AGENDA

Monday, January 27th, 2020

3:00 PM - onwards — **Arrival and Hotel Check-in** [Click Here for Map from Airport]
Caribe Hilton
1 Calle San Geronimo
San Juan, PR 00901

8:30 AM - 4:00 PM — **Scientific Advisory Board Meeting—Conference Rooms 8-10**

8:30 AM - 4:30 PM — **Write Winning Grant Proposals Workshop—San Geronimo B**

7th BioXFEL International Conference

Tuesday, January 28th, 2020

8:30 AM – 8:40 AM — **Conference Welcome - San Geronimo B**
Conference Co-Chairs: Emina Stojkovic (NEIU) and Marius Schmidt (UWM)

8:40 AM – 11:00 AM — **Spectroscopy—San Geronimo B**
Session Chairs: Sarah Bowman (HWI) & Lysmarie Santos Velazquez (UPR)

8:40 AM – 9:00 AM — John Kennis (Vrije Universiteit Amsterdam)
Photoactive yellow protein in crystal and solution phases show distinct rates and intermediates

9:00 AM – 9:10 AM — Discussion

9:10 AM – 9:30 AM — Roseanne Sension (U Michigan)
Femtosecond X-ray absorption reveals excited state structural dynamics in B12 coenzymes

9:30 AM – 9:40 AM — Discussion

9:40 AM – 10:00 AM — Till Stensitzki (FU-Berlin)
Comparison of the initial photoreaction of Bacteriorhodopsin in microcrystals and in solution by fs-pump-probe spectroscopy

10:00 AM – 10:10 AM — Discussion

10:10 AM –10:40 AM — **Coffee Break**

10:40 AM – 11:40 AM — **Methods I—San Geronimo B**
Session Chairs: George Phillips (Rice) & Hazel Borges (UPR)

10:40 AM – 11:00 AM — Yaroslav Gevorkov (CFEL)
Two new indexing algorithms for Serial Crystallography

11:00 AM – 11:10 AM — Discussion

11:10 AM – 11:30 AM — Kanupriya Pande (LBL)
Recent developments in fluctuation X-ray scattering at X-FELs

11:30 AM – 11:40 AM — Discussion

11:40 AM – 12:45 PM **LUNCH GENERAL GROUP—LAS OLAS**

11:40 AM - 12:45 PM **LUNCH ASSESSMENT MEETING FOR BIOXFEL POSTDOCTORAL SCHOLARS—SALON DEL MAR**

11:40 AM – 12:45 PM **LUNCH PRESENTATION ON EDUCATIONAL OPPORTUNITIES FOR TRAVEL SCHOLARS - CONFERENCE ROOMS 8-10**

12:45 PM - 2:15 PM **KEYNOTE—SAN GERONIMO B**
NICOLE WOITOWICH (NORTHWESTERN UNIVERSITY)
JEANNE GARBARINO (ROCKEFELLER UNIVERSITY)
Cultivating an inclusive community through science outreach
Session Chair: Emina Stojkovic (NEIU) and Josue Benjamin (UPR)

2:15 PM - 2:45 PM **POSTDOCTORAL AWARD RECIPIENT TALK: AHMAD HOSSEINIZADEH (UWM) - SAN GERONIMO B**
Atomic-level structural dynamics with femtosecond time-resolution
Session Chairs: Edward Snell (HWI) & Jessika Pazol (UPR)

2:45 PM – 5:00 PM **METHODS I (CONTINUED) - SAN GERONIMO B**
Session Chairs: George Phillips (Rice) & Hazel Borges (UPR)

2:45 PM – 3:05 PM Elspeth Garman (Oxford)
RADDOSE-XFEL: Femtosecond time-resolved dose estimates for macromolecular XFEL experiments
3:05 PM – 3:15 PM Discussion

3:15 PM– 3:35 PM Guillermo Calero (U Pittsburgh)
Transcription with a Laser: Towards a Molecular Movie of Nucleotide Addition
3:35 PM – 3:45 PM Discussion

3:45 PM – 4:15 PM **COFFEE BREAK**

4:15 PM – 4:35 PM Sabine Botha (ASU)
Selecting the optimal data for Single-wavelength anomalous dispersion phasing for Serial Millisecond Snapshot Crystallography
4:35 PM – 4:45 PM Discussion

4:45 PM – 5:00 PM Saha Sarthak (U of Massachusetts-Amherst)
X-ray Compatible Microfluidics for Advanced Protein Crystallography
5:00 PM – 5:05 PM Discussion

5:05 PM — 5:30 PM **POSTER BLITZ—SAN GERONIMO B**
Session Chair: Petra Fromme

7:00 PM - 9:00 PM **POSTER SESSION—SAN GERONIMO ABC**

WEDNESDAY, JANUARY 29TH, 2020

8:30 AM – 11:10 PM **METHODS II—SAN GERONIMO B**
Session Chairs: Lois Pollack (Cornell) & Rafael Maldonado-Hernandez (UPR)

8:30 AM – 8:50 AM Chris Milne (PSI)
SwissFEL: First experiments and future outlook
8:50 AM – 9:00 AM Discussion

9:00 AM – 9:20 AM Peter Schwander (UWM)
Single-particle diffraction with the X-Ray free electron laser: new opportunities to study structure and function in biology
9:20 AM – 9:30 AM Discussion

9:30 AM – 9:45 AM Saminathan Ramakrishnan (NCI)
Visualizing ligand triggered conformation change and phase transitions in riboswitch crystals using Atomic Force Microscopy
9:45 AM – 9:50 AM Discussion

9:50 AM – 10:15 AM **COFFEE BREAK**

10:15 AM – 10:30 AM Amit Samanta (DESY)
Creating and controlling cryogenically-cooled beams of shock-frozen, isolated, biological and artificial nanoparticles
10:35 AM – 10:40 AM Discussion

10:40 AM - 11:00 AM Cecilia Casadei (PSI)
Recent developments in computational methods for two-dimensional serial femtosecond crystallography: paving the way to the time-resolved study of large-scale movements in membrane proteins
11:00 AM - 11:10 AM Discussion

11:10 AM - 11:30 AM Abbas Ourmazd (UWM)
What can we learn from Machine Learning?
11:30 AM - 11:40 AM Discussion

11:40 AM – 12:30 PM **LUNCH GENERAL GROUP—LAS OLAS**

11:40 PM – 12:30 PM **LUNCH ASSESSMENT MEETING FOR NEW BIOXFEL GRADUATE STUDENTS (AS OF 2017) - SALON DEL MAR**

12:30 PM – 3:20 PM **TR– SFX—SAN GERONIMO B**
Session Chairs: Keith Moffat (U Chicago) & Frances Heredia (UPR)

12:30 PM – 12:50 PM Michael Thompson (UCSF)
Turning up the heat on dynamic proteins with multi-temperature and temperature-jump structural biology
12:50 PM – 1:00 PM Discussion

1:00 PM – 1:20 PM Kyle Sutherlin (LBL)
The water oxidation reaction in natural photosynthesis
1:20 PM – 1:30 PM Discussion

1:30 PM – 1:50 PM Denis Rousseau (Albert Einstein)
Snapshot of an oxygen intermediate in the catalytic reaction of Cytochrome C Oxidase
1:50 PM – 2:00 PM Discussion

2:00 PM – 2:30 PM COFFEE BREAK

2:30PM – 2:50 PM George Phillips (Rice)
The study of phytochrome photoconversion by XFEL and trapped synchrotron methods
2:50 PM – 3:00 PM Discussion

3:00 PM – 3:15 PM Henrike Müller-Werkmeister (University of Potsdam)
Watching an enzyme at work: Time-resolved serial crystallography with fixed targets and photocaged substrate from milliseconds to seconds
3:15 PM – 3:20 PM Discussion

6:00 PM DINNER - BEACH AREA

THURSDAY, JANUARY 30, 2020

9:00 AM – 12:00 PM POSTER SESSION CLEAN UP
*If you do not remove your poster you risk it being thrown away.

9:15 AM – 11:35 AM PHOTORECEPTORS—SAN GERONIMO B
Session Chair: Henrike Müller-Werkmeister (Potsdam) &
Jose Julian Del Toro Dominguez(UPR)

9:15 AM – 9:35 AM Sebastian Westenhoff (Gothenburg) c/o Emina Stojkovic (NEIU)
Photoactivation of bacterial phytochromes studied by time-resolved crystallography
9:35 AM – 9:45 AM Discussion

9:45 AM – 10:05 AM Suraj Pandey (UWM)
Time-resolved serial femtosecond crystallography at the European XFEL
10:05 AM – 10:15 AM Discussion

10:15 AM – 10:40 AM COFFEE BREAK

10:40 AM – 11:00 AM	Valerie Panneels (PSI) Mammalian rhodopsin dynamics using an X-ray free electron laser
11:00 AM – 11:10 AM	Discussion
11:10 AM – 11:30 AM	Tobias Weinert (PSI) Cryo-crystallography is dead—long live dynamic serial crystallography
11:30 AM – 11:40 AM	Discussion
11:45 PM - 12:00 PM	**GROUP PHOTO**
12:00 PM	**LUNCH—LAS OLAS**
1:00 PM – 3:00 PM	**BIOXFEL STC MEETING - FOR CENTER MEMBERS ONLY - SAN GERONIMO B**

KEYNOTE SPEAKERS

NICOLE WOITOWICH (NORTHWESTERN UNIVERSITY) & JEANNE GARBARINO (THE ROCKEFELLER UNIVERSITY)

CULTIVATING AN INCLUSIVE COMMUNITY THROUGH SCIENCE OUTREACH

While science outreach is typically contextualized as a valid strategy to promote broad, accessible engagement with science, the full value and impact of science outreach on research communities is yet to be fully realized. When executed effectively, science outreach can help individuals and organizations craft a tailored set of culturally inclusive best practices for improving the clear and present equity gaps within the scientific enterprise, while also serving as a path to gain broad support of scientific research through funding, policy, or other relevant avenues. Yet, institutions of science are slow to invest in the centralization of science outreach, cementing into place a set of barriers for the many scientists who wish to participate in community engagement activities.

In this informational workshop, we will define the core elements of the science outreach framework, and how it relates to building more culturally inclusive communities in science. With this foundation, workshop participants will take part in small group discussions to identify key lessons from social justice case studies, and spend time thinking about how to bring elements of the science outreach framework into their own work.

BIOGRAPHIES

Nicole C. Woitowich, PhD, Associate Director, Center for Reproductive Science, Feinberg School of Medicine, Northwestern University

Nicole (Niki) Woitowich, PhD, is the Associate Director of the Center for Reproductive Science at Northwestern University. She is actively transforming the landscape of women's health through her research, advocacy, and outreach activities. While formally-trained as a biochemist, her current research focuses on the evaluation of the biomedical research enterprise through the lense of sex- and gender-inclusion. She has significant expertise in science policy and public engagement with science, and as such, was named a Presidential Management Fellow in 2016 and currently serves as chair for the American Society for Biochemistry and Molecular Biology – Science Outreach and Communication Committee.

Jeanne Garbarino, PhD, Director of RockEDU Science Outreach, The Rockefeller University

Jeanne is the Director of RockEDU Science Outreach at The Rockefeller University. Along with the RockEDU team, she works to promote and support science outreach within the scientific community, open channels for community members to develop an appreciation for science as a human endeavor, and to provide equitable access to scientific resources and opportunities that genuinely reflect the process of science. She is deeply interested in how to build meaningful professional connections within the science outreach and engagement professional space, and to promote the continued professionalization of the science outreach field. Before becoming a practitioner of science outreach, Jeanne kicked off her scientific career as a lipid biochemist, earning her PhD in metabolic biology from Columbia University, then conducting postdoctoral studies on cholesterol transport at Rockefeller. You can find Jeanne on Twitter, Instagram, and in the woods (whenever possible).

John Kennis (Vrije Universiteit Amsterdam)

Primary isomerization reactions in Photoactive Yellow Protein and solution phases show distinct rates and intermediates

John T.M. Kennis[1], Patrick E. Konold[§1], Enis Arik[§1], Jörn Weissenborn[1], Jos C. Arents[2], Klaas J. Hellingwerf[2], Ivo H.M. Van Stokkum[1], Marie Louise Groot[1]

[1]Department of Physics and Astronomy and LaserLaB, Faculty of Science, Vrije Universiteit, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands

[2]Laboratory for Microbiology, Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park, 1098 XH Amsterdam, The Netherlands

Femtosecond time-resolved crystallography (TRC) on proteins enables resolving the spatial structure of short-lived intermediates populated during biological signaling and catalysis. An open question is whether confinement and lower hydration of the proteins in the crystalline state affect the structural events. Here, we measured the full photocycle dynamics of a signal transduction protein often used as model system in TRC, Photoactive Yellow Protein, in the crystalline state and compared those to the dynamics in solution, utilizing electronic and vibrational transient absorption measurements from 100 femtoseconds over 12 decades in time. We find that the photocycle kinetics and structural dynamics of PYP in the crystalline form deviate from those in solution, from the very first steps following photon absorption. This work illustrates that TRC results must be considered with utmost caution when extrapolated to *in vivo* function.

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Roseanne Sension (University of Michigan)

Femtosecond X-ray absorption reveals excited state structural dynamics in B12 Coenzymes

Department of Chemistry, University of Michigan, Ann Arbor, MI 48109 USA

The fate of a photoactive molecule is determined by the electronic and structural rearrangements that follow excitation. Femtosecond (fs) X-ray free electron lasers (XFELs) have made it possible to use X-ray absorption spectroscopy to probe changes in electronic configuration and atomic structure as a function of time, beginning from the initial excited state. Both 'movies' of coherent or ballistic motion and 'snaphots' of local minima or kinetic intermediates are possible. Polarization anisotropy, long exploited in ultrafast optical measurements, permits decomposition of the X-ray transient difference signal into contributions along the direction parallel to the transition dipole initially pumped, and perpendicular to this transition dipole. This decomposition allows the analysis of asymmetric sequential structural changes of photoexcited molecules in isotropic solution.

We have used femtosecond X-ray absorption near edge structure (XANES) at the Co K-edge to characterize the excited state dynamics of B_{12} coenzymes, adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl) (Figure 1). These data are compared with measurements on several other B_{12} compounds with varied upper axial ligands. The initial subpicosecond dynamics are found to be ballistic rather than kinetic, with sequential structural changes in the plane of the corrin ring first polarized in the x-direction, then in the y-direction, followed by expansion of the axial bonds perpendicular to the ring (the z-direction). A change in electronic configuration accompanies the axial bond elongation as the molecule evolves from the initial 'bright' (i.e. fluorescent) state to a 'dark' state within a few hundred femtoseconds. Subsequent structural intermediates prior to bond dissociation or ground state recovery are also identified and characterized.

The Finite Difference Method Near Edge Structure (FDMNES) program is used to calculate the XANES spectrum for both the ground and excited states. The FDMNES method is used for simulations because it avoids the muffin tin approximation of FEFF and provides reasonable results for cobalamin ground states. The simulations are used to extract more detailed static and dynamical information from the time- and polarization-resolved XANES difference spectra.

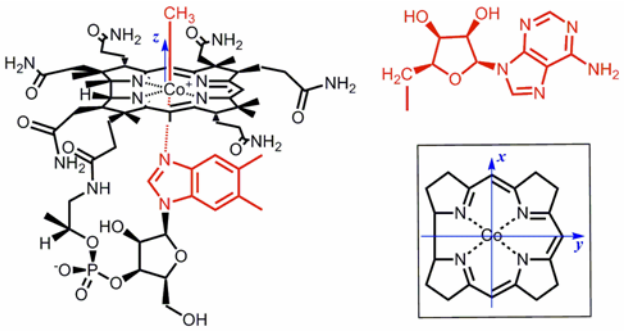

Figure 1. Schematic structure of methylcobalamin indicating the directions assigned as x, y, and z. For adenosylcobalamin the methyl group is replaced by a 5'-deoxyadenosyl group, shown to the right.

Acknowledgments

This work was supported by the NSF through NSF-CHE 1464584 and NSF-CHE 1836435. Use of the Linac Coherent Light Source (LCLS), SLAC National Accelerator Laboratory, is supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. DE-AC02-76SF00515. In addition, many collaborators at Michigan (A. Deb, K.J. Kubarych, A. Kanishiro, J. Meadows, L. B. Michocki, N. A. Miller, J.E. Penner-Hahn, D. L. Sofferman) the University of Louisville (B. Garbato, M. Toda, P.M. Kozlowski) and at LCLS (R. Alonso-Mori, A. Britz, D. DePonte, J. M. Glownia, J. Koralek, S. Song, T.B. van Driel, D. Zhu) contributed to the success of this work.

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Ahmad Hosseinizadeh (U of Wisconsin-Milwaukee)

Atomic-level structural dynamics with femtosecond time-resolution

A. Hosseinizadeh, R. Fung, P. Schwander, M. Schmidt, and A. Ourmazd

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

Understanding the atomic-level structural dynamics of photo-activated biological processes is of fundamental interest, and may lead to the ultimate control of such processes. Using a collection of diffraction snapshots from a serial femtosecond X-ray scattering experiment with ~100fs time resolution[1], we have determined the photon-induced femtosecond structural dynamics of the Photoactive Yellow Protein, which involves a trans-to-cis isomerization. Our results include molecular movies of the isomerization reaction with femtosecond time- and 1.6A spatial-resolution.

1. K. Pande et al., Science **352** (6268), 725-729 (2016).

This research was supported by the US Department of Energy, Office of Science, Basic Energy Sciences under award DE-SC0002164, and by the US National Science Foundation under award STC 1231306. We also acknowledge scientific contributions from R. Sepehr.

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Yaroslav Gevorkov (CFEL)

Two new indexing algorithms for Serial Crystallography

Yaroslav, G[1,2], Yefanov, O.[1], Barty, A.[1], White, T.A.[1], Mariani, V.[1], Brehm, W.[1], Tolstikova A.[1], Wiedorn, M.O.[1,3,4], Meents, A.[1], Grigat, R.R.[2] and Chapman, H.N.[1,3,4]

1. Center for Free-Electron Laser Science, Deutsches Elektronen-Synchrotron DESY, Notkestraße 85, 22607 Hamburg, Germany; 2. Vision Systems, Hamburg University of Technology, 21071 Hamburg, Germany; 3. Department of Physics, Universität Hamburg, Luruper Chaussee 149, 22761 Hamburg, Germany; 4. The Hamburg Center for Ultrafast Imaging, Universität Hamburg, Luruper Chaussee 149, 22761 Hamburg, Germany.

Indexing is a key step in the serial crystallography data analysis pipeline. In serial crystallography, hundreds or thousands still diffraction patterns from randomly oriented crystals are merged to form a complete data set. To process the data, the diffraction patterns must first be indexed, which is equivalent to determining the orientation of each crystal. Only successfully indexed diffraction patterns can be utilized by further stages of the data processing pipeline. We present two novel automatic indexing algorithms that usually significantly increase the indexing rates over current state-of-the-art programs.

The first algorithm, XGANDALF [1], is an extension of the well-known Fourier methods. It allows better indexing rates and better refinement, while being fast enough for real-time analysis. The algorithm does not require prior knowledge of the lattice parameters but can make use of that information if provided. Cases with a small number of Bragg spots per pattern appear to particularly benefit from the new approach. Importantly, the algorithm performs well on diffraction patterns generated by several crystals in the beam enabling the indexing of multi-crystal hits.

The second algorithm, pinkIndexer [2], can be used in a variety of contexts including measurements made with a monochromatic radiation source, a polychromatic source, or with radiation of very short wavelength. As such, the algorithm is particularly suited to automated data processing for two emerging measurement techniques for macromolecular structure determination: serial pink-beam X-ray crystallography and serial electron crystallography, which until now lacked reliable programs for analysing many individual diffraction patterns from crystals of uncorrelated orientation. The algorithm requires approximate knowledge of the unit-cell parameters of the crystal, but not the wavelengths associated with each Bragg spot.

Both algorithms are implemented in an open source C++ library and distributed under the LGPLv3 license. An interface to it has been added to the CrystFEL software suite.

[1] Gevorkov, Yaroslav, et al. "XGANDALF–extended gradient descent algorithm for lattice finding." Acta Crystallographica Section A: Foundations and Advances 75.5 (2019): 694-704.

[2] Gevorkov, Yaroslav, et al. "PinkIndexer - A universal indexer for pink-beam X-ray and electron diffraction snapshots." Acta Crystallographica Section A: Foundations and Advances (accepted)

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Kanupriva Pande (LBL)

Recent Developments in Fluctuation X-ray scattering at x-fels

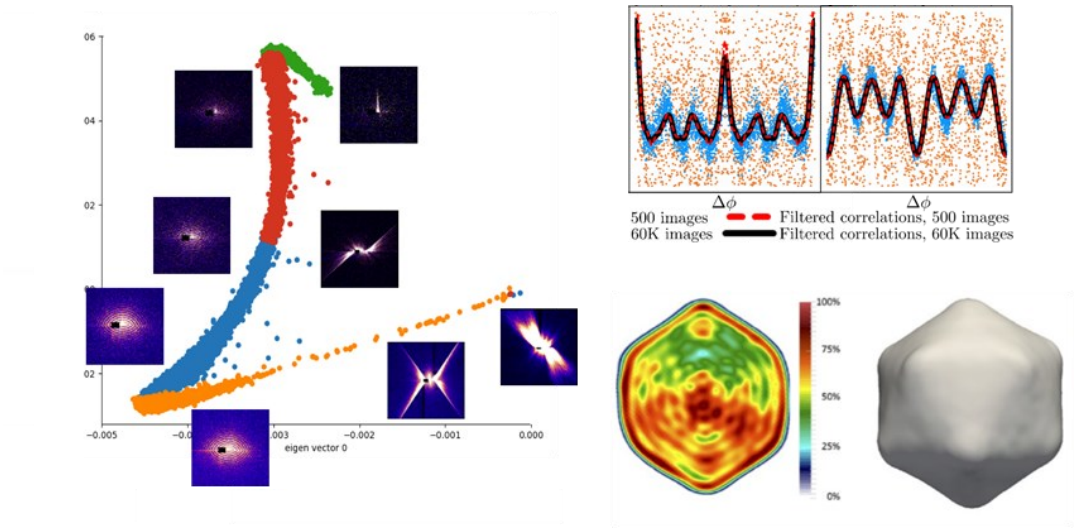
K. Pande[1,2], J. J. Donatelli[2,3], C.Gati[4], M. Hunter[4], R. A. Kirian[5], P. H. Zwart[1,2]

1. Molecular Biophysics and Integrated Bio-Imaging Division, LBNL, Berkeley, CA, USA; 2. Center for Advanced Mathematics for Energy Research Applications, LBNL, Berkeley, CA, USA; 3. Department of Mathematics, Computational Research Division, LBNL, Berkeley, CA, USA; 4. SLAC National Accelerator Laboratory, Menlo Park, CA, USA; 5. Arizona State University, Tempe, AZ, USA

Fluctuation X-ray scattering (FXS) is a biophysical technique that overcomes the low data- to-parameter ratios encountered in traditional X-ray scattering methods used for studying non- crystalline samples [1]. In an FXS experiment solution scattering data are collected from particles in solution using ultrashort X-rays of pulse lengths shorter than the rotational diffusion time of the particles. The resulting data contains angularly varying information that yields structures with a greater level of detail than those obtained using tradition SAXS [2].

After successful application of FXS to single-particle data [3], we have recently demonstrated the experimental feasibility of this technique on data from an ensemble of PBCV-1 virus particles in solution [4, 5]. We found that by using advanced noise-filtering methods the required number of images to obtain decent correlation data is far lower than originally expected, thereby reducing the required data collection time to less than a few minutes. In this talk I will outline the data processing techniques for analysis of FXS data, and present an assessment of the effect of concentration and various sources of noise on experimental data.

This research was supported by the Advanced Scientific Computing Research and the Basic Energy Sciences programs of the Office of Science of the US Department of Energy (DOE), and the National Institute of General Medical Sciences of the National Institutes of Health (NIH).



References

1. Kam Z, Koch MH, Bordas J. (1981). PNAS 78 (6), 3559–62.
2. Donatelli JJ, Zwart PH, Sethian JA. (2015). PNAS 112 (33), 10286–10291.
3. Kurta RP, et al. (2017). Phys Rev Lett., 119 (15), 158102.
4. Pande K, Donatelli JJ, Malmerberg E, Foucar L, Bostedt C, Schlichting I, Zwart PH. (2018). PNAS, 114 (46), 11772-11777.
5. Pande K, et al. (2018). Scientific Data, 5, 180201.

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Elspeth Garman (University of Oxford)

RADDOSE-XFEL: Femtosecond time-resolved dose estimates for macromolecular XFEL experiments

Garman, E.F., McCubbin, P.T.N., Dickerson, J.L.
Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, UK

For macromolecular structure determination at synchrotron sources, radiation damage remains a major limiting factor. Estimation of the absorbed dose (J/kg) during data collection at these sources by programs such as RADDOSE-3D has allowed direct comparison of radiation damage between experiments carried out with different samples and beam parameters. This has enabled prediction of roughly when radiation damage will manifest so it can potentially be avoided. X-ray free electron lasers (XFELs), which produce intense X-ray pulses only a few femtoseconds in duration, can be used to generate diffraction patterns before most of the radiation damage processes have occurred, and hence hypothetically enable the determination of damage free atomic resolution structures. In spite of this, several experimental and theoretical studies have suggested that structures from XFELs are not always free of radiation damage. There are currently no freely available programs designed to calculate the dose absorbed during XFEL data collection, as such a code would need to track photons and photoelectrons on the femtosecond timescale. We present here an extension to RADDOSE-3D [1,2] called RADDOSE-XFEL [3], which uses Monte Carlo simulations to calculate the time-resolved dose during XFEL experiments. As a result of neglecting energy deposited after the end of the pulse, RADDOSE-XFEL produces much lower dose estimates than those calculated by previous methods. We anticipate that RADDOSE□XFEL could be used to facilitate the study of radiation damage at XFELs and ultimately be used prior to data collection so that experimenters can plan their experiments to avoid radiation damage manifesting in their structures.

References

[1] RADDOSE-3D: time- and space-resolved modeling of dose in macromolecular crystallography. Zeldin, O. B., Gerstel, M. & Garman, E. F. (2013). *J. Appl. Crystallogr.* **46**, 1225–1230.
[2] Estimate your dose: RADDOSE-3D. Bury, C. S., Brooks-Bartlett, J. C., Walsh, S. P. & Garman, E. F. (2018). *Protein Sci.* **27**, 217–228.
[3] RADDOSE-XFEL: Femtosecond time-resolved dose estimates for macromolecular XFEL experiments. Dickerson, J.L., McCubbin, P.T.N. & Garman, E.F. *J. Appl. Crystallogr.* (submitted)

Grant information:
We thank the Oxford Laidlaw Scholars Programme (JLD) and the EPSRC Vacation Placement Programme (PTNM) for funding this work.

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Guillermo Calero (University of Pittsburgh)

Transcription with a laser: Towards a molecular movie of Nucleotide addition

Lin G[1*], Weiss S[1*], Aina Cohen[3], Kaplan C[#2] and **Calero G[#1]**

[1] Department of Structural Biology, University of Pittsburgh School of Medicine

[2] Department of Biological Sciences, University of Pittsburgh

[3] Structural Molecular Biology, Stanford Synchrotron Radiation Light Source, Stanford University

DNA-directed RNA Polymerase II (Pol II) is a highly-conserved protein among eukaryotic organisms and plays a fundamental role in cellular life, specifically the transcription of genes into messenger RNA (mRNA). Structural studies of Pol II, traditionally X-ray but more recently single particle cryo-electron microscopy have provided snapshots of initiation, elongation, and interactions with factors, DNA damage, and chromatin. Due to the nature of previous experiments (structural snapshots) and limited resolution, mechanisms of Pol II catalysis and the role of the essential "trigger loop" (TL, Rpb1 residues 1077-1096) have been limited. Specifically, by the inability to capture the TL in its closed or "on" conformational state, which to date has been achieved in essentially two datasets published in 2006. In this seminal work, the TL is stabilized in the closed conformation by the presence of a template-specified matched nucleotide. This structure represented a clear breakthrough in our knowledge of the mechanisms of transcription. However, this study was limited by the low resolution of the data (4.0 Å). Furthermore, electron density for the two catalytic Mg^{2+} ions (A- and B-site) was not clearly discernable, meaning the catalytic conformation has not yet been directly observed. Using a newly discovered crystallization condition, and an automated protocol for fixed goniometer free electron laser data collection, we have obtained a radiation-damage free dataset of wild-type Pol II (WT) at 3.0 Å, which is the highest resolution of WT elongation complex yet recorded. Our experiments show *the first static picture* of a pre-inserted nucleotide in the catalytic pocket; the TL in the "on" conformation; strong electron density for sites A and B metals; and a putative third Mg^{2+} (C-site) never before observed. Given that our crystallization condition allowed consistent visualization of all the structural elements involved in the process of transcription by Pol II, we performed time resolved X-ray crystallographic experiments *to elucidate the time evolution* of the molecular events during Pol II nucleotide addition cycle. Our data shows the first molecular movie of Pol II in the act of transcription.

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Sabine Botha (Arizona State University)

Selecting the optimal data for Single-wavelength anomalous dispersion phasing in Serial Millisecond Snapshot Crystallography

Sabine Botha[1,2], Natasha Stander [1,2,3], José Martin-Garcia[2,3], Hao Hu[1,2], Uwe Weierstall[1,2], Martin Fuchs[4], Wuxian Shi[4], Babak Andi[4], John Skinner[4], Hebert Bernstein[5], Petra Fromme[2,3], John Spence[1] Nadia Zatsepin[1, 2, 6]

1. Department of physics, Arizona State University, Tempe, AZ 85287, USA; 2. Biodesign Center for Applied Structural Discovery, Arizona State University, Tempe, AZ 85287, USA; 3. School of Molecular Sciences, Arizona State University, Tempe, AZ 85287, USA; 4. Energy & Photon Sciences Directorate, Brookhaven National Laboratory, Upton, NY 11973, USA; 5. School of Chemistry and Materials Science, Rochester Institute of Technology, Rochester, NY, USA; 6. Department of Chemistry and Physics, La Trobe University, Melbourne, 3086, Victoria, Australia

Serial femtosecond crystallography (SFX) is an up and coming method for room-temperature protein structure determination and it has been shown that SFX data can be phased *de novo* [1]. This method of data collection has been adapted for synchrotron sources, termed serial millisecond crystallography (SMX) [2]. SMX substantially reduces radiation damage incurred by the individual protein crystals compared to conventional, oscillation data collection approaches, facilitating room-temperature structure solution from micrometer sized crystals at synchrotrons. However, *de novo* phase retrieval remains difficult and is rarely applied to serially-collected SFX and SMX data.
Using DatView [3], a graphical user interface for data visualization that allows users to quickly see trends, correlations, and statistics in large, multi-dimensional datasets such as unmerged serial crystallography (SMX and SFX) datasets, we explore the factors influencing the anomalous signal for single-wavelength anomalous dispersion (SAD) *de novo* phasing of SMX data collected on model system microcrystals in a systematic study at NSLS-II, delivered in a lipidic cubic phase (LCP) jet.

DatView is written in python and uses PyQt, matpotlib, NumPy, SciPy, h5py, pyqtgraph, and lxml.
It is freely available from ***https://github.com/nstander/DatView****, with documentation at* ***https://zatsepinlab.atlassian.net/wiki/spaces/DAT/overview****.*

Acknowledgements:
This research was supported by NSF award #1565180 and BioXFEL STC Award #1231306. AMX (17-ID-1) and FMX (17-ID -2) beamlines are supported by the NIH National Institute of General Medical Sciences (P41GM111244), and by the DOE Office of Biological and Environmental Research (KP1605010), and the NSLS-II at BNL is supported by the DOE Office of Basic Energy Sciences (DE-SC0012704(KC0401040)). We would further like to acknowledge Stu Myers and Jean Jakoncic.

References:
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[2] Martin-Garcia J. M. et al., IUCrJ (2017); 4:4
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Cecilia Casadei (PSI)

Recent developments in computational methods for two-dimensional serial femtosecond crystallography: paving the way to time-resolved study of large-scale movements in membrane proteins

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Serial diffraction images can be recorded from radiation-sensitive membrane protein two-dimensional (2D) crystals using ultra-short and ultra-bright free electron laser X-ray pulses focused to the sub-µm and a low background environment. The interest in this exotic and demanding data collection mode resides in that membrane proteins arranged periodically in a monolayer maintain their physiological dynamics.

A dedicated processing pipeline was developed for the analysis of serial femtosecond crystallography (SFX) data from 2D crystals. 2D-SFX data present common features with well established methods, in particular serial crystallography from three-dimensional crystals and 2D electron diffraction. Yet there are intrinsic differences with each of these techniques, requiring the development of customized code. On one hand, unlike diffraction intensities from 3D crystals, 2D-SFX intensities are continuous in the out-of-plane direction of reciprocal space. On the other hand, the need of merging techniques that account for indexing ambiguity in serial images complicates the analysis with respect to single-crystal methods[1]. Our processing method deals with such peculiarities and includes an algorithm that allows to extend the resolution limit of the usable data by improving the signal to noise ratio of the measured intensities, which is inherently poor due to the weak scattering power of monolayers[2].

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Abbas Ourmazd (University of Wisconsin-Milwaukee)

What can we learn from Machine Learning?

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The advent of high repetition-rate XFELs is generating a torrent of data. Will machine learning conquer the deluge?

Machine learning, a branch of Artificial Intelligence, perform tasks typically reserved for humans. Most machine-learning tasks involve some kind of "recognition". Examples include recognizing individuals (facial recognition), obstacles (self-driving vehicles), or patterns (stock-market fluctuations).

Recognition tasks are, in essence, labeling exercises. Recognizing a face, for example, involves attaching a name to it. Most machine-learning approaches, such as "Deep Learning", provide little or no insight into the principles by which the labels are generated. The ability to perform a task does not require understanding the underlying processes. You do not have to understand the workings of the brain to recognize your spouse.

Scientific knowledge, in contrast, entails understanding the underlying processes. A deep understanding of facial recognition, for example, must elucidate the structures and processes by which the brain recognizes faces.

Traditionally, scientific understanding proceeds by assimilating a few experimental clues into a (mathematically sound) theory. This theory is then buttressed by a succession of carefully designed observations. Such discovery processes are designed to make the best use of limited data. The data deluge is undermining this approach.

I will describe how machine learning can help extract scientific understanding from the data deluge.

This work was supported by the US Department of Energy, Office of Science, Basic Energy Sciences under award DE-SC0002164 (underlying dynamical techniques), and by the US National Science Foundation under award STC 1231306 (underlying data analytical techniques).

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Chris Milne (Paul Scherrer Institute)

SwissFEL: First experiments and future outlook

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The Swiss X-ray free electron laser SwissFEL[1] (Paul Scherrer Institute, Villigen, Switzerland) recently completed its first year of user operation. This seminar will present an overview of the project, how it fits into the current global XFEL landscape, and some details on the research that has been performed since it achieved first light in 2017, with specific examples presented of serial femtosecond crystallography and ultrafast photobiology experiments. Finally, an outlook for the future will be presented with details on the planned soft X-ray branch and experimental stations that will come online in the coming years.

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Peter Schwander (University of Wisconsin-Milwaukee)

Single-Particle Diffraction with the X-Ray Free Electron Laser: New opportunities to study structure and function in biology

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The invention of the X-Ray Free Electron Laser (XFEL) enabled to collect diffraction patterns from individual biomolecules. The obvious advantage is that no crystals are required. But the true potential of single-particle diffraction unfolds in the presence of structural variability, associated with biological function. Manifold-based machine learning applied to a large ensemble of single-particle snapshots allows to reveal the concerted structural changes exercised by the sample. This enables to map conformational spectra and energy landscapes, determine functional pathways and compile 3D molecular movies. The quality of such analysis depends largely on the number of snapshots that can be collected during an experiment. This sets challenging requirements for sample delivery, beamline alignment and data acquisition. With the new generation of high-repetition XFEL, high-speed X-ray detectors and advances in sample injection, we expect to collect tens of millions of snapshots during a single shift. This could allow to access the rare, high-energy conformations close to transition states. Performing single-particle diffraction in time-resolved mode can further advance these opportunities for understanding biological function and bring structural biology to a new level.

This work was funded by the US National Science Foundation STC 1231306.

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Michael Thompson (UC-San Francisco)

Turning up the heat on dynamic proteins with multi-temperature and temperature-jump structural biology

Thompson, M.C.[1], Wolff, A.M.[2], Nango, E.[3,4], Kubo, M.[5], Young, I.D.[1], Cho, H.S.[6], Nakane, T.,[7] Sugahara, M.[3,4], Tanaka, R.[3,4], Ito, K.[8], Brewster, A.S.[9], Sierra, R.G.[10], Schotte, F.[6], Yumoto, F.[11], Nomura, T.[3], Owada, S.[3], Hino, T.[12], Tosha, T.[3], Tanaka, T.[3,4], Im, D.[4], Aquila, A.[10], Carbajo, S.[10], Koralek, J.[10], Yamashita, A.[3], Luo, F.[13], Boutet, S.[10], Sauter, N.K.[9], Tono, K.[14], Iwata, S.[3,4], Anfinrud, P.A.[6], and Fraser, J.S.[1.]

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Protein dynamics are critical for function, but it remains challenging to understand, in atomic detail, how a molecule's biological activity is enabled by the physical coupling of its conformational fluctuations across varied length and time scales. In this seminar, I will describe how temperature- and time-dependent X-ray crystallographic measurements of molecular structure can overcome some of the limitations of traditional structural biology and yield deep insight into protein conformational landscapes by providing additional experimental variables that can be manipulated. I will demonstrate how multi-temperature crystallography experiments can yield information about the link between a protein's function and its conformational ensemble under physiological conditions, describe how observations from multi-temperature structural measurements motivated the development of time-resolved temperature-jump (T-jump) crystallography, and summarize the results of our early T-jump experiments. Finally, I will discuss ongoing efforts to improve the quantity and quality of structural information that can be extracted from the combination of temperature perturbations with X-ray crystallography, as well as efforts to apply these experiments to increasingly complex biological systems.

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Kyle Sutherlin (Lawrence Berkeley National Laboratory)

The water oxidation reaction in natural photosynthesis

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Many of the catalytic reactions in inorganic systems and natural enzymes involve multiple electrons and proceed through several intermediate steps. For example, photosynthetic water oxidation in nature is catalyzed by the metal center that consists of oxo-bridged four Mn and one Ca atoms, which is located in multi-subunit membrane protein, Photosystem II (PSII). This is one of the most important, life-sustaining chemical processes occurring in the biosphere. The oxygen-evolving complex (OEC) in PSII, which contains the heteronuclear Mn_4CaO_5 cluster, catalyses the reaction

$$2H_2O \rightarrow O_2 + 4e^- + 4H^+$$

that couples the four-electron oxidation of water with the one-electron photochemistry occurring at the PSII reaction center. The OEC cycles through five intermediate S-states (S_0 to S_4) corresponding to the abstraction of four successive electrons from the OEC. Once four oxidizing equivalents are accumulated (S_4-state), a spontaneous reaction occurs that results in the release of O_2 and the formation of the S_0-state.

Recently, the development of X-ray Free Electron Lasers (XFELs) has opened up opportunities for studying the dynamics of biological systems. Intense XFEL pulses enable us to apply both X-ray diffraction and X-ray spectroscopic techniques to dilute systems or small protein crystals. By taking advantage of ultra-bright femtosecond X-ray pulses, one can also collect the data under functional conditions of temperature and pressure in a time-resolved manner, after initiating reactions, and follow the chemical dynamics during catalytic reactions and electron transfer. Such an approach is particularly beneficial for biological materials and aqueous solution samples that are susceptible to X-ray radiation damage.

We have developed the spectroscopy and diffraction techniques necessary to fully utilize the capability of the XFEL x-rays for a wide-variety of metalloenzymes, like Photosystem II, and to study their chemistry under functional conditions. One such is the simultaneous data collection for x-ray crystallography and x-ray spectroscopy, which allows us to look at overall structural changes of proteins and chemical changes at metal catalytic sites. We have used this technique to study the water oxidation reaction of Photosystem II, in which the Mn_4CaO_5 cluster catalyzes the reaction. The current status of this research and the mechanistic understanding of the water oxidation reaction based on the X-ray techniques is presented.

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Denis Rousseau, Albert Einstein College of Medicine

Snapshot of an oxygen intermediate in the catalytic reaction of Cytochrome C Oxidase

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Cytochrome *c* oxidase (C*c*O), the terminal enzyme in the electron transfer chain, translocates protons across the inner mitochondrial membrane by harnessing the free energy generated by the reduction of oxygen to water. Time-resolved
spectroscopic studies indicate that the dioxygen reduction reaction has several intermediates and follows a sequential mechanism. However, how the oxygen reaction is coupled to proton translocation remains unproven. By using time-resolved serial femtosecond crystallography, we trapped and identified a key oxygen intermediate of bovine C*c*O. It is assigned to the **PR**-intermediate, which is characterized by specific redox states of the metal centers and a distinct protein conformation. The heme *a*3 iron atom is in a ferryl ($Fe^{4+}=O^{2-}$) configuration and heme *a* and CuB are oxidized while CuA is reduced. A Helix-X segment is poised in an open conformational state; the heme *a* farnesyl sidechain is H-bonded to S382; and Loop-I-II adopts a distinct
structure. These data offer new insights into the mechanism by which the oxygen chemistry is coupled to unidirectional proton translocation.

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George Phillips (Rice University)

The study of phytochrome photoconversion by XFEL and trapped synchrotron methods

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A major barrier to defining the structural intermediates that arise during the reversible photo-interconversion of phytochromes between their biologically inactive and active states has been the lack of crystals that faithfully undergo this transition within the crystal lattice. Here, we describe a crystalline form of the cyclicGMP phosphodiesterases/adenylyl cyclase/FhlA (GAF) domain from the cyanobacteriochrome PixJ in *Thermosynechococcus elongatus* assembled with phycocyanobilin that permits reversible photoconversion between the blue light-absorbing Pb and green light absorbing Pg states, as well as thermal reversion of Pg back to Pb. Cryo-crystallography at 150 K, which compared diffraction data from a single crystal as Pb or after illumination with blue light, detected photoconversion product(s) based on Fobs − Fobs difference maps that were consistent with rotation of the bonds connecting pyrrole rings C and D. Further spectroscopic analyses showed that phycoviolobilin is susceptible to X-ray radiation damage, especially as Pg, during single-crystal X-ray diffraction analyses, which could complicate fine mapping of the various intermediate states. Fortunately, we found that PixJ crystals are amenable to serial femtosecond crystallography (SFX) analyses using XFELs. As proof of principle, we solved by room temperature SFX the GAF domain structure of Pb to 1.55-Å resolution, which was very similar to synchrotron-based models. Analysis of these crystals by SFX should now enable structural characterization of the early events that drive phytochrome photoconversion.

Another barrier to visualizing dynamics is the occurrence of mixtures of states in crystals during time resolved experiments. Sometimes this problem can be overcome by trapping states at low temperatures and slowly raising the temperature to reveal different intermediate populations. Quenching to cryo temperatures after fixed time delays can also be used as a surrogate for time-resolved experiments. These experiments have the advantage of decoupling the diffraction experiment from the biological or chemical reaction, allowing normal synchrotron beamlines to be used for dynamics experiments, as well as the ability to collect full datasets on orders of magnitude less sample. Both of these methods, pump-quench and temperature-scan crystallography, have been used to study photoconversion of PixJ. We have modified a temperature switcher system to incorporate light pulses, allowing us to trap illuminated states of PixJ between 200ms and 10s time delays, allowing us to cover the late intermediates in the photoconversion pathway. By illuminating the PixJ crystals in a scan of temperatures below the glass transition, we have trapped intermediates that we believe correspond to the fast occurring states in the photoconversion pathway. These two experiments in tandem display the whole photoconversion pathway of PixJ.

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Sebastian Westenhoff

Photoactivation of bacterial phytochromes studied by time-resolved crystallography

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Eye-less species use photosensor proteins to collect information about ambient light conditions. Phytochromes are a photosensor superfamily in plants, fungi, bacteria. Upon photoactivation of a biliverdin cofactor, the chromophore and protein undergo a series of structural changes on multiple time- and length scales in order alter the biochemical output activity. The structures of the resting and light-activated states of bacteriophytochromes are known, but the structural mechanism with which light cues are transferred into structural rearrangements are not well understood. In particular, the primary structural response of the chromophore and the surrounding residues remains elusive.

Here, we present a crystallographic investigation of the phytochrome from *D. Radiodurans*. We solved a new room-temperature structure obtained by serial femtosecond X-ray crystallography at the Japanese X-ray free electron laser. We also present a time-resolved snapshot of the protein at 1 ps and discuss its implication for the primary photoresponse of phytochrome proteins.

The snapshot confirms rotation of the D-ring and reveals a concomitant 'liberation' of the chromophore from the protein scaffold. Surprisingly, we find displacement of the pyrrole water at 1ps, which leads to displacement of several conserved amino acids, which point towards the main signaling route, the PHY tongue. We suggest that the pyrrole water plays a hitherto unrecognized role in the photochemistry of bacteriophytochromes.

The results provide a structural basis for understanding the primary photoresponse of phytochromes.

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HAIGUANG LIU (CSRC)

OBSERVING THE MOTION OF CL-IN RHODOPSIN BY TIME RESOLVED SFX AND MOLECULAR DYNAMICS SIMULATIONS

Haiguang Liu[1],Ji-Hye Yun[2], Xuanxuan Li[1], Jae-Hyun Park[2], Zeyu Jin[2], Yingchen Shi[1], Chufeng Li[3], Hao Hu[3], Yang Wang[1], Suraj Pandey[4], Sergio Carbajo[5], Nadia Zatsepin[3], Uwe Weierstall[3], Mark Hunter[5], Meng Liang[5], TJ Lane[5], Chun Hong Yoon[5], Raymond Sierra[5], Wenkai Zhang[6], Marius, Schmidt[4], Weontae Lee[2]

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The chloride ion pumping rhodopsin was studied with the X-ray lasers and supercomputers, to understand the molecular mechanism. The chloride ion pumping rhodopsin (ClR) utilizes energies from light to actively transport Cl- ions through membranes. In this study, we determined atomic structures of ClR at several time point after photoactivation at room temperature using serial femtosecond X-ray Crystallography (SFX) method, using pump-probe method. The femtosecond optical pumping laser and XFEL pulses allow the study of dynamics from sub-picosecond to 100 picosecond range. The dynamics emerged mainly as retinal isomerization, chloride ion movement, and the re-arrangement of retinal neighbouring amino acids. Four buried water molecules were also observed in the crystal structures to be closely coupled with the chloride ion pumping. We found that the Cl- ion pumping process was initiated by retinal isomerization, then a movement of Cl- ion away from the Schiff base, followed by a motion towards the cytoplasmic side of the membrane. Combining the results from time-resolved SFX and atomic MD simulations, we offer the first 3D molecular movie, describing the early stage dynamics of the chloride ion being transported through ClR molecule.

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Suraj Pandey (University of Wisconsin-Milwaukee)

Time-resolved serial femtosecond crystallography at the European XFEL

Suraj Pandey[1], Richard Bean[2], Tokushi Sato[2], Ishwor Poudyal[1], Johan Bielecki[2], Jorvani Cruz Villarreal[3], Oleksandr Yefanov[4], Valerio Mariani[4], Thomas A. White[4], Christopher Kupitz[5], Mark Hunter[5], Mohamed H. Abdellatif[4], Saša Bajt[6], Valerii Bondar[2], Austin Echelmeier[3], Diandra Doppler[3], Moritz Emons[2], Matthias Frank[7], Raimund Fromme[3], Yaroslav Gevorkov[4,8], Gabriele Giovanetti[2], Man Jiang[2], Daihyun Kim[3], Yoonhee Kim[2], Henry Kirkwood[2], Anna Klimovskaia[2], Juraj Knoska[4], Faisal H. M. Koua[4], Romain Letrun[2], Stella Lisova[9], Luis Maia[2], Victoria Mazalova[4], Domingo Meza[10], Thomas Michelat[2], Abbas Ourmazd[1], Guido Palmer[2], Marco Ramilli[2], Robin Schubert[10], Peter Schwander[1], Alessandro Silenzi[2], Jolanta Sztuk-Dambietz[2], Alexandra Tolstikova[4], Henry N. Chapman[4,11,12], Alexandra Ros[3], Anton Barty[4], Petra Fromme[3], Adrian P. Mancuso[2,13], Marius Schmidt[1]

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The advent of X-ray Free Electron Lasers (XFEL) has started a new era in Macromolecular Crystallography. Previously impossible reaction dynamics between macromolecules can now be explored by using time-resolved serial femtosecond crystallography (TR-SFX) at XFELs. In TR-SFX, a reaction in microcrystals is initiated with an optical laser pulse during sample delivery in the X-ray interaction region and the progress of the reaction is probed after a certain time delay by the pulsed X-ray beam. TR-SFX has been pioneered at XFELs with X-ray pulse repetition rates between 30 and 120 Hz. Similarly, TR-SFX has the potential to take advantage of the megahertz pulse rate at the European XFEL (EuXFEL) to structurally map multiple states of a reaction with a single experiment.

The EuXFEL near Hamburg, Germany, produces femtosecond, ultra-brilliant and spatially coherent X-ray pulses. At the EuXFEL, X-rays arrive in pulse trains repeating at 10 Hz. Each train consists of bursts of X-ray pulses with an intra-train rate of up to 4.5 MHz. Due to the MHz pulse repetition rates, an experiment at the EuXFEL can be challenging. Here, we investigate the picosecond time range in the photocycle of photoactive yellow protein (PYP). We show that difference electron density maps of excellent quality can be obtained. Results connect the previously explored femtosecond dynamics of PYP to time scales accessible at synchrotrons. This opens the door to a wide range of time-resolved studies at the EuXFEL.

Reference:

Pandey, S., Bean, R., Sato, T. et al. Time-Resolved Serial Femtosecond Crystallography at The European XFEL. Nat Methods (2019) DOI:10.1038/s41592-019-0628-z

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Valerie Panneels

Mammalian rhodopsin dynamics using pump probe serial femtosecond crystallography

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Mammalian Rhodopsin, a prototype of the largest druggable G Protein-Coupled Receptors family (GPCRs), is our light receptor for night vision. Upon photon absorption, its chromophore 11-cis retinal undergoes one of the fastest events in biology, which happens in the femtosecond range, the isomerisation into all-trans. The whole rhodopsin photoactivation process lasts over about 10 orders of magnitude on a logarithmic time scale, until the coupling to the G protein transducin occurs. Our study aims at determining the structure of rhodopsin intermediates using time-resolved serial femtosecond crystallography (TR-SFX), which has been successfully used for the prokaryotic proton pump bacteriorhodopsin [1-3]. Rhodopsin microcrystals grown in the dark are successively injected in the light of a pump laser and directly probed after various time-delays from femtoseconds to milliseconds using an X-ray free electron laser. Several 'static' structures of dark and active states of rhodopsin have been characterized by X-ray crystallography in cryogenic conditions [4]. However, obtaining high-resolution structures of photoactivated intermediates in a time-resolved manner and at room temperature would provide important insights on the detailed mechanism of rhodopsin activation, e.g. retinal isomerization, changes in protonation states, rearrangement of amino acid side chains and water molecules.

We have now prepared and characterized crystals of wild-type mammalian rhodopsin diffracting to a resolution of 2 Å. The crystals were obtained for the first time in a lipidic cubic phase, which offers various advantages, including an optimal constant speed of sample delivery. Pilot SFX tests at the SACLA (SPring-8 Angstrom Compact Free Electron Laser) and a TR-SFX test at the LCLS (Linac Coherent Light Source) showed a satisfactory hit rate. Data were collected at the SACLA and SwissFEL. A preliminary map shows, as a proof of principle, rhodopsin with the retinal in a batho conformation at the correctly earlier-predicted time-delay. Time-resolved serial femtosecond crystallography on rhodopsin will not only give details on the molecular activation of a class A GPCR, but will also give insights into the photophysical trigger of retinal excitation upon photon absorption.

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Tobias Weinert (Paul Scherrer Institute)

Cryo-crystallography is dead— Long live dynamic serial crystallography

Protein crystallography at cryogenic temperatures is currently one of the most successful scientific techniques at synchrotrons. However, highly challenging targets like big complexes or mammalian membrane proteins are now preferentially investigated using cryo-electron microscopy, freeing up beamtime and shifting the focus of synchrotrons worldwide. Time resolved crystallography (TRX) at synchrotrons has a long and successful history[1]. However, these classical methods are technically challenging and were never adopted by a large community. The widespread availability of fast detectors has now made it possible to achieve biologically relevant time resolutions in standard synchrotron beamlines. Serial crystallography invented for Linac Coherent Light Source (LCLS)[2] has removed the need to collect data on single crystals also at synchrotrons, overcoming experimental limitations that previously prevented dynamic studies of small protein crystals at ambient temperatures.

We implemented the high viscosity injector setup developed for LCLS[3] at a standard 3rd generation synchrotron beamline (X06SA at Swiss Light Source) that has a high frame rate EIGER 16M detector. We were able to show that most crystallographic experiments, including native-SAD phasing, can be readily carried out at ambient temperatures using our setup[4]. Ambient temperatures allow to study protein dynamics not only by TRX, but also by integrative structural approaches to reveal protein dynamics[5].

By supplementing our ambient temperature setup with a $200-laser diode, we were able to obtain excellent time-resolved data of the proton pump Bacteriorhodopsin with a time resolution of 5 ms. The data revealed large conformational changes and a water chain that allows re-protonation of the Schiff base and finalizes the pumping cycle[6]. Since the diode's implementation, we have used the setup with a large variety of targets, some of which we have also studied at XFELs.

Having simple dynamic serial crystallography setups at synchrotrons worldwide will allow users to prepare experiments, samples and spend precious beamtimes at the few XFELs most efficiently. Making TRX readily accessible to all structural biologists will shift the focus of TRX towards biological questions and expand the field. This will provide data and opportunities for standardization and improvement of the current methods.

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Saminathan Ramakrishnan (National Cancer Institute)

Visualizing ligand triggered conformation change and phase Transitions in riboswitch crystals using atomic force microscopy

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Martensitic phase transition in crystals is an important phenomenon in material science and biology. Particularly, solid to solid (SS) phase transition in crystals have critical biological functions, for instance docking of T4 bacteriophage on the bacterial host and motion control in bacteria. However, the martensitic transition has been studied extensively only in organic, inorganic materials and polymers. Here we present a new approach to record time-lapsed ligand induced phase transition in adenine riboswitch crystals using Atomic Force Microscopy (AFM). Under optimized ligand concentration and temperature, the crystal lattice transition from initial apo state to trans state and final bound state was recorded along with multiple intermediate structures at the rate of 23 seconds per frame and 100nm scan size. Moreover, our time-resolved serial femtosecond crystallography (SFX) data obtained using an XFEL, which also illustrate the phase transition in adenine riboswitch crystals, are consistent with our AFM observations. AFM data could provide a preliminary understanding of the phase transition, and observed lattice parameters throughout the transition could be useful in the SFX data analysis.

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Amit Samanta (DESY)

Creating and controlling cryogenically-cooled beams of shock-frozen, isolated, biological and artificial nanoparticles

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Single-particle diffractive imaging (SPI) is emerging as a new technique for 3D imaging of aerosolized nanoparticles at x-ray free-electron lasers (XFELs). However, one of the primary bottlenecks in realizing SPI is the efficient delivery of isolated, reproducible target particles into the x-ray focus [1]. Here, we present novel approaches for the production of cold and high-density beams [2] of a broad variety of biological nanoparticles, ranging from single-domain proteins, including membrane proteins, to multi- subunit protein complexes and molecular machines, designed for use in XFEL experiments. This will also enable us to gain a better understanding of the ultrafast dynamics across extended biological systems. Fast freezing from ambient temperature to 10 K in less than 10 µs will help freezing room-temperature equilibrium state distribution and even trapping reaction intermediates.

Furthermore, we have developed a numerical simulation infrastructure that allows quantitative simulation of isolated particle trajectories throughout the setup [3]. This allowed us to improve injection geometries and build aerosol injection systems optimized for specific particle sizes in order to produce the highest-density particle beams [2, 4]. We propose an optimised setup with cooling rates for few- nanometers particles on nanoseconds timescales. The produced beams of shockfrozen, isolated nanoparticles provide a breakthrough in sample delivery, e.g., for diffractive imaging and microscopy or low-temperature nanoscience. The produced cryogenically-cooled particle beams can subsequently be further manipulated and controlled using electric [5] or optical fields, such as hollow-core vortex laser beams [6].

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Henrike Müller-Werkmeister (University of Potsdam)

Watching an enzyme at work: Time-resolved serial crystallography with fixed targets and photocaged substrate from milliseconds to seconds

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To fully capture the dynamics and structural changes during biochemical reactions over several orders of magnitude in time, is a major experimental challenge and became recently accessible by time- resolved serial femtosecond crystallography (TR-SFX), driven by the availability of XFEL sources.

The sample delivery tools originally developed for those studies at XFELs on the ultrafast time scale [I. Martiel, H. M. Müller-Werkmeister, A. E. Cohen, *Acta Cryst. D*, 2019, D75, 160–177] start to inspire similar experiments at synchrotrons [R. L. Owen, D. Axford, D. A. Sherrell, A. Kuo, O. P. Ernst, E. C. Schulz, R. J. D. Miller, H. M. Müller-Werkmeister, *Acta Cryst. D*, 2017, D73, 373-378.], thereby enabling time- resolved serial synchrotron crystallography (TR-SSX), which allows the study of protein dynamics on time- scales >us at ambient conditions.

We have recently demonstrated the *hit-and return* (HARE) approach [E. C. Schulz*, P. Mehrabi*, H. M. Müller-Werkmeister*, F. Tellkamp, A. Jha, W. Stuart, E. Persch, R. De Gasparo, F. Diederich, E. F. Pai, R. J. D. Miller, *Nature Methods*, 2018, 15 (11), 901-904.], which uses fixed targets for sample delivery of up to 25.000 protein crystals on an individual crystallography chip and allows the efficient data collection for several time points during a single synchrotron beamtime. Using the HARE approach, we were able to follow the full reaction cycle of an enzyme, fluoroacetate dehalogenase, and captured 18 time points from 30 milliseconds to 30 seconds during the non-reversible turnover.

The experimental details reveal four catalytic turnovers and show the entire reaction mechanism, including the formation of the covalent intermediate. [P. Mehrabi*, E. C. Schulz*, R. Dsouza, H. M. Müller- Werkmeister, F. Tellkamp, R. J. D. Miller, E. F. Pai, *Science* 365 (6458), 1167-1170, 2019.] The time-resolved structures further reveal the allosteric mechanism leading to the previously observed half-the-sites reactivity. While the secondary structure shows no conformational changes during the enzymatic reaction, the local water structure both on the protein surface and at the dimer interface shows a strong asymmetry between the subunits, revealing a “molecular phone wire” of water molecules, which seem to transmit the allosteric signal.

Crucial for this experiment was the efficient triggering of the enzymatic reaction using a photocaged substrate. TR-SX experiments mainly focus on protein dynamics that can be light-triggered, however the majority of proteins lack a native chromophore and time-resolved studies of these systems rely on external triggers, like photocages, for reaction initiation, thereby allowing to study light- insensitive, irreversible processes [J. J. Zaitsev-Doyle, A. Puchert, Y. Pfeifer, H. Yan, B. A. Yorke, H. M. Müller-Werkmeister, C. Uetrecht, J. Rehbein, N. Huse, A. R. Pearson, M. Sans, *RSC Adv.*, 2019, 9 (15), 8695– 8699.].

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Saha Sarthak (University of Massachusetts-Amherst)

X-ray compatible microfluidics for advanced protein crystallography

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Serial femtosecond crystallography requires the use of upwards of a million crystals for a single data set. Microfluidic and microfabricated devices are a powerful strategy to facilitate the efficient mounting of a large number of crystals. In particular, our efforts are focused on the design and fabrication of polymer-based fixed target devices for serial crystallography. The use of polymeric materials provides low background while circumventing challenges associated with stray diffraction signals from crystalline materials. This approach also has the potential to take advantage of low-cost continuous manufacturing strategies and further facilitate translation of these strategies from XFELs to synchrotrons. We have developed photoresist based microfluidic platform designed for high throughput serial crystallography experiments in room temperature and cryogenic conditions, as well as under vacuum. The architecture of the device allows photoresist film to act as an ultra-thin X-ray diffraction window while sealing the device to prevent significant water loss for weeks. The use of cleanroom photolithography enables custom tailoring of these devices based on the requirements, allowing easy integration with various X-ray sources and beamline automation. Our fabrication scheme also allows for the straightforward incorporation of graphene into our devices, opening up a myriad of applications. In particular, we have demonstrated the ability to use integrated graphene sheets as X-ray transparent electrodes to enable electric field based studies on various protein samples. We have also demonstrated the ability to use graphene layers as a gas diffusion barrier to enable the room temperature diffraction analysis of oxygen sensitive targets.

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Till Stensitzki (Freie Universität Berlin)

Comparison of the initial photoreaction of bacteriorhodopsin in solution by fs-pump-probe spectroscopy

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Thee light-activated proton-pump bacteriorhodopsin (BR) has one of the best studied photo-reactions. The initial photoreaction of BR consists of the isomerization of the 13-cis retinal to the all-trans form on a sub-picosecond scale and was studied by almost every kind of available spectroscopic technique: BR is readily available and easily handled, so it is often used as a benchmark system for novel techniques.

Recently technological advances made time-resolved x-ray crystallography in the femtosecond regime possible, therefore several time-resolved x-ray studies on BR were published and it is now possible to compare spectroscopic with structural changes on a femtosecond timescale. This comparison is necessary, since it is not known if initial photoreaction is affected by crystallization of the protein. Furthermore, recent x-ray experiments used critically high excitation powers. How and if this affects the reaction is unclear.

Here we use polarization resolved femtosecond pump-probe spectroscopy, which is sensitive to the structure, to show that the initial photo-reaction in BR in identical in LCP-microcrystals and in the purple membrane. To simulate the conditions used in the XFEL experiments, we repeated the experiments with pump powers approaching XFEL conditions. Under these conditions, we observed drastic signal changes.

A distinct feature of the XFEL results are the low-frequency oscillations, which were not yet observed by optical spectroscopy. Using Vis-pump IR-probe spectroscopy we present evidence linking these atomic oscillations to spectroscopic features.

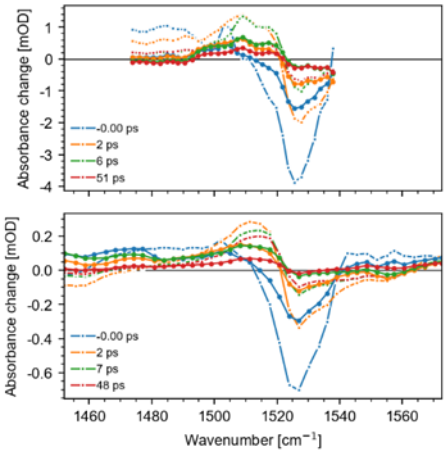

Figure 1: Polarization resolved transient IR spectra in the region of the retinal C=C stretching vibration. Light lines show the difference spectra for parallel pump-probe polarizations, normal lines for perpendicular polarizations. The ratio these two signals depends the structure. *Top:* transient spectra measured on a BR micro-crystal slurry. *Bottom:* transient spectra of BR in the purple membrane. Both the dichroic ratio and the dynamics are identical.

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