GPCRs are targeted by a major share of therapeutic drugs and pose as attractive targets for structure-based drug design. Recent breakthroughs in cryo-electron microscopy and X-ray free electron lasers have accelerated structural studies of difficult-to-crystallize receptors and their signaling complexes, and have opened up new opportunities in understanding conformational dynamics and visualizing the process of receptor activation with unprecedented spatial and temporal resolution. In this presentation, I will summarize major milestones in the field of GPCR structural biology, challenges associated with the application of these techniques and outline future directions in their development with a main focus on membrane protein structural biology.

A. Ishchenko, C. Gati, V. Cherezov, Structural biology of G protein-coupled receptors: new opportunities from XFELs and cryoEM. Current Opinion in Structural Biology, Vol 51, Aug 2018, p 44-52

Vignesh Kasinath, University of California-Berkeley

Epigenetic silencing by PRC2: Visualizing cofactor mediated regulation by Cryo-EM

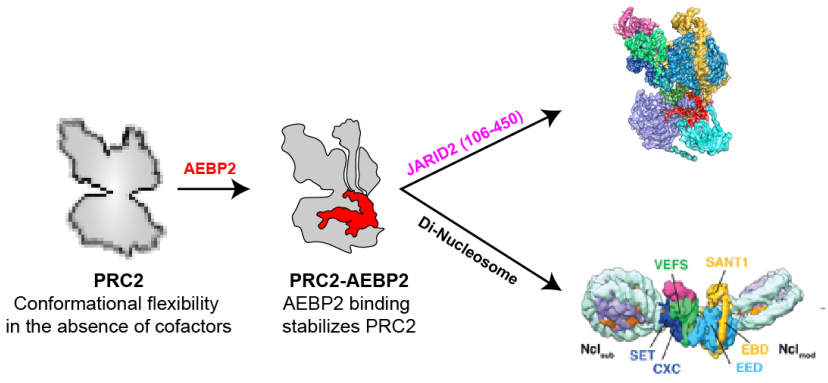
Vignesh Kasinath[1], Simon Poepsel[1], and Eva Nogales[1,2,3]

[1]QB3 Institute, Department of Molecular and Cellular Biology, University of California, Berkeley, California 94720, USA
[2]Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, USA
[3]Howard Hughes Medical Institute, University of California, Berkeley, California 94720, USA

One of the fundamental questions in biology is to understand how cells with identical set of genes differentiate to form diverse cell types, capable of highly specialized function. Polycomb group proteins are transcriptional repressors controlling gene expression patterns and play a pivotal role in cell differentiation and maintaining cell type identity. The chemical modifications of histones and DNA caused by the regulated activity of chromatin-modifying enzymes such as Polycomb help establish and maintain such expression patterns. Polycomb repressive complex 2 (PRC2) is the only known methyltransferase specific for histone H3 lysine 27 (H3K27) and catalyzes its trimethylation leading to the repressive H3K27me3 mark. PRC2 associates with several cofactors *in vivo* that modulate its activity and aid in its recruitment to chromatin. The best characterized cofactors are AEBP2 and JARID2, which coexist in PRC2 complexes. Mechanistically, two aspects need to be addressed in order to understand PRC2 function:

Regulation of PRC2 methyltransferase activity by cofactors JARID2 and AEBP2
Interactions of PRC2 with chromatin resulting in the local spreading of repressive H3K27me3 mark

Here, we have obtained cryo-electron microscopy (cryo-EM) structures of the human PRC2 bound to cofactors JARID2, AEBP2 in two different active states (*1*). In addition, we have also obtained a cryo-EM structure of PRC2 bound to bifunctional di-nucleosome substrate (*2*). These structures, in combination with cross-linking mass spectrometry and biochemical studies, provide detailed insight into the interaction landscape of core PRC2 components, cofactors and chromatin substrates. We find that both cofactors mimic histone H3 tails in their interaction with PRC2, and synergistically stimulate PRC2 activity using distinct mechanisms. We show that the geometry of PRC2: di-nucleosome interaction allows PRC2 to simultaneously interact with histone H3 tail from both nucleosomes, providing a structural basis for the local spreading of H3K27me3 repressive mark.



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Thomas White, DESY

Horror stories from serial crystallography data processing and how CrystFEL can help

White, T. A.[1]

1. Center for Free-Electron Laser Science, Deutsches Elektronen-Synchrotron DESY, Notkestraße 85, 22607 Hamburg, Germany.

Data processing methods for serial crystallography have reached a point where the large volumes of data involved can be handled in a routine manner, with the task of indexing, integrating and merging the diffraction patterns sometimes seen (wrongly, in our opinion!) as something of a chore. CrystFEL [1] has a key role in the routine handling of large serial crystallography data sets, as the most widely-used software for this purpose. Alongside efforts to steadily increase the data quality from any given data set by using, amongst others, better methods for modelling the underlying diffraction physics [2], our aim is to make CrystFEL handle as many scenarios as possible with minimal intervention from the scientist.

However, the real world finds ways to defeat the best laid plans, and over the years a number of "interesting" situations have emerged which needed special handling. These "horror stories" include unfamiliar types of indexing ambiguity, subtleties of facility data storage formats, and outlier measurements due to calibration problems. Though they may need special attention, these cases have led to improvements in our understanding of how best to process data, many of which have been encoded into CrystFEL. This contribution will describe some of these cases, and how you can avoid getting fooled by some of the more subtle crystallographic pitfalls.

Acknowledgements: Code has been contributed to CrystFEL by Valerio Mariani, Helen Ginn, Oleksandr Yefanov, Yaroslav Gevorkov, Steve Aplin, Nicholas Riebesel, Thomas Grant, Mamoru Suzuki, Keitaro Yamashita, Takanori Nakane, Parker de Waal, Wolfgang Brehm, Alexandra Tolstikova, Fedor Chervinskii, Cornelius Gati, Anton Barty, Karol Nass, Nadia Zatsepin, Chun Hong Yoon, Lorenzo Galli, Andrew Martin, Andrew Aquila, Rick Kirian and Kenneth Beyerlein. The design of CrystFEL and its algorithms has been influenced by Henry Chapman, Petra Fromme, James Holton, John Spence and many others. Development of CrystFEL is primarily funded by the Helmholtz Association via programme-oriented funds. Additional funding for CrystFEL is provided by "X-Probe", a project of the European Union's 2020 Research and Innovation Program under the Marie Skłodowska-Curie grant agreement 637295 (2015-2018), and by the BMBF German-Russian Cooperation "SyncFELMed", grant 05K14CHA (2014-2017). Past funding for CrystFEL has been received from BioStruct-X, a project funded by the Seventh Framework Programme (FP7) of the European Commission.

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Ti-Yen Lan, Princeton University

Analysis of sparse serial microcrystal diffraction data collected at a storage-ring

The success of serial femtosecond crystallography at free electron lasers has motivated the adaptation of serial crystallography experiments at existing storage-ring sources because of their wider availability. In order to keep the crystals within the safe radiation dose, however, data frames collected from small crystals are usually discarded because the resolvable Bragg peaks in these `sparse' frames are insufficient for the indexing softwares to determine the crystal orientations. As a result, proteins not bound up in large crystals are wasted for the purpose of structure determination. Using the expand-maximize-compress (EMC) algorithm [1], we have developed an analysis method to solve the protein structure from such discarded sparse frames. This talk will demonstrate this approach through the analysis of sparse crystal diffraction patterns collected at a storage-ring source. It is seen that the protein structure solved from the sparse frames is able to reach comparable quality to that solved from the strong, indexable frames [2].

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Joe Chen, Arizona State University

Shape transform phasing

J. P. J. Chen[1], J. J. Donatelli[2,3], K. E. Schmidt[1] and R. A. Kirian[1]

[1]Department of Physics, Arizona State University, Tempe, AZ, 85287, USA
[2]Department of Applied Mathematics, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA
[3]Center for Advanced Mathematics for Energy Research Applications, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA

We present an interesting algorithmic leap towards the long-standing idea that the crystallographic phase problem in protein crystallography can be solved directly, from the diffracted intensities alone, if intensities between Bragg reflections can be measured.

The idea of recovering the structure of a molecule from measurements of the diffracted intensity between Bragg peaks was put forth over 60 years ago by David Sayre [1], and in 2009 such a diffraction signal was measured clearly for the first time using micron-sized crystals illuminated by an x-ray free-electron laser [2]. In the years that followed, many other datasets were observed to have similar "shape transform" signals between Bragg reflections. Attempts at direct phasing of those datasets have been made, but none have been successful so far, with the exception of experiments performed on artificial, inorganic crystals [3]. The reason for the lack of experimental results on protein crystals is that, for crystals smaller than the coherence width of the x-ray beam, the molecular transform is strongly dependent on how the molecules are arranged at the edge of the crystal, which can be modelled by the occupancy of the molecules at their lattice points. This counterintuitive "edge-truncation problem" is an important obstacle that needs to be overcome for shape transform phasing to succeed [4-7].

In this presentation we discuss a new phase retrieval algorithm that is able to reconstruct the electron density of a molecular asymmetric unit given the averaged coherent diffracted intensity from many crystals of different sizes and shapes, and unlike previous works, we allow for totally arbitrary molecular occupancies. This algorithm solves the edge -truncation problem entirely and constitutes the first time that the shape transform phasing method could be seen to work in principle.

Acknowledgment

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Sebastian Günther, DESY

Roadrunner III & IV: High-speed fixed-target sample delivery

S. Günther[1], P. Fischer[1], T. Pakendorf[1], M. Barthelmess[1], O. Yefanov[1], A. Tolstikova[1], V. Hennicke[1], J. Meyer[2], J. Urbschat[2], J. Lieske[1], G. Kipp[1], M. Liang[3], M. Hunter[3], A. Batyuk[3], S. Boutet[3], M. Levantino[4], H. Chapman[1], and A. Meents[1]

[1] Center for Free Electron Laser Science, DESY, Notkestrasse 85, 22607, Hamburg, Germany.
[2] Deutsches Elektronen Synchrotron, Photon Science, Notkestrasse 85, 22607, Hamburg, Germany.
[3] Linac Coherent Light Source, SLAC National Accelerator Laboratory, Menlo Park, CA 94025, USA.
[4] European Synchrotron Radiation Facility, 71 Avenue des Martyrs, 38000, Grenoble, France.

Reliable sample delivery has remained a challenge for serial crystallography and imaging experiments at XFELs. In particular jet based systems require large amount of samples and the hit rates are typically low. An alternative approach is fixed-target sample delivery, where the sample is immobilized on a solid support, which is then raster-scanned through the X-ray beam [1,2].

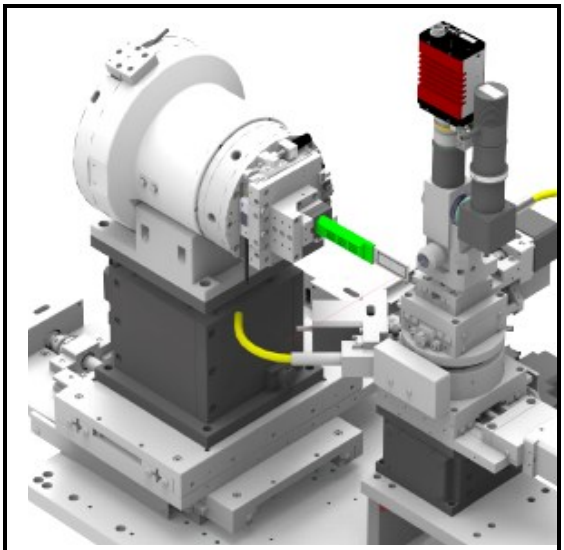

Figure 1: Roadrunner III goniometer for high-speed fixed target serial crystallography experiments.

We have developed the Roadrunner III and IV goniometers for high-speed fixed target diffraction experiments at XFELs and synchrotrons with sample exchange rates of up to 1 kHz. The goniometers are built in a modular way and both feature a high-resolution inline microscope for X-ray beam and sample visualization. Main element is a high precision rotation axis equipped with a centering stage and a very fast linear motor driven translation stage in its center for sample scanning with speeds of up to 100 mm/s.

The Roadrunner III goniometer is designed for serial crystallography experiments at atmospheric pressure for fast and easy sample exchange (figure 1). Using a local helium atmosphere in combination with the concept of the capillary beamstop allows achieving very low background scattering levels [3]. Data collection for a complete structure determination with about 10000 indexed diffraction patterns requires the measurement of one chip and can be performed in about 10 minutes. The setup is further well suited for laser pump-probe experiments.

The Roadrunner IV (In-Vacuum) goniometer is designed for in-vacuum diffraction experiments of mainly non-crystalline samples. Samples are ideally placed on windows in the solid supports, which are covered with ultrathin graphene or amorphous carbon layers. For in-vacuum measurements currently only dried samples or samples sandwiched between to protective layers can be measured.

The presentation will cover technical aspects of the goniometers itself, describe sample preparation and loading procedures for both goniometer types and will provide recent applications of the Roadrunner III and IV goniometers.

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DOMINIK OBERTHUER, CFEL

IMPROVING AND RE-INVENTING SAMPLE DELIVERY FOR MINIMAL SAMPLE CONSUMPTION AND TIME-RESOLVED SFX

An overview will be given on recent developments in XFEL- and synchrotron based serial crystallography sample delivery concepts. Methods for more efficient sample delivery methods for time resolved crystallography and fragment-based drug design will be presented and discussed, as well as limitations and future prospects.

JURAJ KNOSKA, CFEL

NOVEL 3D PRINTED MIXERS AND INJECTORS FOR TIME-RESOLVED STRUCTURAL BIOLOGY

Time-resolved serial crystallography (TR-SX) provides insights into the dynamics of life on an atomic level. To date, most TR-SX experiments use a pump-probe scheme, where a reaction is triggered by a light pulse. However, most biochemical reactions are not triggered by light but are initiated by binding. Mix-and-inject TR-SX approach exploits substrate diffusion into sample crystals for reaction initiation. We employ two-photon polymerization for sub-micron precise 3D printing of microfluidic mixers and nozzles for efficient mixing by placing static mixing elements into the flow path of liquids. These static elements introduce stirring of the fluids by splitting and stretching process. We image the mixing process and benchmark our mixers at various flow conditions and diffusion coefficients using X-ray microtomography. Additionally, we explore different nozzle tip designs for reliable generation of thin (low background) and fast (megahertz SX) liquid jets. We have used these injectors successfully during mix-and-inject serial crystallography experiments at X-ray free-electron lasers.

Martin Trebbin, University at Buffalo

Microfluidic reaction control for time-resolved structure determination at XFELs and synchrotrons

Diana C. F. Monteiro[1], Godfrey Beddard[2] and Martin Trebbin[3,*]

University of Hamburg, Centre for Ultrafast Imaging (CUI), D-22761, Germany.
University of Edinburgh, School of Chemistry, EH9 3FJ, UK
Department of Chemistry, The State University of New York at Buffalo, 760 Natural Sciences Complex, Buffalo, New York 14260-3000, USA

A driving question in time-resolved structural biology is to unravel the structure-function relationships of bio macromolecules on the molecular level. [1] However, time-resolved serial femtosecond crystallography experiments are challenging to design and execute due to the requirement for synchronous triggering of protein molecules across the microcrystal before exposure to the X-ray pulse for diffraction. For high-resolution to be achieved, complete triggering of the molecules, either by light-induced changes or by delivery of ligands by rapid-mixing, is a required key element. [2] Since only a handful of interesting biological targets are naturally photoactivatable, the fast delivery of ligands by rapid-mixing offers a powerful alternative and widely applicable route to triggering for investigating a broad range of biological processes in small crystals *in situ*. To address this challenge, [3,4] we developed microfluidic rapid mixing sample environments allowing for mixing times within 100s of microseconds with a very well-defined time-zero point and short dead time from mixing to the X-ray probe. These mixing times have been determined using finite-element method-based computational fluid dynamic simulations (CFD, see Fig.1). Using our three-dimensional two-phase flow liquid jet CFD-model, we could track the concentration field of along the flow lines of tracer particles in a time-resolved flow simulation to determine the precise mixing times. These results have been confirmed experimentally by fluorescence microscopy using rapid dye quenching. The here described principles and insights for time-resolved experiments, reaction triggering and (3D-) microchannel design can also be applied for analyzing, optimizing and triggering the chemical kinetics of other samples, such as the controlled synthesis of polymers and micro-/nanoparticles.

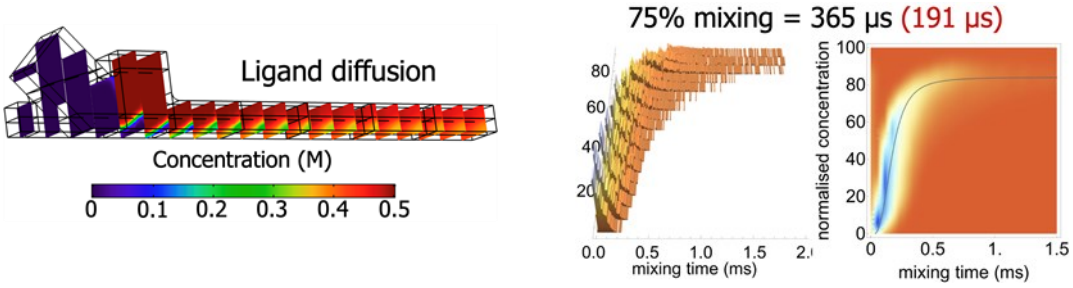

Fig. 1. TR-CFD simulation of microfluidic rapid mixing geometry. (*Top*) 3D-flow simulation of interdiffusion and (*bottom*) the resulting mixing time distribution

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