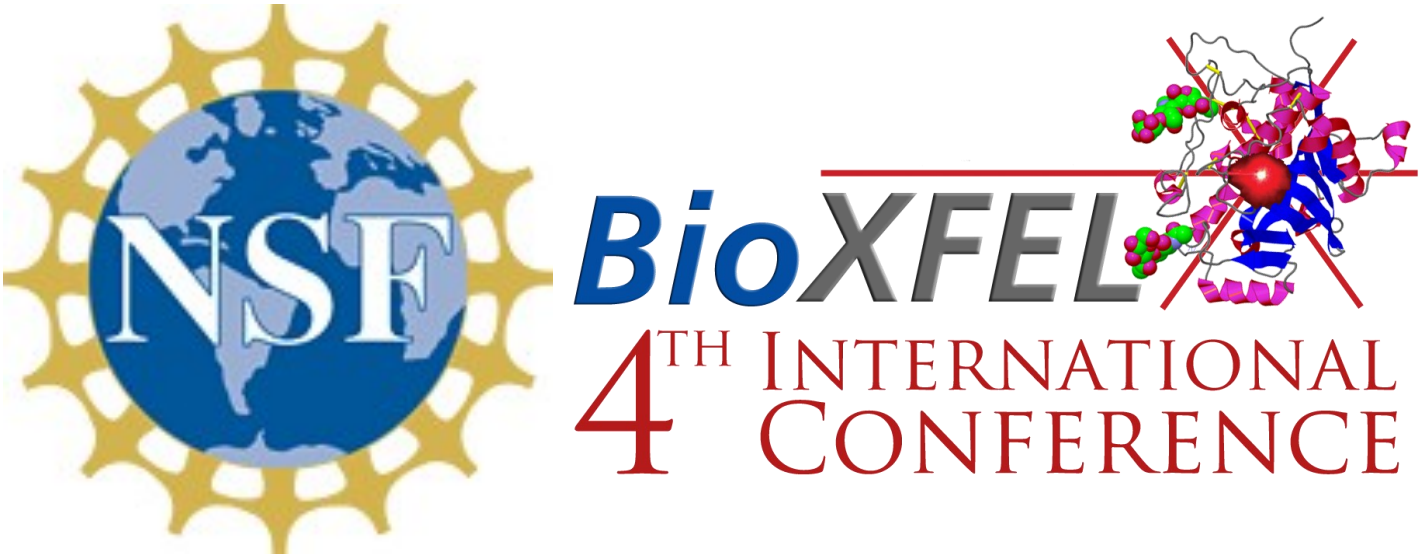
Poster Competition Abstracts



A National Science Foundation Science and Technology Center

BioXFEL 4TH INTERNATIONAL CONFERENCE

POSTER BLITZ

TUESDAY 5:20-5:45 ROMAN I

CHAIR: MITCH MILLER (RICE UNIVERSITY)

#	Name	Title
73	Bill Bauer	XFEL: Crystal Blaster
49	Matthew Coleman	Cell-free translation systems for production of membrane bound protein complexes for BioXFEL studies.
23	Eugenio De La	Time-resolved SFX on acetylcholinesterases
33	Izumi Ishigami	Serial Femtosecond X-ray Crystallography of Carbon Monoxide-Bound Cytochrome c Oxidase
55	Olof Jonnson	Hit detection in Serial Femtosecond Crystallography using photon spectroscopy of plasma emission
74	Yashas Rao	Visualizing Antibiotic Mechanisms Targeting the Ribosome at a XFEL

BioXFEL 4TH INTERNATIONAL CONFERENCE

POSTER SESSION

TUESDAY 8:00—10:00 ROMAN II & IV

JUDGING COMMITTEE: ANNE STONE (MOLECULAR DIMENSIONS), KEITH MOFFAT (U OF CHICAGO), ANDREA KATZ (CORNELL), AINA COHEN (SLAC), THOMAS BARENDS (MPI)

#	Name	Poster Title
1	Adalberto Diaz	Molecular Biophysical Studies and Crystallization Screening of the interaction between Centrin and Prp40Ap
2	Aina Cohen	New Opportunities for Structural Biology Research at SSRL and LCLS
3	Alani Aldarondo-Torres	Hydrogen Sulfide (H2S) as an Inhibitor of Insulin (IBP) Amyloid Fibrils
4	Alberto Pietrini	Cleaning up data for Flash X-ray Imaging: Noise Reduction and Artifact Correction in CsPad detectors at the LCLS CXI beamline
5	Alexander Wolff	Reliable Extraction of the Diffuse Signal from X-ray Diffraction Images
6	Ali Dashti	Conformational movies and energy landscapes of the RyR Ca-Channel
7	Alina Roman-Hubers	Concepts on the Stability of Sulfheme
8	Allen Orville	On-Demand Acoustic Injectors: time-resolved SFX correlated with complementary methods
9	Andrei Rode	Steering particles with optical forces
10	Andrew Shevchuk	A definition of resolution for reconstructions from single-particle imaging data using an XFEL
11	Ariel Lewis	Protein Conformational Dynamics Induced by Tryptophan Binding to Human Trytophan Dioxygenase
12	Aslin Rodriguez-Nassif	Stability of KH Domain GXXG Loop in the Presence and Absence of TFA
13	Austin Echelmeier	Microfluidic Tools for Serial Crystallography
14	Benedikt Daurer	Experimental strategies for imaging small bioparticles with femtosecond hard X-ray pulses
15	Benjamin von Ardenne	Structure Determination from Few Photon Single Molecule X-Ray Scattering Experiments using Photon Correlations
16	Brandon Hayes	Monitoring time-resolved mixing reaction in isocyanide hydratase crystals using UV-vis spectroscopy
17	Carolin Seuring	Single layer graphene as a low background support for femtosecond X-ray coherent diffraction imaging of aligned fibres
18	Chufeng Li	Improving auto-indexing for sparse patterns in Serial Crystallography
19	David von Stetten	Serial Synchrotron Crystallography using a High Viscosity Extrusion injector device on beamline MASSIF-3 at the ESRF

20	Donghyun Ka	Structure analysis of Cas1-Csn2 complex in Streptococcus pyogenes type II CRISPR-Cas system
21	Edward Snell	The High-Throughput Crystallization Screening Center
22	Elin Claesson	Elucidating the structure of a dark form phytochrome at biologically relevant temperatures.
23	Eugenio de la Mora	Time-resolved SFX on acetylcholinesterases
24	Fabiola Moreno-Echevarria	Sulfmyoglobin and Hydrogen Sulfide Metmyoglobin formation in Metmyoglobin Crystals
25	Fatemehsadat Jamalidinan	Determining the Structure of K channel-Proteins In Situ
26	Frances Heredia-Negron	Validation of Methods for Predictions of Tertiary Structure of DNA Aptamers
27	Gabriela Casanova	Micro-crystal Sorting by Size-Exclusion Chromatography for XFEL experiments
28	Ganesh Subramanian	From picosecond to microsecond: Time-resolved X-ray absorption highlights intermediates formed during the photolysis of Methylcobalamin
29	Garrett Nelson	3D Printing for Nozzle Fabrication
30	Hazel Borges & Alfredo Reyes-Oliveras	Hemoglobin I as a novel protein donor of hydrogen sulfide in biological system
31	Hernan Machado	Formation of sulfmyoglobin upon addition of H_2S to crystallized oxymyoglobin
32	Indra Gonzalez	Hydrogen Sulfide (H2S) Limits Lysozyme Amyloid Development and Enhances Soft Matter Assembly
33	Izumi Ishigami	Serial Femtosecond X-ray Crystallography of Carbon Monoxide-Bound Cytochrome c Oxidase
34	Jennifer Vargas-Santiago	Hydrogen Sulfide (H2S) Trapment by Hemoglobin I From Lucina pectinata Encapsulated in Sol-gels.
35	Jesse Coe	Time-resolved Crystallography at XFELs: Advantages, Challenges and Potential
36	Jochen Kupper	COMOTION - Controlling the motion of large molecules and particles
37	John Westbrook	Extending PDB data architecture to support XFEL experiments
38	Jose Carmona	Crystallographic Studies of Ferrocene-Estrogen Derivatives Complexed with Human Serum Albumin and Estrogen Receptor alpha as Potential Drug Delivery Targets
39	Jose Manuel Martin Garcia	Monochromatic and Pink Beam Serial Millisecond Crystallography at the Advanced Photon Source
40	Jose Olmos & Christopher Kupitz	Mix-and-Inject: a ßlaC Story

41	Josiris Rodriguez	Isolation and primary structure of a novel protein associated with HbI from Phacoides pectinatus
42	Juan Valentin Goyco	Nanodisc Assembly and Characterization
43	Junhyung Kim	Crystal structure of psychrophilic adenylate kinase from Antarctic fish
44	Lars Redecke	Serial crystallography in cellulo
45	Louise Lassalle	Structure of photosystem II and substrate binding at room temperature
46	Manoj Saxena	Ti-IV bond transferrin X-ray crystal structure reveals citrate's synergistic role in Ti-transferrin binding
47	Margaret Gabanyi	BioSync: An Online Resource for X-ray Facilities Worldwide
48	Martin Mechelke	Bayesian structure reconstruction from single molecule scattering data
49	Matthew Coleman	Cell-free translation systems for production of membrane bound protein complexes for BioXFEL studies.
50	Matthias Frank	Solid sample support approaches for biological imaging at XFELs
51	Max Hantke	Smaller droplets for bigger success
52	Max Wiedorn	High speed fixed target serial crystallography
53	Mitchel Miller	Progress in the Time-Resolved Crystallographic Analysis of Photosensory Proteins Delivered by an Acoustical Drop on Demand Conveyer Belt System
54	Natasha Stander	Pattern Selection for Continuous Diffuse Scattering 3D Merges of Photosystem II Crystals
55	Olof Jonsson	Hit detection in Serial Femtosecond Crystallography using photon spectroscopy of plasma emission
56	Osamu Miyashita	3D Reconstruction from X-ray Free Electron Laser Single Particle Analysis of Biomolecular Systems
57	Oskar Berntsson	Signal Transduction in Photosensory Proteins Resolved by X-ray Solution Scattering
58	Peter Schwander	Three-dimensional Structure by Single-particle X-ray Scattering
59	Prakash Nepal	Difference structures from time-resolved SAXS/WAXS
60	Reyhaneh Sepehr	Deterministic method for resolving indexing ambiguity in serial crystallography
61	Reza Nazari	Three-Dimensional Simulation of an Aerodynamics Lens System Particle Focusing.
62	Romain Letrun	Combined Two-Dimensional Electronic Spectroscopy and X-ray Crystallography Study of Photosynthetic Reaction Centers in AXSIS
63	Shangji Zhang & Jay-How Yang	Structural Study of F-type ATP Synthase from Spinach Chloroplasts and Heliobacterium modesticaldum
64	Shatabdi Roy-Chowdhury	Studying the oxidation of water: The Photosystem II story
65	Simone Sala	XFEL wavefront characterization using ptychography at LCLS

66	Simon Weiss	Structure determination of the Au146 cluster using Micro-ED and X-ray diffraction
67	Stewart Malave-Ramos	Investigating the Role of an Atypical Variant Surface Glycoprotein (aVSG) in Developmental Progression to Infectivity in Trypanosoma brucei
68	Suraj Pandey & Ishwor Pyoudal	X-RAY FREE ELECTRON LASER (XFEL) IN PROTEIN CRYSTALLOGRAPHY
69	Thomas Grant	Live Monitoring of Time-resolved Solution Scattering Data at XFELs
70	Timothy Stachowski	Initial studies on the dynamics of TGFβ-1 latent protein dissociation
71	Tyler Norwood	Analysis of Protein Reaction Kinetics by Time-Resolved Absorption Spectroscopy
72	Victoria Mazalova	X-ray Emission Spectroscopy within the AXSIS project: Electronic Dynamics and Undamaged Electronic Structure Study of Photosystem II.
73	William Bauer	XFEL: Crystal Blaster
74	Yashas Rao	Visualizing Antibiotic Mechanisms Targeting the Ribosome at a XFEL
75	Zachary Dobson	Discovery and Isolation of a New, High-Light Tolerant Cyanobacteria

Adalberto Diaz, University of Puerto Rico

Molecular Biophysical Studies and Crystallization Screeing of the Interaction between Centrin and Prp40Ap

Díaz-Casas, A.,[1,2] Bauer, W.[3] and Pastrana-Ríos, B.[1,2]

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Centrin is a protein of ~20 KDa that belongs to the superfamily of Ca^{2+}-binding proteins. This multifunctional protein is one of the "eukaryotic signature proteins", therefore, centrin is considered critical for the structure and function of the eukaryotic cell. In this research, we focused on *Homo sapiens* centrin 2 (*Hs*cen2) and *Chlamydomonas reinhardtii* centrin (*Cr*cen). Both proteins shares approximately 70% sequence identity, yet exhibit different affinities for Ca^{2+}. Within the nucleus, *Hs*cen2 is a component of XPC-RAD23-*Hs*cen2 complex, which is involved in the nucleotide excision repair. Also, *Hs*cen2 is a component of the TREX-2 complex, which plays an important role in mRNA export. Recently, we found that *Homo sapiens* Prp40 Homolog A (*Hs*Prp40A) possess a hydrophobic triad $W_1L_4L_8$ which is found in other centrin-binding proteins. Herein we present the interaction of centrin with a novel biological target called Prp40 Homolog A (*Hs*Prp40A) which is involved in pre-mRNA splicing. Specifically, we studied a Prp40 Homolog A peptide (*Hs*Prp40Ap) comprised of the following sequence for fragment based drug design (FBDD): $_{524}$KQLRKRNWEALKNILDNMANVTYSTTWSEAQQY$_{556}$ which possess the hydrophobic triad ($W_1L_4L_8$). Here we carried out isothermal titration calorimetry (ITC) to determine the thermodynamics governing binding between centrin and Prp40A. Also, two-dimensional infrared (2D IR) correlation spectroscopy was performed to determine the molecular changes that occurred in both proteins during complex formation. Our next step is to obtain a high resolution structure of centrin-Prp40Ap complex by X-Ray crystallography. Therefore, a crystal screening was performed in collaboration with the high-throughput crystallization screening lab (HTSlab) in order to find optimal conditions for protein crystal growth

This research material is supported by grant NIH-R25GM088023 from the National Institute of General Medical Sciences. We also thank the Alfred P. Sloan Foundation for its support in this research project.

AINA COHEN, SLAC

NEW OPPORTUNITIES FOR STRUCTURAL BIOLOGY RESEARCH AT SSRL AND LCLS

Aina E. Cohen and Thomas M. Weiss, representing the entire SSRL-SMB team.

SLAC National Accelerator Laboratory, Stanford, California, 94025, USA.

The Structural Molecular Biology (SMB) program at the Stanford Synchrotron Radiation Lightsource (SSRL) provides an integrated suite of macromolecular crystallography (MC) and small angle x-ray scattering (SAXS) beam lines and facilities enabling studies on the most challenging problems in structural biology. These include the undulator station BL12-2 optimized for micro-beam MC experiments, BL9-2, BL7-1 and BL14-1 optimized for high-throughput MC screening and data collection, and BL4-2, a small angle x-ray scattering/diffraction station that provides state-of-the-art experimental facilities for equilibrium and time-resolved structural studies on non-crystalline biological materials in solution. The SMB program also includes x-ray absorption, emission and imaging spectroscopy facilities. A next-generation undulator microfocus beam line, BL12-1, which will provide a preeminent capability for MC research in the US, is under development. BL12-1 will be outfitted with a broad bandpass capability which will provide exceptional brightness, smaller microbeams and a high number of reflections when rastering on the fly or using crystal injectors. It will be equipped with a high speed EIGER PAD detector and a high speed multi-axis goniometer, enabling new approaches for data collection and phasing. Similarities in instrumentation, existing and new sample delivery systems, and software environments will form the foundation of a synergistic relationship between the SSRL BL12-1 and a new Macromolecular Femtosecond crystallography instrument (MFX) at LCLS, through a Gateway approach.

LCLS-MFX began experiments with the start of the user program on July 1, 2016. Various sample delivery and data acquisition systems are currently being implemented at MFX, which will enable serial crystallography and in-situ spectroscopic monitoring and promise to reveal the structures and dynamics of complex biomolecules. The standard sample environment available to the scientific general user community at MFX is a highly automated goniometer setup for diffraction experiments, developed and supported by the SMB group. The experimental front-end is based on developments at SSRL and LCLS XPP to provide an efficient framework to carry out goniometer-based FX experiments using automated strategies tailored to handle a variety of sample requirements, crystal sizes and experimental goals. These developments coupled with improvements in data processing algorithms make it possible to derive high resolution crystal structures using only 100 to 1000 still diffraction images.

Alani Aldarondo-Torres, University of Puerto Rico

Hydrogen Sulfide (H_2S) as an Inhibitor of Insulin (IBP) Amyloid Fibrils

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Amyloid fibrils are formed by soluble proteins that, through the process of protein unfolding, assemble into undegradable insoluble fibers. These amyloid fibrils are associated to many types of diseases, like Diabetes Type 2, that are characterized by the specific protein or peptide chain that aggregates extracellular to a body tissue. The process of protein unfolding and fibrillation can be promoted by applying extreme conditions of high temperature and low pH to the desired protein. It has been proven that by adding a compound X, hen egg white lysozyme (HEWL) amyloid fibrils can be inhibited (Rosario, 2014). Due to the fact that lysozyme possesses structural similarities with insulin, it was considered a biological model to carry out the experimentation with Insulin from Bovine Pancreas (IBP). With the goal of thoroughly studying the interactions between insulin and the X compound, 10 mg/mL IBP solutions were prepared with different ratios of the compound. The existence of amyloid fibers was determined by qualitative methods, such as the careful examination of the samples at different time intervals, and by quantitative methods, such as ThT Fluorescence and Raman Spectroscopy. The samples without the compound of interest and the samples with a one on one molar ratio (IBP:X) showed a fibril-like structure characteristic of amyloid aggregates. Contrary to the results with lysozyme, the samples with small ratios of the compound suggest the presence of amyloid fibrils. Furthermore, HEWL and IBP protein samples express the same behavior when exposed to high concentrations of the X compound and extreme temperature; both promote gelification. When performing ThT Fluorescence, all samples presented fibril formation, although a decrease in fluorescence was observed as the X compound concentration increased. Future analyses like Circular Dichroism, Atomic Force Microscopy (AFM), and Scanning Electron Microscopy (SEM) will be performed to study the structure and kinetics of these substances.

Acknowledgements:
Thanks to INBRE Program for sponsoring and funding this research project.

Alberto Pietrini, Uppsala University

Cleaning Up Data for Flash X-ray Imaging: Noise Reduction and Artifact Correction in CsPad Detectors at the LCLS CXI Beamline

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† Laboratory of Molecular Biophysics, Department of Cell and Molecular Biology, Uppsala University, Sweden; ‡ Division of Scientific Computing, Department of Information Technology, Science for Life Laboratory, Uppsala University, Sweden.

Flash X-ray Imaging (FXI) is a novel and promising technique for imaging biological particles smaller than 100 nm in diameter. When fully developed, it will allow the recovery of the 3D structure of bioparticles by analyzing diffraction from highly brilliant x-ray pulses hitting *single particles*, thanks to the *diffraction-before-destruction* principle [1]. Here, we report the existence of persistent detector noise and artifacts in data collected during the L730 and L867 beamtimes at the CXI Instrument at the LCLS (the beamline originally devoted to the FXI purpose), which are major obstacles to the proper analysis of signals from weak scatterers. The detectors taken into account are based on CsPad (Cornell-SLAC hybrid Pixel Array Detector) technology. We focus on CsPad-140K camera [2] (L730) and the CsPad large camera (L867), which share the same fundamental unit (CsPad 2x1 module), in silicon revision 1.6. We discuss remedies to correct for detector errors, specifically analyzing a per-column per-ASIC correction in the CsPad raw signal: despite an overall small improvement in the width of the zero-photon peak (about 2%), we decrease the false positive photon detection rate (respectively from $1.70 \cdot 10^{-4}$ to $1.58 \cdot 10^{-4}$ and from $5.57 \cdot 10^{-5}$ to $5.32 \cdot 10^{-5}$ photon counts/pixel/frame), thus getting an appreciable noise level reduction (**Figure**). We also conclude that the severity of the artifact varies between frames and across the panel. The improvements suggested are critical in a strictly photon counting regime (as in the single particle imaging case), but they can also be relevant for serial nano-crystallography, especially if ab initio phasing from the diffuse scattering is considered [3]. Moreover, even though what stated here has been proved only at the CXI Instrument, it has a general validity for every detector based on CsPad modules.

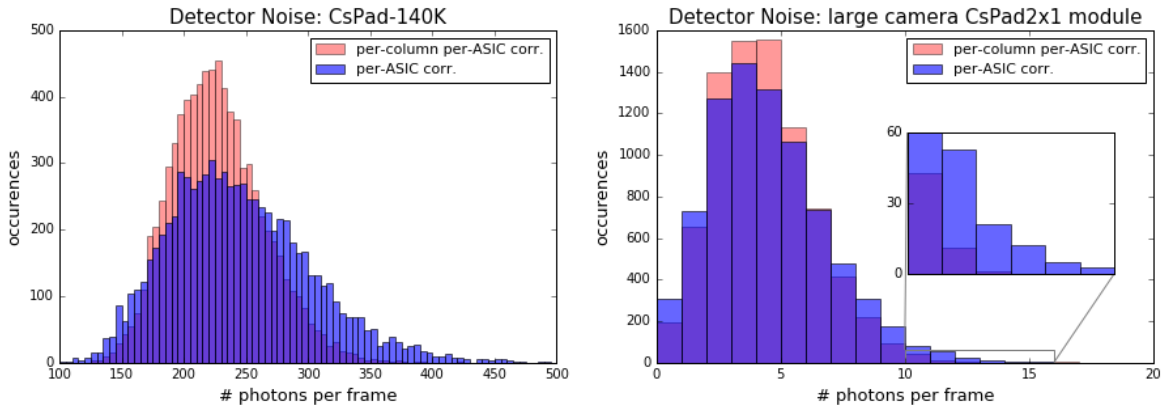

Figure – Detector noise histograms for data collected during L730 beamtime on the CsPad-140K detector (to the left) and for data collected during L867 beamtime on one CsPad 2x1 module in the CsPad large camera (to the right). For both the plots, on the x-axis there is the number of photons in each dark frame (representing the detector noise); on the y-axis there is the number of occurrences of each value in that dataset. The correction for a per-ASIC offset is displayed in blue; the one for per-column per-ASIC offset is depicted in red. The inset on the right figure shows a larger tail for the per-ASIC offset correction.

1. Richard Neutze, et al., "Potential for biomolecular imaging with femtosecond X-ray pulses", *Nature* **406**, 752-757, (2000).
2. Sven Herrmann, et al., "CSPAD-140k - a Versatile Detector for LCLS Experiments", NIMA proceedings on the 12th Pisa Meeting on Advanced Detectors, (2012).

3. Kartik Ayyer et al., "Macromolecular diffractive imaging using imperfect crystals", *Nature* **530**, 202–206, (2016).

Alexander Wolff, University of California-San Francisco

Reliable Extraction of the Diffuse Signal from X-ray Diffraction Images

Wolff, A.M.[1]; Van Benschoten, A.H.[1]; Sauter, N.K.[2]; Wall, M.E.[3]; Fraser, J.S.[4]

1. Graduate Program in Biophysics, University of California, San Francisco, San Francisco, CA, USA; 2. Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA; 3. Computer, Computational, and Statistical Sciences Division, Los Alamos National Laboratory, Los Alamos, NM, USA; 4. Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, CA, USA.

X-ray diffraction images contain a wealth of information about the biomolecules that compose a crystal. The Bragg peaks report on the averaged structure across unit cells, while diffuse features are generated by correlated displacements within the crystal. Due to the challenges of properly measuring and extracting the diffuse scattering signal, protein crystallographers have historically focused on the data contained within the Bragg peaks. Thus there is a shortage of carefully collected diffuse scattering data, as well as a lack of analysis of the resulting signal in images showing strong diffuse features. Our recent work has shown that it is possible to measure diffuse scattering during the routine collection of Bragg diffraction data, and that the resulting diffuse signal can inform models of protein motion. Our current goal is to automate extraction of the diffuse signal from X-ray diffraction images so that all protein crystallographers can access this information in their data. Initial results show that our pipeline is robust with regards to datasets collected at synchrotron sources. With the enhanced flux available at FEL light sources, diffuse features in the diffraction images will be even stronger. We are currently working to generalize the extraction of diffuse signal from SFX-XFEL data. This analysis will be crucial to make full use of the data from time-resolved pump-probe experiments. The diffuse signal will enable development of unique multi-conformer models to represent the perturbed structures. We hope that wide availability and ease of access will open the door for researchers to investigate structural dynamics using diffuse X-ray scattering.

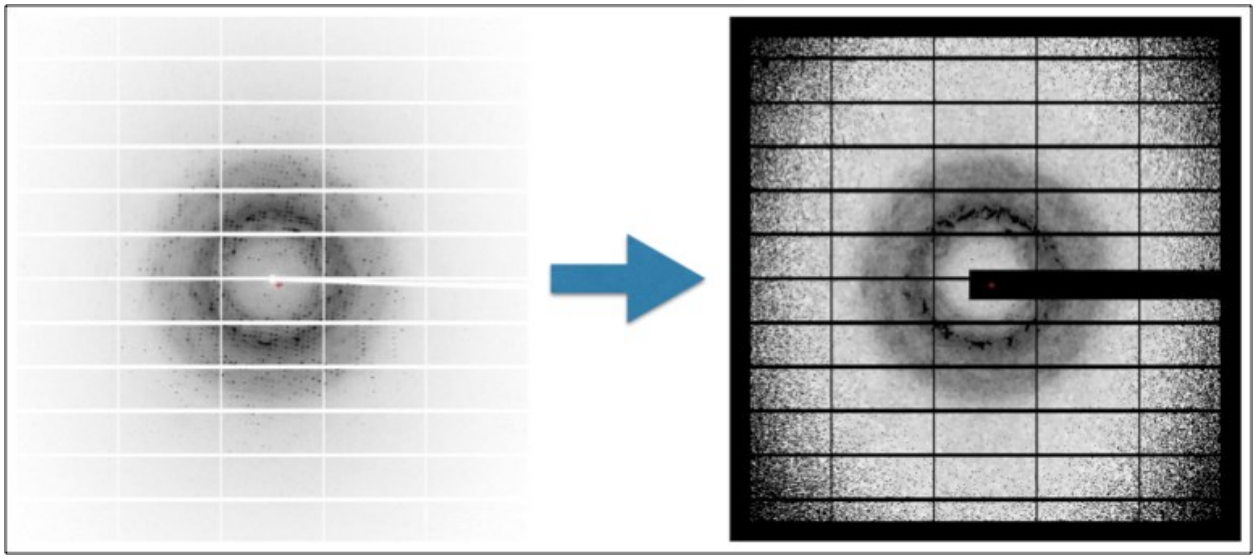

Figure 1. Isolation of the diffuse signal from a single Cyclophilin A diffraction image.

Ali Dashti, University of Wisconsin-Milwaukee

Conformational Movies and Energy Landscapes of the RyR Ca-Channel

A. Dashti[1], P. Schwander[1], G. Mashayekhi[1], A. des Georges[2], J. Frank[3,4], and A. Ourmazd[1]

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Using cryo-EM snapshots of the intracellular Ca++ channel RyR1, we present the trajectories traversed over the energy landscape in the course of continuous conformational changes of RyR1 exposed to Ca++, caffeine and ATP ligands. By a consistent description of the conformational energy landscapes with and without ligands, we directly observe the effects of ligand binding on the conformational trajectories of the channel, and shed new light on competing ligand-binding mechanisms.

Alina Roman-Hubers, University of Puerto Rico

Concepts on the Stability of Sulfheme

Román, A.T.; Santos, L. and Garriga, J.

Department of Chemistry, Recinto Universitari de Mayagüez, University of Puerto Rico, Mayagüez, Puerto Rico 00681

Easily detected by its characteristic repelling odor hydrogen sulfide (H_2S) has been historically known for its toxicity. Recent studies, now recognize H_2S as a distinguished biological molecule involved in many physiological processes. Hydrogen sulfide is synthesized in human and mammalian tissue and has the ability to modulate biological and physiological processes. In the presence of heme proteins, such as hemoglobin (Hb) and myoglobin (Mb); H_2S has the capacity of binding and heme modification resulting in the heme-derivatives named in 1866 by Hoppe-Seyler as sulfhemoglobin and sulfmyoglobin. The formation of these sulfheme derivatives and their life cycle by the physiological turnover of red blood cells is considered a pathway of H_2S degradation in human tissue. This work evaluates the concepts of stability of sulfheme complexes over a period of time using UV-Vis spectrophotometry. Hemoglobin in the presence of hydrogen peroxide (H_2O_2) and H_2S was monitored for two hundred fifty-three hours to measure sulfhemoglobin stability by analyzing the visible fluctuations at spectrum spectrophotometry. The evaluation of sulfhemoglobin absorbance over a prolonged period of time demonstrates a plausible H_2S concentration reduction in the sample along a period of time.

Allen Orville, Diamond Light Source

On-Demand Acoustic Injectors: Time-Resolved SFX Correlated with Complementary Methods

Acoustic droplet ejection (ADE) is a general, touchless, on-demand method that use focused sound waves to eject picoliter to nanoliter volume droplets from one place to another. In our first applications, we collected serial femtosecond crystallography (SFX) datasets from droplets launched in synchrony to intersect each XFEL pulse. We have also coupled ADE methods with a conveyor belt drive to enable time-resolved SFX (tr-SFX) at the LCLS. Our system is optimized for crystallography and X-ray emission spectroscopy (XES) measurements of photochemical reactions over a wide-range of time scales and illumination schemes. Indeed, the combination of tr-SFX + XES, wherein both types of data are obtained from the same sample and X-ray pulse, provides important complementary information that impacts mechanistic insights. We are also exploiting the region on the belt between the ADE transducer and the X-ray interaction point to introduce additional experimental perturbations that initiate a range of enzyme reactions. I will discuss the potential impact to tr-SFX studies of many macromolecules operating under physiological temperature and pressure.

Supported in part by grant 102593 from the Wellcome Trust and the BBSRC

Christian G. Roessler, Rakhi Agarwal, Marc Allaire*, Roberto Alonso-Mori, Babak Andi, José F. R. Bachega, Martin Bommer, Aaron S. Brewster, Michael C. Browne, Ruchira Chatterjee, Eunsun Cho, Aina E. Cohen, Matthew Cowan, Sammy Datwani, Victor L. Davidson, Jim Defever, Brent Eaton, Richard Ellson, Yiping Feng, Lucien P. Ghislain, James M. Glownia, Guangye Han, Johan Hattne, Julia Hellmich, Annie Héroux, Mohamed Ibrahim, Jan Kern, Anthony Kuczewski, Henrik T. Lemke, Pinghua Liu, Lars Majlof, William M. McClintock, Stuart Myers, Silke Nelsen, Joe Olechno, **Allen M. Orville***, Nicholas K. Sauter, Alexei S. Soares*, S. Michael Soltis, Heng Song, Richard G. Stearns, Rosalie Tran, Yingssu Tsai, Monarin Uervirojnangkoorn, Carrie M. Wilmot, Vittal Yachandra, Junko Yano, Erik T. Yukl, Diling Zhu, Athina Zouni; "Acoustic injectors for drop-on-demand serial femtosecond crystallography" (2016) *Structure* 24, 631-640

Iris D. Young, Mohamed Ibrahim, Ruchira Chatterjee, Sheraz Gul, Franklin Fuller, Sergey Koroidov, Aaron S. Brewster, Rosalie Tran, Roberto Alonso-Mori, Thomas Kroll, Tara Michels-Clark, Hartawan Laksmono, Raymond G. Sierra, Claudiu A. Stan, Rana Hussein, Miao Zhang, Lacey Douthit, Markus Kubin, Casper de Lichtenberg, Long Vo Pham, Håkan Nilsson, Mun Hon Cheah, Dmitriy Shevela, Claudio Saracini, Mackenzie A. Bean, Ina Seuffert, Dimosthenis Sokaras, Tsu-Chien Weng, Ernest Pastor, Clemens Weninger, Thomas Fransson, Louise Lassalle, Philipp Bräuer, Pierre Aller, Peter T. Docker, Babak Andi, **Allen M. Orville**, James M. Glownia, Silke Nelson, Marcin Sikorski, Diling Zhu, Mark S. Hunter, Andy Aquila, Jason E. Koglin, Joseph Robinson, Mengning Liang, Sébastien Boutet, Artem Y. Lyubimov, Monarin Uervirojnangkoorn, Nigel W. Moriarty, Dorothee Liebschner, Pavel V. Afonine, David G. Waterman, Gwyndaf Evans, Philippe Wernet, Holger Dobbek, William I. Weis, Axel T. Brunger, Petrus H. Zwart, Paul D. Adams, Athina Zouni, Johannes Messinger, Uwe Bergmann, Nicholas K. Sauter, Jan Kern, Vittal K. Yachandra, Junko Yano, "Structure of photosystem II and substrate binding at room temperature" (2016) *Nature* (in press)

Andrei Rode, Australian National University

Steering Particles with Optical Forces

Niko Eckerskorn,[1] Woei Ming Lee,[2] Salah Awel,[3,4] Daniel Horke,[3,4] Richard A. Kirian,[5,3] Jochen Küpper,[3,4,6] Henry N. Chapman,[3,4,6] and Andrei V. Rode[1]

[1]Laser Physics Centre, Research School of Physics and Engineering, and [2]Research School of Engineering, The Australian National University, Canberra ACT 2601 Australia; [3]Center for Free-Electron Laser Science, DESY, 22607 Hamburg, Germany; [4]Center for Ultrafast Imaging, Universität Hamburg, 22761 Hamburg, Germany; [5]Department of Physics, Arizona State University, Tempe, AZ, USA; [6]Department of Physics, Universität Hamburg, 22761 Hamburg, Germany

The manipulation of airborne particles by light is governed by photophoresis, a thermal force due to uneven illumination of a particle. We built a funnel-shaped hollow-core laser beam to trap graphite particles at different heights, depending on their masses. The particles were stably trapped at different heights inside the optical funnel depending on their mass, showing the direct relationship with the force that acted on the particles at each position in the laser beam. This allows for calibration, and prediction of the trajectories of objects in the optical funnel depending on laser intensity, size and air pressure [1].

We combined a spatial light modulator (SLM) and an electrically tunable lens to construct a variable-divergence vortex beam providing dynamic and stable axial and azimuthal positioning of optically levitated micrometer-size particles under normal atmospheric pressure. In addition, modulation of the beam divergency with a predicted oscillatory movement and resulted particle move into a different equilibrium position provides a measure of the mass of a single, isolated particle [2]. Such touch-free positioning could be used to place a biomolecule in the focus of an x-ray free electron laser. The driving signal of oscillatory motion can potentially be phase-locked to an external timing signal enabling synchronization of particle delivery into the x-ray focus with XFEL pulse train.

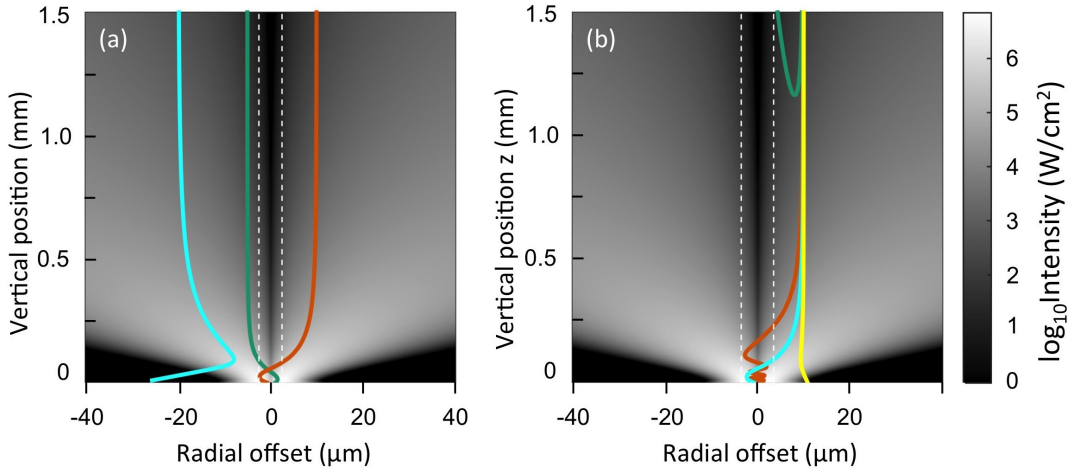

FIG. 1(a,b). Examples of the calculated trajectories for a 1 μm particle entering the optical funnel with a total laser power of 5 W. The dashed lines show the size of the vortex ring in the focus of the beam; the scale on the right shows the logarithm of laser intensity illuminating the particles in various points of the beam. (a) – trajectories of particles entering the trap with a speed of 15 m/s at a transverse offset of 5 μm (green), 10 μm (red), and 20 μm (cyan). (b) – trajectories of particles entering at a fixed offset of 10 μm from the axis with the initial speed of 10 m/s (green), 12 m/s (red), 15 m/s (cyan), and 30 m/s (yellow).

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Andrew Shevchuk, Arizona State University

A Definition of Resolution for Reconstructions from Single-particle Imaging Data Using an XFEL

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XFEL imaging of individual biomolecules has long been a goal of the "diffraction-before-destruction" paradigm, but the reconstruction of high-resolution charge density maps from individual diffraction patterns presents many challenges. Among these is an adequate definition of the resolution of a reconstruction when data from known structures is included in the process. Tuukkanen et al (2016) define resolution in such a case for reconstructions from small-angle X-ray scattering (SAXS) profiles. Their method generates an ensemble of reconstruction models whose simulated scattering profiles provide a good fit to the experimental SAXS profile. The model variability (a measure of the reconstruction's reproducibility) is inversely proportional to the momentum transfer where the ensemble's average Fourier shell correlation (FSC) function falls below 0.5. When the average pairwise FSC function is computed instead between the ensemble and a known structure, the equivalent quantity to the model variability is the model resolution. The variability and resolution are linearly related to each other, with fitting coefficients that depend on the modeling method. By calibrating this relationship using a database of known structures, the resolution of ab initio models can be determined directly from the model variability for an unknown structure. Use of the phase-retrieval transfer function is also useful (Marchesini et al (2005)).

We discuss the application of this methodology to the definition of resolution for reconstructions from single-particle data taken at an XFEL. Single-particle XFEL imaging presents a strictly superior dataset to the spherically symmetric SAXS profiles because each snapshot contains orientation information. This orientation information is present because the pulse duration is significantly shorter than the molecule's rotational diffusion time. This information can nominally reduce the variability of the ensemble of models compatible with the observed scattering pattern, thereby improving the reconstruction resolution beyond that of SAXS models. A naïve summation (i.e., without orientation determination) of all snapshots yields a spherically averaged profile comparable to that achieved by SAXS.

This definition of resolution can be used to tackle other important problems, such as the question of what pulse duration optimally balances signal-to-noise ratio with radiation damage to achieve the most detailed reconstruction. Simulations are underway for dielectric spheres and icosahedral viruses.

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Acknowledgment: STC Award NSF 1231306

Ariel Lewis, Albert Einstein College of Medicine

Protein Conformational Dynamics Induced by Tryptophan Binding to Human Trytophan Dioxygenase

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Human tryptophan dioxygenase (hTDO) is an important heme-containing enzyme that catalyzes the first and rate-limiting step of the kynurenine pathway, the major metabolic route for our dietary tryptophan (Trp). In addition to being responsible for regulating blood Trp level under normal physiological conditions, recently it was found that hTDO is upregulated in many cancers to drive Trp consumption, thereby protecting cancer cells from immune surveillance. As a result, hTDO has been recognized as an important drug target for cancer therapy. The goal of this work is to delineate the mechanism by which the conformational dynamics of heme-based dioxygenases regulate the energy landscapes of their enzymatic reaction. As the first step to achieve the goal, we determined the structural changes in hTDO induced by Trp binding, the first step of the dioxygenase reaction cycle. We used synchrotron radiation and serial femtosecond X-ray crystallography (SFX) to probe the 3D structures of hTDO in substrate-free and Trp-bound states. The data revealed conformational changes to an active site loop induced by Trp binding. They pave the way towards using SFX as a tool to determine protein dynamics critical for hTDO function that is unattainable with synchrotron radiation at cryogenic temperature.

Aslin Rodriguez-Nassif, University of Puerto Rico

Stability of KH Domain GXXG Loop in the Presence and Absence of TFA

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Human tryptophan dioxygenase (hTDO) is an important heme-containing enzyme that catalyzes the first and rate-limiting step of the kynurenine pathway, the major metabolic route for our dietary tryptophan (Trp). In addition to being responsible for regulating blood Trp level under normal physiological conditions, recently it was found that hTDO is upregulated in many cancers to drive Trp consumption, thereby protecting cancer cells from immune surveillance. As a result, hTDO has been recognized as an important drug target for cancer therapy. The goal of this work is to delineate the mechanism by which the conformational dynamics of heme-based dioxygenases regulate the energy landscapes of their enzymatic reaction. As the first step to achieve the goal, we determined the structural changes in hTDO induced by Trp binding, the first step of the dioxygenase reaction cycle. We used synchrotron radiation and serial femtosecond X-ray crystallography (SFX) to probe the 3D structures of hTDO in substrate-free and Trp-bound states. The data revealed conformational changes to an active site loop induced by Trp binding. They pave the way towards using SFX as a tool to determine protein dynamics critical for hTDO function that is unattainable with synchrotron radiation at cryogenic temperature.

Austin Echelmeier, Arizona State University

Microfluidic Tools for Serial Crystallography

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Serial femtosecond crystallography (SFX) with X-Ray Free Electron Lasers (XFEL) has evolved as a powerful technique for crystallography of large protein complexes over the past years. Several limitations of this emerging method have however prevented its general applicability. Among those limitations are the restrictions in growing protein crystals sufficiently small in size (ideally sub-µm) for SFX, the requirement of highly concentrated crystal suspensions of several mL volumes, the lack of tools for substrate based time-resolved crystallography studies as well as the lack of de novo phasing approaches. The field of microfluidics has developed in the past decade as a tool kit allowing prototype device design suitable for providing solutions to current challenges in SFX. To address the loss of precious protein crystals in liquidjet injection technology typically achieved with gas dynamic virtual nozzles (GDVNs), we propose microfluidic droplet generation intermitting the aqueous crystal suspension stream with an oil phase to dramatically reduce sample waste. We demonstrate that microfluidic droplet generation can be coupled to traditional GDVNs and applied this approach to SFX of granulovirus. This approach can also be fully integrated on a microfluidic chip. In addition, we developed a microfluidic device capable of separating protein crystal suspensions into sub-µm size fractions. This concept is based on dielectrophoresis at integrated constrictions allowing to guide crystals of different size into various outlets. Our latest work optimized the sorting principle for high throughput applications required for SFX studies and we demonstrate a yield of up to 500 µL per hour highly concentrated photosystem I crystal suspension which could be directly injected with GDVNs in SFX experiments. Crystal quality after sorting remained excellent and yielded hit rates up to 40%. The data set is currently investigated for novel phasing approaches. Further, we explore microfluidic mixing based on hydrodynamic focusing and fast diffusive mixing for SFX. Mixing devices were developed both with photolithography as well as 3D printing approaches achieving sub-ms mixing times at flow rates compatible with GDVNs. Geometrical optimization of device geometry and channel volume allow the measurement of reaction time points ranging from several ms up to seconds. This mixing approach has been applied to study the reaction of the enzyme 3-deoxy-D-manno- 2-octulosonate-8 phosphate synthase with its substrates phosphoenolpyruvate and arabinose-5-phosphate.

Benedikt Daurer, Uppsala University

Experimental Strategies for Imaging Small Bioparticles with Femtosecond Hard X-ray Pulses

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Flash X-ray Imaging (FXI) using X-ray lasers has been demonstrated on biological particles in the range 0.1 – 2µm, including virus particles [1,2], cell organelles [3] and cells [4]. For smaller objects, this experiment becomes significantly more challenging as signal levels decrease. In this study, the capabilities for imaging sub-100 nm biological samples in the Coherent X-ray Imaging (CXI) instrument at Linac Coherent Light Source were explored. A well-characterized sample, the 40-nm Omono River Virus [5], was delivered to the submicron focus of the CXI instrument. Data was collected at 5.5 keV photon energy and revealed large variations in both particle size and beam intensity which provided an ideal dataset for characterizing important aspects of this experiment, such as sample delivery, signal and background levels, and properties of the X-ray focus. The maximum intensity observed was at $1.9 \cdot 10_{12}$ photons per μm_2. A weighted inversion of the intensity distribution gave a reconstructed average beam profile resembling a Lorentzian function with a full width at half the maximum of 0.5 µm assuming 20% beamline transmission. As an example, a strong diffraction pattern from a sample-sized particle was phased to a full-period resolution of 13.5 nm (Figure 1). Our results suggest that sample delivery could be improved by reducing the size of the initial droplets. Furthermore, a reduction in background scattering at all diffraction angles could give access to higher resolution. With these considerations and new advances in noise and background aware reconstruction algorithms [5], high-resolution FXI on small bioparticles can be brought closer to its potential.

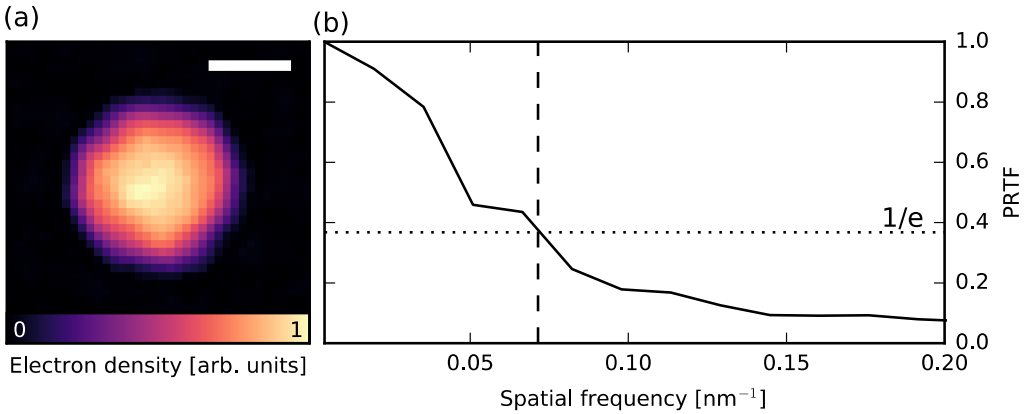

Figure 1. Image reconstruction from a single diffraction pattern of a sample-sized object. (a) Average magnitude based on 5000 independent reconstructions. The scale bar indicates 20 nm. (b) Phase retrieval transfer function (PRTF) dropping below 1/e (dotted line) at a resolution of 13.5 nm (dashed line).

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Benjamin von Ardenne, Max Planck Institute

Structure Determination from Few Photon Single Molecular X-ray Scattering Experiments using Photon Correlations

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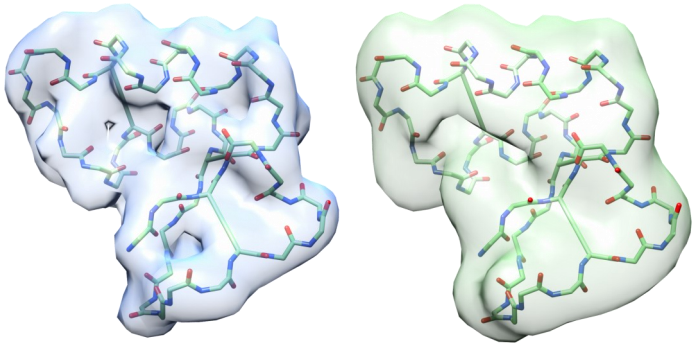

Original Electron Density Retrieved Electron Density

X--ray free electron lasers (XFEL) hold the promise of solving macromolecular structures in single molecule experiments. Scattering images contain few signal photons (as low a 10 photons) but the recording of many pictures is feasible. Signal to noise is estimated to be 1:10 and pictures are taken without the knowledge of the molecule's orientation at the time of the illumination. Existing structure determination methods explicitly or implicitly sort images by their orientation and average them to retrieve the Fourier density. However, this orientation classification breaks down at low photon counts.

Here, we present a *de novo* structure determination method which is based only on three-photon correlations extracted from the scattering patterns. In contrast to previous methods based on photon correlations [1], we make use of the full three-photon correlation. First, we derive the analytic expression of the three-photon correlation as a function of the intensity using a spherical harmonics expansion. Then we solve the inverse problem by a Monte Carlo simulated annealing scheme, in which the correct Fourier density is identified by comparing the expected and experimentally observed triple correlations using the analytic forward model. To reduce the search space, we use information from the analytic inversion of the two-photon correlation. The electron density is eventually retrieved by applying an iterative phase retrieval method [2].

We challenge the method with synthetic diffraction data of Crambin and asses the quality of the densities dependent on the number of detected triplets. From a dataset containing $3.2 \cdot 10^{10}$ triplets we determined the electron density with an estimated resolution of approx. 4 Å (see Figure for comparison).

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Brandon Hayes, SLAC

Monitoring Time-resolved Mixing Reaction in Isocyanice Hydratase Crystals using UV-vis Spectroscopy

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Serial femtosecond crystallography at X-ray free electron lasers has enabled time-resolved mixing experiments, where ligands are added to protein crystals a short time before they are probed with X-rays. Time points are often accessed by estimating ligand mixing and diffusion rates, which, together with flow parameters, determine spatial offsets for the experimental equipment. The time points are then verified after data collection, and offsets are adjusted if necessary, wasting valuable beam time. Here, we applied UV-vis microspectrophotometry as an offline monitoring technique to characterize an enzyme-substrate mixing experiment prior to LCLS beamtime. UV-vis absorption spectroscopy is a useful tool to monitor a reaction cycle by detecting changes in the absorption spectra of substrate and product. While typically used with protein solutions, this technique can be utilized to observe reactions in protein crystals as well. Specialized instrumentation for this purpose is available at SSRL BL11-1 and BL9-2 which is typically used to monitor specific radiation damage effects in metalloenzyme crystals[1].

Isocyanide hydratase (ICH) is an enzyme expressed in *Pseudomonas fluorescens*, which is an abundant and critical bacterial component of the healthy soil microbiome. ICH is one of two known enzymes that hydrates isocyanides, which are cytotoxic to most bacteria. Previous kinetic studies on ICH crystals indicate a turnover number of ~0.3s^{-1} at steady state, with a pre-steady state burst whose rate constant is ~20 s^{-1}. In addition to being a relatively slow reaction, there is a significant difference in absorbance between a model substrate, p-nitrophenyl isocyanide, and the product, p -nitrophenyl formamide. ICH also diffracts to very high resolution in serial femtosecond crystallography experiments. These features make ICH an excellent model system to design and test mixing devices with millisecond resolution using UV-vis spectroscopy. We explore the capabilities of ICH as a model system to characterize mix and probe experiments at XFELs and create a methodology to apply to many systems.

BioXFEL 4TH INTERNATIONAL CONFERENCE

CAROLIN SEURING, UNIVERSITY OF HAMBURG

SINGLE LAYER GRAPHENE AS A LOW BACKGROUND SUPPORT FOR FEMTOSECOND X-RAY COHERENT DIFFRACTION IMAGING OF ALIGNED FIBRES

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The formation and deposition of amyloid aggregates in brain tissues is associated with a variety of serious human diseases, amongst which Alzheimer's disease, type II diabetes, Parkinson's and prion disease are the most prominent ones. Fibers are made from thousands of molecules well aligned in β-sheets around a central axis. Aligning molecules along a single dimension, fibers can be seen as diffracting species bridging the gap between a 3D crystal and the single particle.

At the LCLS, we have acquired X-ray fibre diffraction using a combination of low background graphene supports and a single nanofocused femtosecond X-ray free electron laser pulse. We can show that that the ultrabright XFEL beam allows to record well resolved diffraction up to ~2 Å from only a few fibers of Tobacco Mosaic Virus in a single shot or only a few amyloid fibrils when they are deposited on a free standing graphene substrate. Merging data from multiple measurements using computational alignment techniques from single particle imaging further improves signal levels while avoiding the averaging effects of powder diffraction. The low background provided by a monolayer graphene support shows promise as a sample delivery medium for fibres and larger single particles.

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Chufeng Li, Arizona State University

Improving Auto-indexing for Sparse Patterns in Serial Crystallography

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Superior brilliance and ultra-short pulse duration of X-ray Free Electron Lasers (XFEL) enable the studies of bio-molecular structures and conformational dynamics using the diffract-before-destroy method. Snapshot diffraction patterns from different crystals of various shape, size and orientation are recorded in a typical experiment using XFEL. This new diffraction method termed Serial Crystallography (SX) differs from X-ray crystallography using synchrotron sources where molecular structures are generally solved from a few big crystals in oscillation approach with a goniometer. These “still” diffraction images collected in SX experiments are indexed and merged to obtain a 3-D reconstruction of the reciprocal space that is Fourier transformed to the electron density map eventually. Auto-indexers such as MOSFLM and DIRAX, etc. were initially developed for synchrotron X-ray crystallography and are generally based on 3-D/1-D Fourier transform and projection algorithms. They also prove widely effective and successful for most of the protein SX data sets. However, these auto-indexers require that each diffraction pattern consist of no less than a certain number of Bragg peaks to yield a reliable orientation matrix. Therefore, these traditional auto-indexers become much less effective for sparse patterns that usually take a large portion of the whole data set.

In this work, we propose an auto-indexing algorithm for sparse patterns using the relative positions of 5 Bragg peaks from each pattern. Prior knowledge of the lattice parameters is needed as input for this sparse pattern auto-indexing algorithm. Alternatively, lattice type and constants can be determined from the virtual powder diffraction pattern. This algorithm is tested using a simulated data set from I3C crystals, an experimental data set from GPCR protein crystals data set (5HTB) and an experimental data set from I3C crystals collected at LCLS respectively. The indexing rate for the I3C data set from LCLS is increased by approximately 3-folds compared to that from CrystFEL pipeline. To verify that the new auto-indexing algorithm actually gives the correct solution, orientation matrixes are compared against that from DIRAX for patterns that are successfully indexed by both auto-indexers. Auto-indexing statistics of run 78 of LJ69 data set collected at LCLS is presented, which shows consistency between the solutions from the new algorithm and traditional auto-indexers. Finally, limits of the new auto-indexing algorithm were discussed from the aspects of computation time cost and accuracy.

This work is supported by the STC Program of the National Science Foundation through BioXFEL under Agreement No. 1231306, and ABI Innovation: New Algorithms for Biological X-ray Free Electron Laser Data under NSF grant No. 1565180.

David von Stetten, ESRF

Serial Synchrotron Crystallography using a High Viscosity Extrusion Injector Device on Beamline MASSIF-3 at the ESRF

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Serial Femtosecond Crystallography (SFX) on XFEL sources has provided a lot of new results recently by collecting diffraction images from many microcrystals, using a free-standing liquid jet, a stream of a viscous medium, or a solid mount. Here we present the experimental setup and X-ray diffraction data recorded from protein microcrystals embedded in a grease matrix, using a High Viscosity Extrusion (HVE) injector [Botha *et al.*, Acta Cryst D71, 387-397] at the MASSIF-3 beamline at the ESRF synchrotron. Up to a million still images were recorded within half an hour using an Eiger 4M detector, so that even a moderate hit rate of a few percent easily yielded tens of thousands of diffraction patterns that could be successfully indexed and which were of sufficient quality for structure solution.

Synchrotron sources provide a pseudo-continuous X-ray beam which favors this kind of experiment, as no synchronization is needed to ensure that the X-ray beam hits crystals embedded in the supporting medium. As a proof of concept, microcrystals of insulin were used in an injector experiment and gave diffraction data of sufficient quality to allow the structure to be solved using molecular replacement. Furthermore, experimental phasing of gadolinium-derivatised lysozyme crystals was possible with only a few thousand diffraction still images.

While femtosecond resolution in time-resolved experiments certainly needs an XFEL source, many other experiments can and should be done at synchrotron sites. Also, the availability of such a serial crystallography setup on a more readily available synchrotron allows the preparation and optimization of conditions for XFEL experiments.

DONGHYUN KA, SEOUL NATIONAL UNIVERSITY

STRUCTURE ANALYSIS OF CAS1-CSN2 COMPLEX IN *STREPTOCOCCUS PYOGENES* TYPE II CRISPR-CAS SYSTEM

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Clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated (Cas) proteins constitute an adaptive defense system against invading foreign nucleic acids in bacteria and archaea. Here, we report the crystal structure of *Streptococcus pyogenes* type II Cas1 (SpCas1) and its interaction with *S. pyogenes* Csn2 (SpCsn2) involved in spacer acquisition during CRISPR-mediated microbial immunity. The SpCas1 structure exhibits a unique conformational state distinct from type I homologues. We also show that SpCas1 directly interacts with SpCsn2 by using isothermal titration calorimetry and size-exclusion chromatography. We identified a putative binding interface and key residues for the complex formation. Our results provide structural information for a multi-protein Cas complex which functions in the type II CRISPR-Cas systems.

Edward Snell, Hauptman-Woodward Institute

The High-Throughput Crystallization Screening Center

Getacrystal.org

The High-Throughput Crystallization Screening Center housed at the Hauptman-Woodward Medical Research Institute has been performing crystallization screening experiments for academia and industry since Feb. 2000. Since that time over 16,000 different biological samples have been screened for crystallization.

Users submit a sample which is then screened using the microbatch under oil method against 1,536 different biochemical conditions with visual images recorded and sent to the user. UV and SONICC imaging also takes place. Instructions on how to reproduce the results, scale them up (or even scale them down) are available along with analysis tools to analyze each experiment as a group.

The process is efficient and cost effective with a significant fraction of samples going onto to PDB deposition. The use of the Center, the services it offers users, and the type of analysis that can be carried out on the data provided is described.

Elin Claesson, Gothenburg University

Elucidating the Structure of a Dark Form Phytochrome at Biologically Relevant Temperatures

Claesson E.[1], Edlund. P.[1], Takala. H[1,2,3], Henry. L.[1], Dods. R.[1], Lehtivuori. H.[4], Panman. M.[1], Pande. K.[5], White. T.[5], Nakane. T.[6], Berntsson. O.[1], Gustavsson. E.[1], Båth.P.[1], Modi. V.[7], Roy-Chowdhury.S.[8], Zook.J.[8], Berntsen. P.[9],Pandey. S.[10], Poudyal. I.[10], Tenboer. J.[10], Kupitz. C.[10], Barty. A.[5], Fromme.P.[8], Koralek J. D.[11], Tanaka. T.[12], Spence. J.[13,14,15], Liang. M.[11], Hunter. M. S.[11], Boutet. S.[11], Nango. E.[12], Moffat .K[16,17], Groenhof. G.[7], Ihalainen J. A.[18], Stojković E. A.[19], Schmidt. M.[10], and Westenhoff. S[1].

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Phytochromes are an important family of light-sensing kinases which control diverse cellular processes in plants, bacteria, and fungi. The photoreceptors are sensitive to visible light in the red and far-red region. Upon light absorption, several structural changes appear in the protein which lead to the activation/deactivation of an output domain. These structural changes are known to originate in the chromophore. However, the earliest steps in the structural evolution during signal transduction are still unclear. Serial femtosecond crystallography (SFX) provides atomic resolution on sub-picosecond time scales and is therefore an ideal method with which to study the structural changes in the photosensory core of phytochromes.

In this investigation, the room temperature crystal structure of the resting state of the chromophore binding domain (CBD) from the bacterial phytochrome *Deinococcus Radiodurans* is presented. Data were collected from protein microcrystals at the free electron laser at Linac Coherent Light Source (LCLS). The crystal structure is solved to a resolution of 2.1 Å and the structure agrees well with the existing steady-state structures recorded at cryogenic temperatures. The most significant differences between our results and the existing structures are found within the chromophore and in residues close to the chromophore-protein linkage. The results presented here are important steps in unravelling the sub-picosecond structural dynamics of the phytochrome photocycle.

Eugenio de la Mora, Institut de Biologie Structurale

Towards Time-resolved Serial Femtosecond Crystallography on Acetylcholinesterases Using Caged Compounds

Eugenio de la Mora

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X-ray free electron lasers (XFEL) enable structure determination from nanocrystals and time-resolved (TR) studies down to the fs time scale by use of Serial Femtosecond Crystallography (SFX) (1,2). The success of TR-crystallographic studies relies on efficient and synchronous triggering of a reaction within the crystal. Hence light-driven proteins, whose photodynamics can be triggered efficiently by UV-vis lasers, are most amenable to time-resolved crystallography. However, most proteins are insensitive to light, and therefore defy application of TR-SFX. In the early days of Laue crystallography, it was demonstrated that photolabile precursors of substrates or products, so called-caged compounds (CC), could be complexed with non-inherently photosensitive proteins, transforming these into light -activable molecular machineries (3). However, the crystal size required for high-resolution studies at synchrotrons, and their correspondingly high optical density, reduce uncaging efficiency. SFX on microcrystals is thus ideally suited to study irreversible reactions such as those triggered by uncaging CC.

Acetylcholinesterase (AChE) is an essential enzyme that terminates nerve impulse transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine (4). Its catalytic reaction is one of the fastest known in biology, with a turnover of 1 000-10 000 s^{-1} depending on species. The deeply buried nature of its active site has raised cogent questions as to how traffic of substrate and products, to and from the active site, occurs in such a confined environment. Based on molecular dynamics simulations (5) and temperature-controlled X-ray crystallography (6, 7), it was proposed that the enzymatic product choline could exit the active site by other routes than the active site gorge.

We present the room-temperature structure of *Torpedo californica* AChE determined from serial synchrotron crystallography at the ID13 microfocus beamline of the ESRF and show high-resolution, yet incomplete, static SFX data collected on CXI (LCLS). Since photolabile precursors of the enzymatic product choline are available (8) and have been shown to bind within the enzymatic active site (6), TR-SFX experiments on AChE are now within reach. They will allow obtaining structural snapshots on the microsecond time-scale of substrate and product trafficking within this rapid enzyme.

This research project was conducted by the following scientists: Thomas Barends, Sébastien Boutet, Manfred Burghammer, Jacques Philippe Colletier, Nicolas Coquelle, R. Bruce Doak, Fredrik Ekström, Lutz Foucar, Mark Hunter, Gabriela Kovacsova, Florian Nachon, Karol Nass, Ilme Schlichting, Robert Shoeman, Israel Silman, Joel Susmann, Martin Weik, Joyce Woodhouse.

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Fabiola Moreno, University of Puerto Rico

Sulfmyoglobin and Hydrogen Sulfide Metmyoglobin Formation in Metmyoglobin Crystals

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Hydrogen Sulfide (H_2S) is a colorless gas that can often be associated with multiple known chronic diseases, but can also be beneficial. It is known H_2S interacts with hemeproteins and affects their functionality. To study these events, metMyoglobin (metMb) crystals are grown and the best crystals are chosen to be soaked and exposed to H_2S which will act as a ligand. A similar process is done with other crystals, but instead of only soaking H_2S we will also soak hydrogen peroxide (H_2O_2). Crystals should remain stable after soaking for us to study the effect the H_2S has on the protein structure, whether $metMbSH_2$ or sulfmyoglobin is formed. Crystals are grown in the hanging drop vapor diffusion technique varying several conditions. Protein concentration ranges between 5 mg/mL and 15 mg/mL and pH from 4.6 to 7.5 maintaining a 4 °C temperature. Further research will be possible once we have our results. The crystals will allow us to determine the new structures using the Stanford Synchotron and X-ray Free Electron Laser (XFEL) radiation to obtain the corresponding electron densities. The data will allow to create various tridimensional models that depict the reaction process at the exact moment the formation of sulfmyoglobin and/or $metMbSH_2$ occurs.

This project was funded by the INBRE and the NIH STEP-UP program in collaboration with Stanford University.

Fatemehsadat Jamalidinan, University of WI-Milwaukee

Determining the Structure of K Channel-Proteins In Situ

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There is a remarkable shortage of the detailed knowledge of membrane proteins at atomic resolution despite the fact that they are the targets of many of today's drugs.
The reason is that membrane proteins tend to have large hydrophobic surfaces, which ensure their correct positioning in a membrane. However, this seems to make crystallization difficult, and this makes difficult traditional methods of structure determination by X-ray crystallography. In this paper, we take advantage of this very fact to suggest an alternative method for structure determination by X-ray scattering of the projected structures of membrane proteins in their natural environments within a membrane. Although in such environments, the proteins are not perfectly aligned as in a crystal, we find that the algorithm suggested by Pedrini [1] appears to promise structure determination perhaps down to atomic resolution. We also suggest how the method may be extended to obtain general (non-symmetric) 3D structures by exploiting the curved nature of Ewald spheres at lower energy.

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Frances Heredia Negron, University of Puerto Rico

Validation of Methods for Predictions of Tertiary Structure of DNA Aptamers

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Aptamers are single-stranded (ss) oligonucleotides that can exhibit specific therapeutic functions as well as unambiguous binding affinity to their target. Understanding the mechanism of their function, it requires to know their tertiary (3D) structure. Currently, the computer algorithms available only predict the 3D folding of ssRNA, but not ssDNA. In order to perform docking studies with ssDNA molecules against a target molecule, the results obtained using these current algorithms for ssRNA were modified by deleting the O2' atoms of the ssRNA molecule and uracil (Us) bases were transformed into thymines (**T**s). The main objective of this work is to introduce a novel technique developed for modeling ssDNA. We recreated a calculated 2D structure of a ssDNA, and then, inserted dsDNA parameters for three dimensionality. The reliability of our novel technique were compared with those results obtained using MC-Sym and FarFar, which are two algorithms used by other research groups for the adaptation of RNA aptamers into DNA structures. Finally, we will demonstrate validation experiments that shows how our results are in agreement with the structures of those aptamers elucidated by x-ray crystallography and proton NMR.

Gabriela Casanova, University of Puerto Rico

Micro-Crystal Sorting by Size-Exclusion Chromatography for XFEL Experiments

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University of Puerto Rico- Mayagüez[1], Arizona State University[2]

Serial crystallography at X-ray Free-Electron Lasers (XFELs) is developing to be a standard method for structure determination of proteins and enzymes. Growing the required micro-crystals in a reproducible way to generate a highly monodisperse crystal suspension can be challenging. While injection in a liquid jet does benefit from monodisperse samples, it is imperative for the collection of high-quality data. As the diffraction intensity scales with the crystal volume exposed by the X-rays as well as the X-ray intensity, analysis and data merging becomes difficult. Furthermore, mix-and-inject experiments to probe enzyme kinetics require a reproducible and uniform activation for the reaction.

Here, we present a method to pre-sort crystals by size before using them for an XFEL serial diffraction experiment. Two different batches of Photosystem I (PSI) micro-crystal suspensions were examined with Dynamic Light Scattering (DLS). Their size range was found to be 400 nm to 1 µm. These samples were run through a size exclusion chromatography column with Illustra Sephacryl S-100 Superfine resin and fractions of the sample were collected in 0.5 mL increments. Visibly green colored fractions were expected to contain significant crystal quantities and further analyzed by DLS.

The results of this study have been inconclusive. For one batch, the separation happened as expected with bigger crystals passing through the column faster than small crystals. For the other batch, which contained bigger crystals on average, an inverse behavior was observed. We believe that crystals that were too big were not able to pass through the column uninterrupted causing the separation to be inverted. Pre-filtering or the preparation of a custom resin designed to separate the crystals of specific sizes will help to improve this important method.

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Ganesh Subramanian, Arizona State University

From Picosend to Microsecond: Time-resolved X-ray Absorption Highlights Intermediates Formed During the Photolysis of Methylcobalamin

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Macrocycle based catalysts utilize their axial sites to engage in catalysis. The biologically active cobalamins follow the same mechanism wherein cleavage of the Cobalt-Carbon (Co-C) bond is the most crucial step. In order to fully understand this mechanism, numerous experiments have been performed, typically by optical excitation [1,2]. Concurrent theoretical work comprising of density functional theory (DFT) simulations [3] have also been performed. However, due to the lack of atomic resolution site-specific measurements, there is no conclusive evidence on the intermediate structures and/or the reaction pathways.

In this work, we studied the photolysis of methylcobalamin at the beamline 11-ID-D, at the Advanced Photon Source (APS). The experiments involved an optical laser excitation to initiate the reaction followed by X-ray probing the unoccupied density of states of the cobalt atom. The measurements were performed across timescales from picoseconds to microseconds and two stable intermediate signatures were identified. Electronic and geometric characteristics of the intermediates were assigned by using DFT-FEFF calculations. These computational studies were performed using allocations from the Arizona State University Advanced Computing Center (A2C2).

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Garrett Nelson, Arizona State University

3D Printing for Nozzle Fabrication

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The Gas Dynamic Virtual Nozzle (GDVN) is a microscopic liquid droplet injector that utilizes a gas focusing sheath rather than a solid-walled nozzle to deliver hydrated bioparticles into vacuum continuously for several hours to days without clogging. The crucial role of the injector in new biological imaging techniques such as serial femtosecond crystallography (SFX) prompts ongoing interest in GDVN development. We recently showed that 2-photon polymerization (2PP) can be used to produce a 3D-printed GDVN with submicron printing resolution and comparable performance during data collection at XFELs [1]. 2PP 3D printing has significant advantages over hand-grinding and flame polishing, as it enables rapid prototyping of new nozzle designs for flow focusing, mixing, and sheet jets and has the potential for highly reproducible nozzle production. Here we report improvements to the 3D printed GDVN approach that have enabled faster printing and simplified device preparation. We also report on anticipated improvements of nozzle performance at XFELs through predictable nozzle performance and nozzle arrays.

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HAZEL BORGES & ALFREDO REYES, UNIVERSITY OF PUERTO RICO

HEMOGLOBIN I AS A NOVEL PROTEIN DONOR OF HYDROGEN SULFIDE IN BIOLOGICAL SYSTEMS

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Hydrogen Sulfide is a well-known poisonous gas whose toxic effects have been studied for many years. In recent studies, it was found that H_2S is produced endogenously in humans through enzymatic pathways and has function as vasodilator, anti-inflammatory, antioxidant and smooth muscle relaxant. The tissue specific enzymes responsible for the physiological production of H_2S are: cystathionine-β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfur tranferase (3MST). As result, currently studies of H_2S are focused as signaling molecule involved in various physiological processes like NO and CO. Also, correlation of H_2S concentration in human physiology have been associated with diseases like: hypertension, Alzheimer, cancer, arthritis, diabetes, ulcerative colitis and cardiovascular diseases. Biochemical and physiological studies of H_2S have been performed by using compounds that release or promote production of H_2S in biological samples for its therapeutic attribution known as H_2S donors. Results in these studies had demonstrated that treatment with H_2S could ameliorate pathologies and thus the quality of life of the patients. Some of the disadvantages that have shown H_2S donors during research are: toxicity, stability in aqueous solution, uncontrollable release concentrations of H_2S and short lifetime in tissue. Therefore, it is crucial to find or develop a reliable H_2S-releasing that functions both in vitro and in vivo studies with reliable characteristics and controllable properties for better understanding the attribution of H_2S in physiological processes which still very unclear. Moreover, according to statistics from the American Heart Association in 2013, cardiovascular diseases were the number 1 cause of death in the United States surpassing deaths from cancer. For this reason, it is important to find a novel H_2S donor for the future treatment or therapy of these patients and diabetes patients resulting from these heart diseases. The goal of this research is to study the viability of the protein HbI from *Lucina Pectinata* and its derivatives for the delivery of H_2S in biological systems to obtain a reliable H_2S donor. This delivery would be examined on parameters of physiological relevance as endothelial function, oxidative stress, anti-inflammatory properties, cardiovascular system and disease in normal and diabetic rats. The physiological and pharmacological characterization of these delivery molecules is an essential step for the development of new drugs and therapies based on H_2S. If these H_2S-delivery proteins are successful and beneficial in the administration of H_2S, the results could lead us to the generation of new pharmacological strategies for the treatment of cardiovascular diseases and other types of therapy.

Hernan Machado, University of Puerto Rico

Formation of Sulmyoglobin Upon Addition of H_2S to Crystallized Oxymyoglobin

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Hydrogen sulfide (H_2S), a poisonous gas, is produced in small amounts in the human body. A correlation between H_2S quantities in the body and chronic diseases has been observed, and although there is no absolute certainty of this correlation, it is imperative to better understand the H_2S cycle. It has been proposed that the interaction between H_2S and myoglobin (Mb) may be a pathway to remove H_2S from the cellular space by changing oxymyoglobin (oxyMb) to sulfmyoglobin (sulfMb) as an intermediate step to finally produce sulfate. However, the details of this reaction are only partially known. This research project centered on studying the possibility of changing oxyMb crystals to sulfMb crystals by an H_2S soaking method. The following conditions were used to grow the Mb crystals, which were grown using the hanging drop vapor diffusion technique, used for the H_2S soaking: [Mb]= 13 mg/mL, 93% saturated solution of $(NH4)_2SO_4$ and 0.1M Tris-HCl buffer pH 7.5. The crystals were then soaked with H_2S to observe if a reaction between H_2S and crystallized oxyMb is possible, and the process resulted in a change of color, to green, of the crystals, indicating the presence of sulfMb. This provides evidence to conduct an X-ray Free Electron Laser (XFEL) test to measure electron densities of the crystallized oxyMb as it is reacting with H_2S. In this way, a complete understanding of the reaction between oxyMb and H_2S could be obtained.

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Indra Gonzalez, University of Puerto Rico

Hydrogen Sulfide (H_2S) Limits Lysozyme Amyloid Development and Enhances soft Matter Assembly

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Amyloid fibrils are conformations of misfolded proteins with a stable β-sheet structure. They are believed to be the main cause of neurodegenerative disorders such as Alzheimer's and Parkinson's disease. We have shown that by adding sulfhydric acid (H_2S) to hen egg white lysozyme (HEWL) the amyloid fibers are inhibited (UPR-Mayaguez Patent). This inhibition results in small spherical aggregates of unordered protein that exhibit almost no cytotoxicity. In the work presented here, the concentration of H_2S in samples of HEWL was varied to explore the associated soft matter assembly and changes in protein conformation. These changes were followed by ThT fluorescence to determine the behavior of the β-sheet structure. Samples without H_2S yielded high concentrations of β-sheet conformations, consistent with amyloid aggregates. However, the samples in the presence of H2S show little to no β-sheet structures. This indicates that the newly formed structures have a vastly different conformation to those of amyloid fibers. Therefore, the results show that the concentration of H_2S influences the protein's soft matter assembly whilst limiting the fibrillation structures. This discovery opens the door to further research into the procedure responsible for fibril inhibition and brings the possibility of applying these procedures as possible therapies for amyloidosis or in industrial processes, like insulin manufacturing and storage.

Izumi Ishigami, Albert Einstein College of Medicine

Serial Femtosecond X-ray Crystallography of Carbon Monoxide-Bound Cytochromoe *c* Oxidase

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Cytochrome *c* oxidase (C*c*O), the terminal enzyme in the electron transfer chain, tranlocates protons across the inner mitochondrial membrane by harnessing the free energy generated by the reduction of oxygen to water. Several mechanisms for this redox coupling have been proposed but they lack confirmation, in part from uncertainties in the reported crystal structures due to radiation damage effects caused by the intense synchrotron radiation. Here we report the damage-free structure of the carbon monoxide (CO) bound derivative of bovine C*c*O (CO-bC*c*O) obtained by serial femtosecond X-ray crystallography (SFX) with an X-ray free electron laser and we compare it to a CO-photodissociated derivative obtained at a synchrotron light source. In the SFX structure resolved at 2.3Å, the CO is coordinated to the heme a_3 iron atom and is bent to 134^o from the heme plane, whereas in the structure of CO-bC*c*O obtained by synchrotron radiation, at a resolution of 1.95Å, the Fe-CO bond is cleaved and the CO has moved to a position near Cu_B. Associated with CO dissociation from heme a_3, the distance between the heme a_3 iron atom and Cu_B changed from 5.27 to 4.91Å, primarily owing to a change in position of Cu_B. Surprisingly, an allosteric transition involving a large movement of a section of the Helix-X polypeptide that lies between the two hemes, is triggered by the change in ligation state. This transition plays a critical role in postulated mechanisms of proton translocation in mammalian C*c*Os.

Jennifer Vargas-Santiago, University of Puerto Rico

Hydrogen Sulfide (H_2S) Trapment by Hemoglobin I from *Lucina pectinato* Encapsulated in Sol-gels

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The scientific community has shown interest in H_2S due to numerous physiological effects and implications in various diseases. This work describes a protocol to scavenge H_2S gas or aqueous state by using tetramethylorthosilicate gel and Hemoglobin I from *Lucina pectinata.* It possesses high affinity for H_2S and the bond does not affect his protoporphirine as occur in human hemoglobin and myoglobin. Here, we focus on determining the association and dissociation rate constants for the rHbI-H_2S complex encapsulated using a simple kinetic model. Results show that the H_2S complex affinity when protein is encapsulated in sol gel was smaller than the corresponding value in solution, as a consequence of hydrogen bond interactions between H_2S diffusion through of the tetramethylorthosilicate structure. This affinity was not affected by presence of ROS. Therefore, these results show that the encapsulated HbI is a good prototype to trap efficiently H_2S. Nevertheless, further improvements in the HbI immobilization system are necessary before future applications.

Jesse Coe, Arizona State University

Time-resolved Crystallography at XFELs: Advantages, Challenges, and Potential

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As XFELS grow out of their infancy and begin to proliferate, the techniques and methodology mature. While work at synchrotrons and other X-ray light sources will continue to contribute heavily towards the growing knowledge base of macromolecular structures, investigating dynamics using these instruments remains highly restricted. Through serial femtosecond crystallography (SFX), XFELs offer the ability to probe structural dynamics both in the ultrafast regime and at a much finer time scale, pushing towards the realization of true "molecular movies" where conformational and enzymatic dynamics can be understood in the time regimes that they actually occur.

This advantage is underscored by the ultra-compact femtosecond pulses and nano- and micron sized crystals utilized in SFX. The ultrafast time regime allows higher temporal resolution along a reaction timeline as well as the ability to probe much shorter delay times than can be realized with other structural techniques. The exploitation of small crystals compliments this, allowing a tighter temporal reaction homogeneity which is particularly important for short lived intermediates. Many pump-probe style experiments have been successfully achieved using XFELs to date, extending from validating proof-of-principle and boundary establishing techniques to novel understanding of macromolecular dynamics, comprising the first true "molecular movies". More recently, diffusion based "on-the-fly" mixing experiments have been successfully conducted, paving the way for a whole new level of understanding in enzymatic structure-function relationships. As structural biology at XFELs emerges as an established technique, time-resolved studies exemplify the incredible potential of what this field can offer to the scientific community at large.

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JOCHEN KÜPPER, DESY-CFEL & UHH

COMMOTION - CONTROLLING THE MOTION OF LARGE MOLECULES AND PARTICLES

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Introduction:
Recent years saw the development of several techniques to control and confine various degrees of freedom of neutral molecules. We can now routinely select single structural isomers of small molecules, disperse rotational quantum-states, and in certain cases create single-quantum-state samples [1]. These are ideally suited for further manipulation using alignment and orientation techniques [2], allowing experiments to be carried out within the molecular frame of reference. Thus, direct information on the nuclear and electronic structure can be obtained. Here we report on our efforts to extend the available techniques to larger systems, from (poly-)peptide molecules to entire cells or viruses.

Methods:
Using soft vaporization techniques, such as laser or acoustic desorption, allows the production of large volatile (bio) molecules in the gas-phase. These can subsequently be cooled using supersonic expansions or cryogenic buffer-gas cells. Different approaches are needed for the production of nanoparticle-sized systems. While these can be introduced into the gas-phase using liquid microjet injectors, achieved densities are typically low and particles encased in solvent. We are working on overcoming these issues through the use of aerodynamic lens stacks [3] and convergent-nozzle focusing injectors [4] to produce controlled particle beams. These controlled particle beams can subsequently be manipulated using optical techniques, such as tractor beams or optical pipelines [5], to focus particles further and steer them into a well-defined interaction volume.

Results:
We present first results from our new laser desorption and laser-induced acoustic desorption setups. These have been designed specifically for incorporation into x-ray-free-electron-laser experiments and provide the required experimental flexibility and necessary long data collection times. For aerosol particle injectors we present novel characterization techniques that allow direct on-the-fly visualization of the particle stream, as well as determination of the particle concentration and velocity within the formed beam [6]. These tools significantly aid in alignment of tightly focused or collimated particle beams with nano-focused X-ray beams in single-particle diffractive imaging experiments. We, furthermore, report on progress of controlling the translational degrees of freedom of aerosol particles in vacuum using shaped laser beams, in particular by utilizing photophoretic forces and trapping particles in light minima, working towards an optical funnel for delivering particles with micrometer precision into an interaction region.

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John Westbrook, Rutgers University

Extending PDB Data Architecture to Support XFEL Experiments

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By enabling measurements of submicron crystalline samples, X-ray Free Electron Laser (XFEL) experiments provide significant new opportunities to study structure, function, and dynamics of biological materials. Capturing and archiving data from these groundbreaking experiments represent an important goal of the Worldwide Protein Data Bank (wwPDB; wwpdb.org) organization. Through its global collaboration, the wwPDB has developed OneDep, a unified platform for deposition, biocuration, and validation of 3D biological macromolecules experimentally determined by X-ray crystallography, NMR spectroscopy, and 3D Electron Microscopy. Data are submitted to the PDB archive *via* this OneDep system. OneDep is designed to help the wwPDB and the global structural biology research community meet the challenges of rapidly changing technologies, such as XFEL, and keep pace with evolving data archiving needs over the coming decades. The OneDep system is underpinned by a flexible data architecture based on the PDBx/mmCIF dictionary (mmcif.wwpdb.org).

The Crystallographic Information Framework (CIF) was an early data and publication standard of the IUCr for diffraction experiments on small molecules. During the 1990s, CIF was extended to describe diffraction experiments with macromolecules. The resulting mmCIF data standard was adopted by the wwPDB for metadata management and data exchange (PDBx/mmCIF) in 2003. By engaging community experts in workshops and targeted working groups, the wwPDB subsequently worked with the NMR, 3DEM, and small-angle scattering research communities to extend the metadata framework (PDBx/mmCIF) to represent and exchange the data required to archive and validate structures obtained from these experimental methods.

Recent efforts of the wwPDB PDBx/mmCIF Working Group have focused on developing recommendations for data extensions for XFEL experiments, including unmerged intensity data. In this poster, we highlight extensions to the PDBx/mmCIF data specification designed to support deposition of structures determined by XFEL methods together with planned content extensions for X-ray diffraction experimental data.

wwPDB Members: RCSB PDB (supported by NSF DBI-1338415, NIH, and DOE); PDBe (EMBL-EBI, Wellcome Trust, BBSRC, EU, and MRC); PDBj (JST-NBDC); and BMRB (NIGMS).

BioXFEL 4TH INTERNATIONAL CONFERENCE

Jose Carmona, University of Puerto Rico

Crystallographic Studies of Ferrocene-Estrogen Derivatives Complexed with Human Serum Albumin and Estrogen Receptor Alpa as Potential Drug Delivery Targets

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Unlike common drugs that consist of entirely organic groups, the incorporation of metal in organic frames provides a new range of not only structure and bonding properties but also reactivity. A new chapter in bioinorganic chemistry was open after cisplatin antineoplastic activity was discovered and introduced as a chemotherapeutic agent 30 years ago. However, secondary side effects caused by a lack of selectivity between healthy and cancer tissues rendered the drug useless. Recently, ferrocene has been introduced for biological applications due its antineoplastic properties on Erlich ascite tumor. In order to develop a new class of metal-based therapeutic drugs with high selective index for hormone dependent breast cancer, a series of estrogen hormones have been functionalized with ferrocenes as pendent groups. These drugs will be used to target estrogen dependent breast cancer (ER+), which account more than the 50% percent among the different breast cancers. We have synthesized a series of ferrocene-estrogen complex derivatives that demonstrate micromolar antiproliferative activity on hormone dependent MCF-7 breast cancer cell lines. Computational studies of the interaction of the ferrocene complex with estrogen receptor protein were performed, and demonstrated the possibilities of docking interaction of these drugs in the ligand binding pocket of the estrogen receptor alpha (ERα). In this work we will present our ongoing efforts to elucidate and validate the effectiveness of the hormone moiety part of the drug that is serving as a vector to target a hormone-dependent MCF-7 cell line as well as specific physiological targets. Two main proteins were used as models: Human Serum Albumin (HSA), a protein carrier in blood serum plasma, and Estrogen Receptor alpha (ERα), a nuclear protein. Elucidation of specific drug-receptor interactions will contribute to our understanding of drug delivery and inhibition and will allow the prediction of future novel drug actions.

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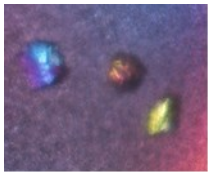
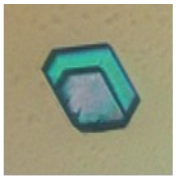
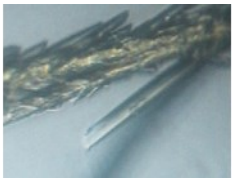
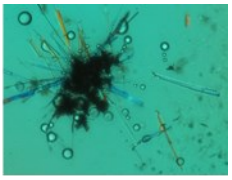

Jose Manuel martin Garcia, Arizona State University

Monochromatic and Pink Beam Serial Millisecond Crystallography at the Advanced Photon Source

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The scarcity of XFEL facilities severely limits the use of serial femtosecond crystallography (SFX) while synchrotron sources are becoming viable options as a real alternative for serial millisecond crystallography (SMX) experiments. As a result, the number of SMX experiments is rapidly growing and, so far, ten experiments have been reported. Here, we present the first injector-based SMX experiments carried out at a U.S. synchrotron source, the Advanced Photon Source (APS). Monochromatic beam experiments were conducted at the GM/CA 23-ID-D beamline. Micro-crystals of a wide variety of proteins including lysozyme, thaumatin, PSII, phycocyanin, human adenosine A_{2A} receptor (A_{2A}AR), beta -2 adrenergic receptor (β_2AR), KDO8PS, FLPP3, and Proteinase K were screened. The 5 - 20 µm crystals were delivered to the beam suspended in lipidic cubic phase, agarose or a high molecular weight PEG (MW=8,000,000), using a high viscosity injector. For each protein target, tens to hundreds of thousands of diffraction patterns were collected by a Pilatus 6M detector in shutterless mode at a repetition rate of 10 Hz, using a photon energy of 12 keV and 10 µm diameter (FWHM) beam size. In-house hit-finding software developed at APS and SFX data-reduction and analysis software suites, Cheetah and CrystFEL, enabled efficient SMX data monitoring, reduction and processing. Although hits were found for almost all proteins tested, the best diffracting crystals were from the A_{2A}AR, proteinase K, phycocyanin, FLPP3, thaumatin, and lysozyme with hit rates of 3.0%, 4.2%, 5.0%, 11.6%, 15.1%, and 34.2%, respectively, and corresponding indexing rates of 36.0%, 18.0%, 23.1%, 23.6%, 3.3%, 15.0%, respectively. The structures of the A_{2A}AR, phycocyanin, FLPP3 and lysozyme were determined to 3.2 Å, 3.1 Å, 3.0 Å, and 2.2 Å resolution, respectively.

Pink beam experiments were conducted at the BioCARS beamline (14-ID-B) on micro-crystals in which each crystal was exposed to the X-rays from only a single ~100 picosecond bunch or 4 consecutive bunches separated by 153 ns for a total exposure time of 560 ns. The broad bandwidth (5%) yielded full reflections and thus only a few thousand diffraction patterns were required to build a complete dataset of phycocyanin to 2.7 Å resolution. Preliminary experiments were carried out with the high viscosity injector for A_{2A}AR, lysozyme, phycocyanin and proteinase K. In order to collect complete data sets more data will be collected for these four proteins at an upcoming beamtime.

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.

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Jose Olmos & Christopher Kupitz, Rice U & ASU

Mix and Inject: a βLaC Story

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ß-lactam antibiotics are antibiotics containing a lactam ring, many of which are used to treat diseases worldwide including tuberculosis, syphilis, and staphylococcus. Unfortunately, the bacteria responsible for these diseases are becoming resistant to such forms of antibiotics. Bacteria have developed an effective defense mechanism using special enzymes known as ß-lactamases. Regrettably, this defense mechanism is causing ß-lactams to rapidly become less effective and in some cases, almost obsolete. For example, tuberculosis, despite significant advancements in treatment, remains a major cause of human morbidity, killing over 1.5 million people worldwide in 2014. Successful time-resolved experiments with ß-lactamases could revolutionize the treatment of this disease and other deadly diseases by providing critical information of protein dynamics. These insights could lead to the creation of inhibitors that block ß-lactamases or antibiotics that avoid the need for blockage altogether. Time-resolved serial femtosecond crystallography (TR-SFX) offers an approach to determine the uncharacterized structural intermediates of enzymes during these irreversible reactions by employing the extremely brilliant femtosecond X-ray pulses produced by free electron lasers (FELs), protein crystals on the micrometer length scale (microcrystals), and the mix-and-inject technique.

Mix-and-inject crystallography (MIC) is an emerging method being developed specifically for studying the reactions catalyzed by enzymes in which microcrystals are mixed with a substrate just prior to being probed by an ultrafast X-ray pulse. By comparison with mixing cell solution-scattering studies at synchrotrons, this new approach offers three advantages – it gives atomic resolution images, while providing adequate time resolution, and a homogeneous reaction, through the use of micron-sized crystals, throughout which diffusion of the substrate is rapid compared with the reaction time. Finally the method out-runs radiation damage by using femtosecond X-ray pulses. We present results that demonstrate time-resolved structure-based enzymology is feasible using MIC and FELs. In this TR-SFX experiment we studied the reaction of ß-lactamase microcrystals from *M. tuberculosis* mixed with a ceftriaxone antibiotic solution. Electron density maps at 2.4 Å resolution of the ß-lactamase, ceftriaxone product were determined, showing there is an additional electron density feature visible in the binding pockets of ß-lactamase. This difference electron density feature corresponds with models of ceftriaxone, demonstrating that mixing was successful. These results pave the way to further studies of irreversible enzymatic reactions and represent an entirely new field of time-resolved structural dynamics for enzymological systems.

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Josiris Rodriguez, University of Puerto Rico

Isolation and Primary Structure of a Novel Protein Associated with HbI from *Phacoides pectinatus*

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Cell protection from harmful substances can be a response modulated by the type of habitat of an organism. Some marine organisms contain highly specialized proteins that allow resistance to a high concentrations of hydrogen sulfide. *Phacoides pectinatus* harbor an uncultivable symbiont along with a high concentration of an unnamed cysteine rich protein (CRP) and a high concentration of hemeproteins. Protein interactions in the presence of hydrogen sulfide remain to this day unclear. CRP can play an important role in both transport and metabolize mechanisms of hydrogen sulfide, hence this project aims to obtain the primary structure of CRP. An initial batch of recently purified protein was obtained from native source. Protein purity was monitored using SDS page electrophoresis and concentration was determined using UV-Vis characteristics bands with previously published extinction coefficients. Di novo sequencing was performed combining MS/MS with Edman degradation. Data showed that CRP is a monomer. It contains 254 amino acids with a corresponding average molecular weight of 26,198 Da. MS data suggest the correlation of CRP with hemoglobin I (HbI). The function of CRP remains to be determined.

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Juan Valentin Goyco, University of Puerto Rico

Nanodisc Assembly and Characterization

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Nanodiscs are self-assembled membranes that are derived from phospholipids and genetically engineered “membrane scaffold proteins” (MSP). They provide an efficient way of characterizing membrane proteins, as they provide a native-like environment that stabilizes the target. Hence, this technology proves useful when carrying out biophysical and chemical techniques to study these proteins. Thus, the further characterization of nanodiscs proves useful for the development of such studies. Here we report the method to express and purify MSP and assemble nanodiscs. Their assembly was successfully validated through Size Exclusion Chromatography (SEC) and Dynamic Light Scattering (DLS).

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JUNHYUNG KIM, SEOUL NATIONAL UNIVERSITY

CRYSTAL STRUCTURE OF PSYCHROPHILIC ADENYLATE KINASE FROM ANTARCTIC FISH

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Psychrophiles are extremophilic organisms capable of thriving in cold environments. Proteins from these cold-adapted organisms can remain physiologically functional at low temperatures, but are structurally unstable even at moderate temperatures. Here, we report the crystal structure of a psychrophilic adenylate kinase (AK) from Antarctic fish, *Notothenia coriiceps*, and identify a key residue substitution responsible for its reduced stability compared to mesophilic homologues. In its central core domain, the psychrophilic protein displays suboptimal hydrophobic packing caused by a Val residue, which is replaced with Ile residues in homologues from tropical and subtropical fishes. The Val-to-Ile mutation resulted in a substantial enhancement in thermal stability of the *N. coriiceps* AK.

Lars Redecke, University of Lübeck

Serial Crystallography *In Cellulo*

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X-ray crystallography requires the growth of well-ordered, sufficiently sized protein crystals to obtain structural insights at atomic resolution. In addition to routinely performed parameter screening *in vitro,* protein crystallization in living cells, referred to as *in vivo* crystallization, holds the possibility to grow a huge number of micron-sized protein crystals with comparable properties and of high quality in a short time, with an enormous potential for structural biology (1-3). In this context, we are currently establishing an easy-to-use pipeline to exploit living cells as native bioreactors for the production of micro- and nano-crystals of recombinant proteins. These efforts resulted in the successful crystallization of more than 25 different proteins so far. However, the number of crystal containing cells varies between more than 70 % and less than 1 %, depending on the recombinant protein itself. If large amounts of crystals that exhibit sufficient stability after isolation from the cell are obtained, high-resolution structural information can be extracted applying serial diffraction data collection at free-electron lasers (XFEL) or highly brilliant synchrotron radiation sources, as already reported (4-5).

To avoid any loss of crystal quality due to changes of environmental conditions during cell lysis and crystal purification, which will restrict the resolution of the diffraction data, we recently established different approaches for diffraction data collection directly within the living cell, denoted as *in cellulo* crystallography. As a model protein we used inosine-5'-mono phosphate dehydrogenase (IMPDH) of *Trypanosoma brucei*, the pathogen causing sleeping sickness, which is an attractive potential drug target. Within insect cells, IMPDH forms needle-shaped crystals with a diameter of approximately 3 µm and a length of up to 30 µm. Applying serial synchrotron crystallography (SSX) at EMBL beamline P14 of the PETRAIII synchrotron source at DESY (Hamburg, Germany), a complete dataset was recorded *in cellulo* under cryo-conditions. Inside the intact cellular environment IMPDH crystals diffracted up to 1.6 Å, representing a gain of ~ 1 Å in resolution compared to diffraction of isolated IMPDH *in vivo* crystals previously collected by applying SFX techniques at an XFEL.

Moreover, we established an *in situ* approach for serial diffraction data collection at room temperature using adherent living insect cells directly in the cell culture plate, again resulting in an improved resolution of the IMPDH *in vivo* crystal diffraction data without significant diffraction of the cellular background. This innovative approach avoids any transfer of the living, crystal containing cells. More importantly, direct screening of insect cell cultures for successful *in vivo* crystallization of recombinant proteins using the X-ray beam proposes overcoming limitations in data collection due to low intracellular crystallization efficiency. Thus, the implementation of *in cellulo* diffraction data collection will further improve the current *in vivo* crystallization pipeline. Moreover, our results pave the way to use crystal containing cells as suitable targets for serial diffraction data collection at an XFEL in the future.

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Louise Lassalle, LBNL

Structure of Photosystem II and Substrate Binding at Room Temperature

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Photosynthesis converts light energy into biologically useful chemical energy vital to life on Earth. This process involved photosystem II (PSII) membrane protein, a 700 kilodalton homodimeric protein complex. PSII catalyzes the photo oxidation of water into dioxygen through at the Mn4CaO5 cluster in the oxygen-evolving complex (OEC). Under illumination, the OEC cycles through five intermediate S-states (S0 to S4) (1), where S1 is the dark-stable state and S3 is the last semi-stable state before O-O bond formation and O2 evolution2,3. The structure of PSII in the dark state has been solved by X-ray diffraction and X-ray free electron laser (XFEL). To understand the O-O bond formation mechanism, elucidating the structures of the OEC in the different S-states is prerequisite.
Previously, we perform a extend study with ammonia to investigate the water-binding site(s) and discriminate between mechanisms proposed in the literature.

Here, we report the *room temperature* structures of Photosystem II in the illuminated S_3-state at 2.8 Å and the dark S_1-state at 3.0 Å resolution obtained using an X-ray free electron laser. Major structural changes are not observed either in the peptide backbone or the Mn_4CaO_5 cluster between the dark and illuminated states, precluding mechanisms that require large changes in the S_3 state. The potential binding sites for NH_3, a water analog, are discussed, based on the 2-flash illuminated state data, which has important implications for the mechanism of water oxidation.

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Ti-IV Bond Transferrin X-ray Crystal Structure Reveals Citrate's Synergistic Role in Ti-Transferrin Binding

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Metals play an important role in human biology as they act as co-factors in many enzymes. Although transport and uptake of the dietary important metals like iron and zinc are well understood, the mechanism of uptake of non-essential metals like titanium (Ti) is obscure. Humans are exposed to titanium(IV) through its presence in food, cosmetic additives, and by bone implants. Studies have shown that Ti concentrations greater than 100µM show cytotoxicity. However, the dominant soluble Ti(IV) species in blood exhibits no cytotoxicity even at 100 µM concentration. Serum Transferrin (sTf) and citrate are supposed to be responsible for maintaining nontoxic Ti(IV) speciation in the blood. Our X-ray structure of Ti(IV) bound sTf reveals the molecular details of Ti(IV) interaction with serum transferrin (sTf) where sTf binds Ti(IV) in an unusual open conformation with citrate as a synergistic anion. Cellular studies of Ti uptake in A549 cells show that contrary to some previous reports, open conformation of transferrin is capable of transporting Ti(IV) into cells. Our results provide the first glimpse into citrate-transferrin synergism in Ti(IV) transport and how the citrate anion impacts Ti speciation, providing an explanation for the failure of anticancer Ti drugs like titanocene dichloride in clinical trials.

Margaret Gabanyi, RCSB Protein Data Bank

BioSync: An Online Resource for X-ray Facilities Worldwide

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Since 1990, the BioSync website (biosync.sbkb.org) serves as central technical resource for the structural biology community. BioSync provides up-to-date information for over 150 synchrotron radiation beamlines available at 31 high -energy facilities around the world. BioSync also serves as an important annotation resource for the PDB by helping connect released PDB structures to the facilities from which the data was collected. BioSync has been a central resource to beamline facility scientists, researchers and funding agencies. Features of BioSync include:

- Links to existing synchrotron facilities, now including XFEL facilities and beamlines
- Beamline characteristics (energies, flux, wavelengths supported, etc.)
- Information about available services such as remote data collection, mail-in, crystallization and structure solution services, robotics for crystal screening and mounting, microfocus beams and facilities for collecting data under extreme conditions.
- 'Contact Beamline' mechanism so that users can email beamline support staff with inquiries
- Progress on future facilities is tracked and information on decommissioned sites is maintained for historical purposes.
- Summary statistics, based on PDB depositions, are updated weekly.
- At the beamline level, galleries of structures, tables of citations and general information are also available.

With the rise of serial femtosecond nanocrystallography (SFX), BioSync is in the process of adding all of the worldwide X-ray Free Electron Laser facilities and beamlines to our catalog. Our curated database contains the latest status and information about existing, future, and also decommissioned beamlines. The recently updated database contains new support for XFELs, and describes the various beamline characteristics, and special facility capabilities to inform any prospective facility user. We have also redesigned our beamline pages so that researchers can find the right synchrotron, XFEL, or other high energy beamlines at-a-glance.

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Martin Mechelke, Max Planck Institute

Bayesian Structure Reconstruction from Single Molecule Scattering Data

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Single molecule X-ray free electron laser (XFEL) scattering experiments promise to alleviate many of the problems typically associated with X-ray crystallography; it does not require crystals and makes time resolved structure determination possible. In single molecule scattering experiments, hundred thousands of noisy scattering patterns need to be combined into a single coherent 3D density to derive at a 3D molecular structure with atomic resolution. A major difficulty in the determination process is that the orientation of each pattern cannot be measured and needs to be estimated along with additional unknown parameters like incident fluence fluctuations and non-uniform background distributions.

Here, we state the reconstruction problem as Bayesian inference problem, where the orientations R and auxiliary parameters ξ are treated in the same way as 3D structure X. The resulting in the posterior over the data D $p(X, R, \xi|D) \propto p(D|X, \xi, R)\, p(X)\, p(\xi)\, p(R)$ factors into several components: the likelihood function $p(D|X, \xi, R)$ quantifies the agreement between observed data D and structure X and the priors on X, ξ and R the encode our prior knowledge and help to prevent overfitting and help to exclude spurious local minima.

We employ a Gibbs sampling scheme in which we iteratively sample from each variable's conditional distribution to generate samples from the joint posterior distribution. To keep sampling from $p(X|R,\xi,D)$ computational tractable, we use modern stochastic gradient techniques [1].

We evaluate our method on an experimental dataset of the Mimivirus particle[2]. After phase reconstruction from the 3D reciprocal density with RAAR [3] the icosahedral shape is clearly recognizable. We achieved a resolution of 100nm, estimated by Fourier shell correlation. Furthermore, we validated the method on simulated scattering data of Ubiquitin. For the simulated data, we were able to reconstruct the electron density with a resolution of 8Å based on 40000 sparse scattering images, with on average 50 scattered photons each. Our results suggest that structure reconstruction even from challenging data can be solved in a probabilistic framework.

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Matthew Coleman, LLNL - UC Davis

Cell-free Translation Systems for Production of Membrane Bound Protein Complexes for BioXFEL Studies

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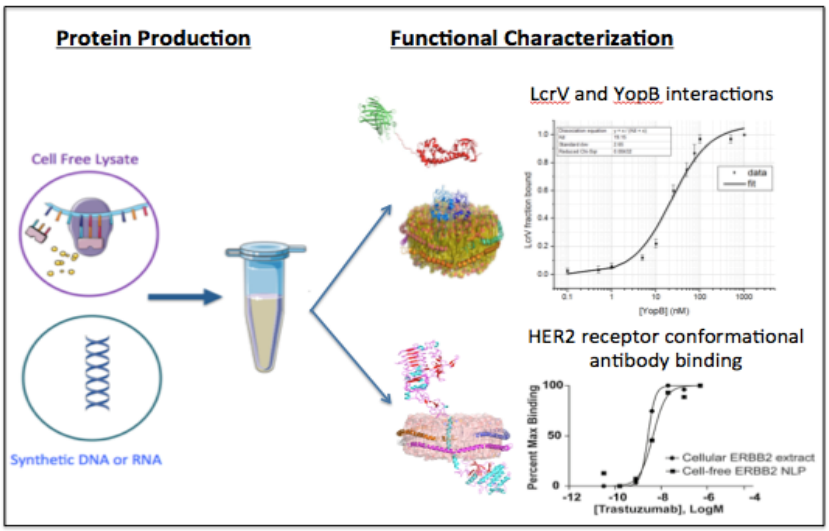
Cell-free expression systems have been utilized to produce "difficult" proteins, such as toxic and membrane bound proteins. Specifically, we have focused on using nanolipoprotein particles (NLPs), a.k.a. nanodiscs, as a support scaffold during cell-free expression for producing membrane proteins (See Figure below). NLPs are 10-20 nm particles containing an apolipoprotein that surrounds a lipid bilayer and represents a membrane mimetic. Here, we discuss how our laboratory has incorporated NLPs with cell-free technologies to produce protein complexes, and trans-membrane proteins with high yields that result in an abundance of material that may be suitable for future XFEL-based studies. We have used cell-free coexpression with NLPs to produce a wide range of active enzymes and receptors such as rhodopsins, G-protein coupled receptors (GPCRs), kinases, cytokines, antibodies, proteases, cell wall hydrolases, and secretion system complexes. After purification, proteins and protein complexes were used for biophysical and biochemical characterization by fluorescence correlation spectroscopy (FCS), circular dichroism (CD), electron microscopy (EM) and small-angle X-ray and neutron scattering (SAXS/SANS) analysis. The NLPs and NLP-membrane protein complexes are also amenable to crystallization techniques. Our process may provide a path forward for single particle analysis using XFELs based on the homogeneity of nanoparticles. Overall, cell-free expression represents a unique solution to address multiple bottlenecks in the production, purification, and characterization of membrane proteins that were previously difficult to obtain.



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Matthias Frank, LLNL - UC Davis

Solid Sample Support Approaches for Biological Imaging at XFELs

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Serial femtosecond nanocrystallography (SFX) and single particle imaging (SPI) of biological nanoparticles have been demonstrated successfully in a number of experiments at LCLS and SACLA over the last years. Most SFX and SPI experiments to date have used liquid-jet or aerosol particle injection based sample introduction approaches, respectively, that require large amounts of sample. Fixed target approaches that allow diffraction measurements on samples supported by solid sample supports at room temperature are a viable alternative to the injection based sample introduction approaches, at least for some types of biological imaging experiments at XFELs. Fixed target approaches are expected to open up opportunities for new types of time-resolved SFX on samples that are not abundant and/or require the sample to be flat (such as 2D crystals). Fixed target approaches may also be useful for SPI. Here, we present fixed-target results from experiments at LCLS that included 2D crystal samples of the membrane protein bacteriorhodopsin and 3D microcrystal samples of REP24, a soluble protein, supported by thin silicon nitride membranes as well as more recent results involving samples supported by graphene layers spanning large arrays of ~10 m holes in silicon wafers. We present strategies for further addressing current challenges for fixed target sample introduction, such as optimizing sample deposition, minimizing background from sample support, maintaining sample hydration, and increasing speed of data acquisition. We also discuss the potential usefulness and limitations of fixed target approaches for SPI.

This work was performed under the auspices of the US Department of Energy by Lawrence Livermore National Laboratory under contract DE-AC52-07NA27344 and Pacific Northwest National Laboratory under Contract DE-AC05-76RL01830. Parts of this research was carried out at the Linac Coherent Light Source (LCLS) at SLAC National Accelerator Laboratory. LCLS is an Office of Science User Facility operated for the US DOE Office of Science by Stanford University. Support provided by LLNL Lab-Directed Research and Development Project 012-ERD-031, NSF STC award 1231306 (BioXFEL Center), the PNNL Chemical Imaging Initiative and NIH grant number 5R01GM117342-02.

BioXFEL 4TH INTERNATIONAL CONFERENCE

Max hantke, Uppsala University

Smaller Droplets for Bigger Success

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Aerosol sample injection with gas-dynamic virtual nozzles (GDVNs) has been demonstrated as an efficient sample delivery method for relatively large objects (100-2000 nm diameter) in flash-diffractive X-ray imaging (FXI)[1–4]. Delivering the sample as an aerosol without any container avoids parasitic scattering from solid supports and allows high-speed data collection[2].

Despite success with big samples, the large droplets of GDVNs create problems when working with smaller samples[5,6]. Non-volatile impurities in the droplets settle on the surface of the samples, and if the droplets are large compared to the sample size, a considerable "caking layer" can form on the surface of small samples. To avoid this, we need to tune the droplet size to approach the sample size. For samples of roughly 100 nm in diameter or smaller, the non-volatile residues from micron-sized droplets of GDVNs can be significant. In-house data from Mie scattering imaging indicates that the caking layer can be as thick as 10 nm from micron-sized droplets when injecting semi-purified carboxysomes of 100 nm diameter. To combat this problem, we replaced the GDVN nebuliser on the injector with a specially-adapted electrospray nebuliser, which produces only about 150 nm droplets as compared to 1000-2000 nm droplets with the GDVN. A simple calculation shows, that the reduction of the droplet volume reduces the thickness of the caking layer to less than one Å. In a recent experiment at the Atomic, Molecular & Optical Science (AMO) end station of the LINAC Coherent Light Source (LCLS), we tested the new system, and demonstrated the successful operation of the modified Uppsala injector with similar hit ratios as with GDVNs and with sample sizes down to 35 nm. This development paves the way for studies of sample particles as small as single protein molecules.

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Max Wiedorn, CFEL

High Speed Fixed Target Serial Crystallography

M. O. Wiedorn, A. Meents and H. N. Chapman – CFEL, Hamburg, Germany

Serial crystallography has become a standard technique for the structure determination of proteins[1] and many structures have been deposited into the PDB using this technique. Still, one of the challenges is posed by efficient sample delivery. Efficiency consists of several parameters: hit rate, sample consumption and data quality. The hit rate measures the fraction of X-ray pulses that intercept a sample crystal. Therefore, it describes whether an experiment makes good use of the available instrument time. Increasing the amount of sample delivered to the X-ray interaction region can improve the hit rate but this ties directly into the sample consumption – a measure of how efficient the sample is used. Many interesting samples are limited in availability. Therefore, efficient use of the often limited amount of sample is crucial for a well designed experiment. Data quality depends on minimizing systematic errors as well as background signal levels introduced by the delivery method.

Methods commonly used for sample delivery for serial diffraction experiments are liquid jets, viscous jets and aerosol streams[2]. All these methods have efficiency imperfections in one or more ways. Liquid jets travel relatively fast and require large amounts of sample (~50 mg of protein for a complete dataset) with hit rates of about 10%. They further cause background scatter from the jetting liquid (few microns in diameter). Viscous jets slow down the sample flow significantly (~0.5 mg), and thereby, provide significantly increased sample efficiency at the cost of an increased background signal caused by 50-100 µm of viscous matrix. Aerosols provide the lowest background levels at the cost of very low hit rates making this method the most inefficient with respect to sample consumption and beam time usage.

A very promising approach is the use of solid sample supports (also known as fix target sample holders). This method allows for very low sample consumption requiring less than 10 µg for a structure determination. For loading, a suspension of micro-crystals is pipetted onto micro-structured single crystalline silicon chips equipped with a periodic pattern of micro-pores. After loading, the liquid is soaked-off through the pores while the crystals are retained by the pores. Ideally, they arrange themselves according to the periodic pore pattern allowing for highly efficient raster scanning of the chips. Drying out of the crystals is prevented by keeping them in an atmosphere at a controlled humidity level. By careful design of the sample environment, including helium flushing of the direct beam path and a novel beamstop concept background scattering could be kept at a similar level as observed for in-vacuum experiments using liquid jets.

Using the Roadrunner II goniometer at the MFX instrument at LCLS for raster scanning of our chips we were able to achieve hit rates of up to 60%. O. Yefanov will present the great results of the most recent experiment at LCLS (LN11).

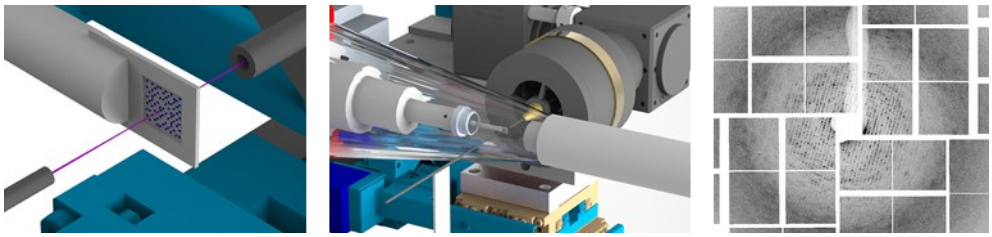

Figure: *Schematic of the sample crystals on a silicon chip (left). The chip is raster scanned across the X-ray beam (entering the interaction region through the collimator tube on the right). Drawing of the experimental environment (middle). The whole chip and the area between the collimator tube and the beamstop is exposed to a humidified helium stream to prevent crystals from dehydration as well as to reduce backgroung induced by air scattering. Example pattern from LN11 (right).*

Mitch Miller, Rice University

Progress in the Time-Resolved Crystallographic Analysis of Photosensory Proteins Delivered by an Acoustical Drop on Demand Conveyer Belt System

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Signaling proteins react to a stimulus in their receptor domain and change the conformation in a distant region. In collaboration with several other groups, we are studying the dynamics of photoconversion in bacterial photoreceptors. A combination of time resolved spectroscopy and structural experiments allows us to probe the structural photo-cycle. This can then be used as a model for other systems in understanding how proteins transmit information to different signaling regions within the same polypeptide. Here we report on progress in the analysis of the data collected during LCLS runs 12 and 14 in collaboration with groups from Lawrence Berkeley National Lab, Diamond Light Source, Washington University in St. Louis and Imperial College London. We used an Acoustic Drop on Demand injector coupled with a tape drive, conveyer belt to deliver crystals into the beam. The high drop hit rate of the XFEL triggered injector leads to relatively high crystal hit rates with a modest sample quantity requirement. This combined with the timing flexibility of multiple laser interaction regions makes this system well suited for time resolved SFX. Data were processed with CCTBX.XFEL, which allowed for rapid feed back of crystal indexing rates to ensure efficient use of beam time.

This material is based upon work supported in part by the STC Program of the National Science Foundation through BioXFEL under Agreement No. 1231306 and by a training fellowship from the Keck Center of the Gulf Coast Consortia, on the Houston Area Molecular Biophysics Program, National Institute of General Medical Sciences (NIGMS) T32GM008280. 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.

Natasha Stander, Arizona State University

Pattern Selection for Continuous Diffuse Scattering 3D Merges of Phtosystem II Crystals

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The continuous diffuse scattering of translationally disordered crystals can be used to directly determine crystallographic phases. Bragg peaks are used to orient each diffraction pattern into a 3D merge, and then iterative phasing algorithms are applied to produce an electron density. A model is then fitted to the electron density using Phenix.refine. The technique has been successfully applied to photosystem II (PSII) crystals in the dark state, extending the resolution from 4.5 Ångströms to 3.5 Ångströms (Ayyer, 2016).

By selecting an optimal subset of the diffraction patterns to include in the 3D merge, the continuous diffraction signal can be enhanced. It was observed (Ayyer, 2016) that patterns with brighter Bragg peaks tended to have stronger continuous diffuse scattering, which also corresponds to the diffraction resolution of the pattern. In the current work, we investigated the potential to enhance continuous diffraction contrast by splitting an approximately 10,000-pattern dataset into three subsets, sorted by various indexing/crystal quality metrics. Each subset was run through the entire analysis pipeline through refinement. Metrics tested include deviation of cell axis lengths and angles from the target cell (individually and combined), diffraction resolution limit, number of implausibly negative reflections, and the cross correlation and scaling factor calculated by process_hkl (in CrystFEL). Although the merged intensities appeared visually different, the R_{free} values were all similar.

Intermediate metrics in the pipeline were examined to determine which corresponded with visual merges. The voxel-by-voxel cross correlation between half dataset 3D merges in shells matched the visual quality best. Using this metric, we determined the diffraction resolution that best enhanced the continuous diffraction in the merged dataset. A CrystFEL stream file containing roughly 30,000 dark PSII patterns was sorted on diffraction resolution limit. Then, 3D merges were run on half datasets with increasing numbers of patterns from 1,500 to 15,000. The cross correlation between half datasets was calculated in shells to determine the best cutoff for structure refinement : 15,000 total patterns at low resolution and 22,000 total patterns at resolutions greater than 3.4 Ångströms. Continuing work optimizing the subset of diffraction patterns included in 3D merges should improve the achievable electron density resolution.

Ayyer, Kartik, et al. "Macromolecular diffractive imaging using imperfect crystals." *Nature* 530.7589 (2016): 202-206.

Olof Jonsson, Uppsala University

Hit Detection in Serial Femtosecond Crystallography using Photon Spectroscopy of Plasma Emission

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With the upcoming high repetition rate X-ray laser sources like the European XFEL, the amount of collected data in an imaging experiment will rise rapidly. In a typical Serial Femtosecond Crystallography experiment at XFELs, not all frames contain useful diffraction data from protein crystals. Fast hit detection and data sorting will become more important as means to reduce the amount of saved information. In the work presented here, the possibility of using X-ray emission from the heated sample is investigated using a non--local thermodynamic equilibrium (non-LTE) plasma code. Spectra from biological and non-biological samples are modeled as a function of pulse intensity and sample delivery methods. It is found that there are energy regions in the emitted plasma spectrum that are significantly different depending on the presence or absence of proteins in the interaction region. This might open up the possibility for quick rejection of diffraction data that does not contain protein crystals.

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Osamu Miyashita, RIKEN

3D Reconstruction from X-ray Free Electron Laser Single Particle Analysis of biomolecular Systems

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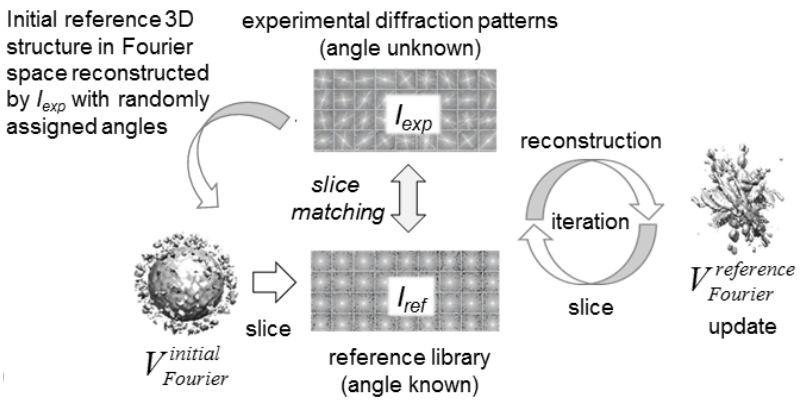
Three-dimensional (3D) structural analysis of single particles using X-ray free electron laser (XFEL) is a new structural biology technique that enables observations of molecules that are hard to be crystallized, such as flexible biomolecular complexes and living tissue in the state close to physiological condition. XFEL can significantly relax the resolution barrier imposed by radiation damage by recording the diffraction pattern before specimen destruction, due to femtosecond-short pulse duration, and also allow to observe the inner structure of biological systems which is difficult with cryo-electron microscopy (cryo-EM). XFEL experimental data are becoming increasingly available and low resolution structures from single-particle approach have been reported (Ekeberg et al., 2015). It has also been shown, theoretically, that high resolution 3D structures could be obtained using millions of diffraction patterns (Loh & Elser, 2009; Tegze & Bortel, 2012; Tokuhisa et al., 2012; Hosseinizadeh et al., 2014) and that dynamics could be directly extracted from 2D data (Tokuhisa et al., 2016).

However, such applications are still far from routine. One of the major problems to be solved in order to reconstruct 3D structures from diffraction patterns obtained in XFEL experiments is the estimation of the orientation of single particles with respect to the laser beam (three Euler angles). This is also a common problem in cryo-EM single particle analysis. In a widely used algorithm to estimate 3D orientation of particles, most consistent combinations of angles for each image and resulting 3D volume are obtained through iterations.



In this study, we aim to examine how the quality of diffraction patterns affects the resolution of 3D reconstruction. We implemented an algorithm for 3D reconstruction (Figure) on top of Xmipp, which is an image processing software package primarily aimed at single-particle 3D cryo-EM (de la Rosa-Trevín et al., 2013). The program suite contains many useful tools for image analysis for analyzing XFEL data. We created synthetic X-ray diffraction pattern dataset of ribosome particle with a variety of conditions, in particular with different beam intensity, oversampling ratio and different number of images, and attempted 3D reconstructions. We will discuss the choice of interpolation parameters and matching protocols for a successful estimation of the incident beam angles and 3D reconstruction.

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Oskar Berntsson, University of Gothenburg

Signal Transduction in Photosensory Proteins Resolved by X-ray Solution Scattering

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Any organism on the face of the earth has to perceive and act in response to light. This is achieved through dedicated photosensory proteins that regulate processes such as phototropism, seed germination, light dependent virulence and the circadian rhythm. Typically, these proteins hold a chromophore within the protein matrix. The chromophore is responsible for the absorption of incoming light. As a photon is absorbed by the chromophore conformational changes are triggered in the surrounding protein in order to relay the signal to downstream processes. Through the use of time-resolved X-ray solution scattering we have studied these rearrangements in both blue and red light photosensors. In the blue light sensing histidine kinase YF1 we identify the conformational changes that arise in the photosensory domain upon formation of the chromophore photoproduct state. We observe how these changes propagate through the protein and cause rearrangements in the kinase output domain. In the red light sensing phytochrome photosensor from *Deinococcus radiodurans* we have connected conformational rearrangements both in the vicinity of the chromophore, as well as several nanometers away, with a single step in the chromophore photocycle. Together these studies advance our understanding of signal transduction in photoreceptor proteins.

This research acknowledges support from the Swedish and European Research Council.

PETER SCHWANDER, UNIVERSITY OF WISCONSIN-MILWAUKEE

THREE-DIMENSIONAL STRUCTURE BY SINGLE-PARTICLE X-RAY SCATTERING

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* The experimental data for this work were obtained by the Single Particle Initiative.

Using a manifold-based approach, we have determined the three-dimensional structure of a virus. The structure was obtained from ~40,000 experimental single-particle diffraction snapshots extracted from a large heterogeneous dataset. The resolution, determined by Fourier shell correlation and R-split, is 9nm, corresponding to the maximum allowed by the detector geometry.

PRAKASH NEPAL, UNIVERSITY OF WISCONSIN-MILWAUKEE

DIFFERENCE STRUCTURES FROM TIME-RESOLVED SAXS/WAXS

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SAXS/WAXS has traditionally been used to determine only molecular shapes as it has been assumed that a SAXS/WAXS curve has not enough information to do so. However, there is an extra piece of information provided in time-resolved SAXS/WAXS, namely information of a nearby (unperturbed) structure which is capable of recovering difference structure directly. It does so because the structural changes in pump-probe detection in a typical time-resolved experiment is generally small enough to be confined to a single residue or group in close proximity which is identified by a method akin to the difference Fourier method of time-resolved crystallography. We show in our work that one may be able to use this extra information to find details of the inside of a molecule from time-resolved SAXS/WAXS without the need for a complementary molecular dynamics simulation.

We demonstrate this with simulations of small time-resolved variations in the structure of photoactive yellow protein. Our plan in the future is to use of a global optimization method such as simulated annealing [1] to identify the actual motion of the individual atoms and to possibly demonstrate our method on experimental SAXS/WAXS data that was the subject of a high-profile recent paper by Neutze et al. [2] on *Blastochloris viridis*, a photosynthesis reaction center. Like the method we have already reported on for time resolved structural changes as derived from differences in the angular pair correlations [3] the deduced difference electron density may be superimposed on a model of the dark structure in a particular orientation despite that fact that SAXS/WAXS comes from an ensemble of randomly oriented molecules.

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Reyhaneh Sepehr, University of Wisconsin-Milwaukee

Deterministic Method for Resolving Indexing Ambiguity in Serial Crystallography

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Crystals with a Bravais symmetry higher than the space-group symmetry can be indexed in more than one way. This so-called twinning ambiguity produces an unknown mixture of more than one indexing convention. Published means for “de-twinning” are iterative and non-deterministic. We present a deterministic, non-iterative approach capable of handling datasets consisting of hundreds of thousands of snapshots, and compare the results with those obtained by standard "de-twinning" method.

Reza Nazari, Arizona State University

Three-Dimensional Structure by Single-particle X-ray Scattering

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One of the most significant challenges of FEL (free-electron laser) research is to optimize the design and operating conditions of aerodynamics lens systems to achieve lower beam diameter, higher transmission efficiency and lower particle terminal axial velocities. As Computational Fluid Dynamics (CFD) modeling of particle trajectories is a powerful tool for this purpose, accurate three-dimensional numerical simulation is developed in our work to analyze particle motions in an existing aerodynamic particle beam focusing system. The flow is assumed to be continuum, laminar and subsonic. Since particle motion does not affect the bulk fluid (Helium) motion significantly, lagrangian particle trajectory analysis with a one-way coupling model assumption is used. Brownian, gravity and drag forces are included in the particle equation of motion. The 3-D model predicted the entire flow field including pressure and velocity distribution, particle terminal axial velocities, particle transmission efficiency, beam divergence angle and particle trajectories. Comparison of numerical results with the available experimental data shows acceptable agreement. Effects of gravity and Brownian motion were also studied by including and excluding the related terms in existing model. Brownian motion does not significantly affect the flow field, particle transmission efficiency and particle contraction ratio. This is due to the large size of particles in this study (one micrometer diameter). Gravity force effect is also observed to be negligible.

ROMAIN LETRUN, DESY-CFEL

COMBINED TWO-DIMENSIONAL ELECTRONIC SPECTROSCOPY AND X-RAY CRYSTALLOGRAPHY STUDY OF PHOTOSYNTHETIC REACTION CENTERS IN AXSIS

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Within the *Frontiers in Attosecond X-ray Science: Imaging and Spectroscopy* (AXSIS) project at CFEL in Hamburg, we are currently developing a new synergistic approach to attosecond serial X-ray crystallography and spectroscopy with the ultimate goal of obtaining information about the structure and dynamics of complex systems with high spatial and temporal resolution to understand their mechanisms [1]. Complementary measurement of X-ray and optical spectra, and X-ray diffraction is envisioned to that end.

This contribution will focus on optical spectroscopy and its implementation at the future AXSIS beamline. Two-dimensional electronic spectroscopy (2DES) has been shown to be a very powerful technique for the investigation of light-harvesting complexes and photosynthetic reaction centers, and has therefore been selected for AXSIS [2]. This method, based on a Fourier transform approach, allows achieving high temporal and spectral resolution simultaneously, while correlating excitation and emission frequencies in the form of a 2D map [3]. Ultrafast light-induced processes can be studied in detail with 2DES, even in complex systems like those involved in photosynthesis that often comprise several identical or very similar pigments. 2D spectra contain a vast amount of information, part of which allows distinguishing the contribution of each individual pigment and following the pathways of energy flow. Therefore, the electronic dynamics of the system can be comprehensively mapped-out down to the femtosecond timescale. The combination of structural information obtained by X-ray diffraction and the electronic energy landscape revealed by 2DES will be key to obtaining a complete picture of photosynthesis.

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Structural Study of F-type ATP Synthase from Spinach Chloroplasts and *Heliobacterium Modesticaldum*

Jay-How Yang, Shangji Zhang, and Petra Fromme

The proposed project aim to use a micro-focus beamline on PETRA III to collect a full data set from crystals of ATP-synthase from chloroplasts (CF1F0) and Heliobacteria (HF1F0), and determine its intact structure.

ATP synthase, one of the most important enzymes on earth, can be found in nearly all organisms from bacteria to humans. The ATP synthase is consisting of a hydrophilic F1 sub-complex and a membrane-bound FO sub-complex. Driven by the electrochemical gradient generated by the respiratory or photosynthetic electron transport chain, the rotation of the FO domain drives movements of the central stalk in response to conformational changes in the F1 domain, in which the physical energy is converted into chemical energy through the condensation of ADP and Pi to ATP.

Structural information is available from the hydrophilic head (the F1 domain, bovine heart and yeast mitochondria, chloroplast, and thermophile *Bacillus* PS3) [1], sub-complexes of the peripheral stalk (*Escherichia coli*) [1] and the FO integral rotor ring (yeast, chloroplast, Spirulina platensis, and thermophilic bacteria *Ilyobacter tartaricus* and *Acetobacterium woodii*) [1]. There is no structure available of the intact ATP-synthase nor the integral FO subcomplex and the exact mechanism of how the ATP synthesis is coupled to proton translocation is not known.

We have successfully crystallized the intact ATP-synthase from spinach chloroplasts and Heliobacteria. The study of Heliobacterial ATP synthase is extremely interesting since the *H. Modesticaldum*, a thermophilic anoxygenic phototrophic bacterium, has played a key role in the evolution of phototrophic bacteria and photosynthesis in general.

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Studying the Oxidation of Water: The Photosystem II Story

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Photosystem II (PSII) is a key enzyme in photosynthesis that drives the synthesis of oxygen by splitting water at the Mn_4O_xCa core of the oxygen-evolving cluster (OEC) in the protein complex. Using time-resolved femtosecond X-ray crystallography (TR-SFX) [1] at an X-ray free-electron laser, conformational changes have been reported both in the OEC and its protein environment [2]. Since 2014, we have optimized the crystallization procedures for improvement of the resolution obtained from nano-crystal diffraction. Combining innovative crystallization techniques with novel techniques for imaging macromolecules from 'imperfect' crystals [3] i.e. continuous diffraction, we have been successful at collecting data beyond the highest resolution of Bragg peaks. Additionally, since this method permits iterative phasing without a need for a structural model or experimental phase information [3], it is pioneering in the field of macromolecular structural studies.

Photo-induced oxidation of water by PSII forms the basis for the development of synthetic water splitting devices [4]. Hence, an improved understanding of the natural process would enhance our efforts towards obtaining sustainable clean energy. Our work presents multi-disciplinary scientific collaboration because it encompasses various research fields of nano-crystallization, liquid sample delivery development, laser optics, along with, crystallographic data processing and evaluation.

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XFEL Wavefront Characterization using Ptychography at LCLS

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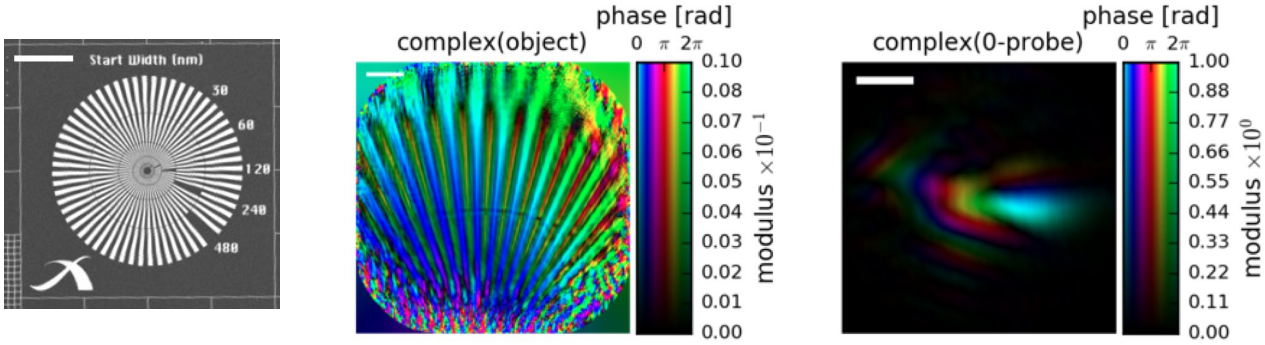
With X-ray free-electron lasers (XFELs) becoming operational worldwide, several techniques experienced fast development and attracted increased interest from the scientific community as it is the case of imaging experiments successfully performed both on nano-crystals and on single particles. For instance, the flash X-ray imaging approach has been proven viable on samples of biological significance such as viruses [1]. However these experiments also raised new challenges, for example, for beam diagnostics. This is key in finding and correcting for optics aberrations which adversely affect many experiments, especially those assuming a flat wavefront and intensity profile as it is the case of flash X-ray imaging experiments.

Among the several and somehow complementary beam characterization techniques applied at XFELs [2-6] ptychography bears the potential for returning the full wavefront of the average beam scanned over an extended sample as well as the wavefront of individual X-ray pulses [7].

We present a study on the application of conventional 2D ptychography on a test sample (Fig. 1a) aimed at the characterization of single pulse wavefronts. The experiment has been performed at the Atomic Optical and Molecular Science (AMO) beamline at the Linac Coherent Light Source (LCLS) at 1.25 keV with the same geometry as that used for flash X-ray imaging experiments. The attenuated X-ray beam was scanned onto the test sample and several diffraction patterns were collected at each position. For the first time at an XFEL experiment, a mixed-state ptychographic algorithm [8] has been applied to obtain a image of the test pattern (Fig. 1b) along with the multimodal decomposition of the complex-valued average illumination function (Fig. 1c), i.e. the wavefront of the average beam. Further processing returns information on the individual pulses contributing to the average beam therefore giving some insight into pulse-to-pulse fluctuations of the wavefront to an unprecedented degree.



a b c

Figure 1. (a) SEM image of the gold test pattern used for the experiment; scalebar 10 µm. (b) Complex-valued transmission function of the test pattern and (c) main mode of the complex-valued illumination function as reconstructed with a mixed-state ptychographic algorithm; scalebars 2 µm each.

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Structure Determination of the Au146 Cluster Using Micro-ED and X-ray Diffraction

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X-ray crystallography has been the most successful method to obtain structural information of biological targets. Crystallization of large multi-protein complexes is challenging, and even if successful, the resulting crystals are usually small, delicate and present multiple challenges. New developments such as serial femtosecond crystallography using a free electron laser (FEL) and electron crystallography or micro-electron diffraction (microED[1]) are alternative approaches to obtain atomic resolution by using nano-meter or low micro-meter sized crystals (nanocrystals). Structure determination using microED has some important distinctions to common X-ray diffraction practices. First, electrons interact much stronger with matter than X-rays and deposit less energy onto the sample. This means that electron diffraction can extract meaningful high-resolution data from crystals that are orders of magnitude smaller in volume than what is needed from a conventional X-ray crystallography experiment. Second, the wavelength of electrons produced in an electron microscope is significantly smaller than for X-ray crystallography. This leads to an Ewald sphere that is much larger with electron diffraction, and essentially flat at the resolutions seen in macromolecular crystallography. Finally, because the scattered electrons can be focused by the microscope, images of the crystals can also be collected and accurate phase information can be recovered. Our lab has pioneered nanocrystal discovery and optimization using transmission electron microscopy (TEM). The former has allowed us to increase significantly the number of potential crystallization conditions amenable for X-ray and microED experiments. The latter has permitted optimization of crystal conditions through direct observation of crystal lattices. Here we present microED and X-ray diffraction data collected for monolayer protected $Au_{146}S_{57}$ nanocrystals. Using the protocols developed in our lab we were able to obtain crystal fragments of at least 1-2 µm diameter for the microED data collection. Negative stain analysis shows lattices with FFT of 4th order. A protocol to cryo-stabilize the sample was developed and microED data was collected on a FEI Tecnai F20 microscope with resolution of up to 0.7 Å. Combining several of microED datasets using XDS,POINTLESS and SCALA software we were able to obtain a 65 % complete data set at a resolution of 1.0 Å. Conducting parallel experiments with X-ray diffraction we were able to collect a complete data set at 1.3 Å at Swiss Lightsource synchrotron. The structural model of $Au_{146}(pMBA)_{57}$ could be build from the X-ray data using XPREP, Phenix and Coot and refined to 1.0 Å using microED data. Indexing of X-ray and microED data shows the same unit cell parameters (space group P2(1)/C1 and dimensions of 56.132, 32.483, 57.311 and 90, 116.61 and 90), though solving the phase problem of the microED data with direct methods using XPREP is still ongoing.

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INVESTIGATING THE ROLE OF AN ATYPICAL VARIANT SURFACE GLYCOPROTEIN (aVSG) IN DEVELOPMENTAL PROGRESSION TO INFECTIVITY IN *TRYPANOSOMA BRUCEI*

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Trypanosoma brucei, a single-celled parasitic protozoa, is the causative agent of African sleeping sickness in humans and of nagana in livestock, which pose a major threat to the health and economic well-being of citizens in Sub-Saharan Africa. *T. brucei* is transmitted by an insect vector, the tsetse fly, where the parasites undergo a series of developmental steps from a non-infectious procyclic stage to an infectious metacyclic form. Although the overall life cycle of the trypanosome has been well-known for more than a century, scientists have been limited in their molecular studies of the mechanisms of differentiation and infectivity in the trypanosome due in part to experimental challenges of studying the parasite in the fly vector. Previous studies have demonstrated that the RNA-binding proteins RBP6 plays a central role in the development of the different stages of the trypanosome life cycle. In particular, over-expressing of RBP6 in non-infectious trypanosomes recapitulated most of the stages leading to infectivity in the insect vector. The *in vitro* process opens numerous research avenues that will further our understanding how the pathogen becomes infectious and, further down the road, will provide an opening for new intervention strategies. To begin to probe the mechanism of action of RBP6, we asked whether RBP6 expression at early stages of differentiation leads to changes in abundance of certain mRNAs. This strategy identified a transcript encoding an atypical VSG (Tb927.5.3990) as being up-regulated over 300-fold. Atypical VSGs are a family of poorly characterized surface proteins and they were named atypical, because they lack cysteine residues in the C terminus. To address the function of this atypical VSG, the mRNA was down-regulated by RNAi in induced RBP6 cells. Although the atypical VSG mRNA was down-regulated about 50%, no effect was observed on the reacquisition of infectivity and similar to control cells, the different developmental stages were observed. Further research will need to be done in order to elucidate the function of the atypical VSG.

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X-ray Free Electron laser (XFEL) in Protein Crystallography

X-RAY FREE ELECTRON LASER (XFEL) IN PROTEIN CRYSTALLOGRAPHY

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X-Ray free electron lasers (XFELs) are an extremely bright coherent X-ray source, which is creating different and new fields of research in important disciplines. Time Resolved serial femtosecond crystallography (TR-SFX) can be broken into two subsets: light-induced and Mix and inject Serial Crystallography (MISC), these are two important disciplines in crystallography. MISC is the newly developed process in which very small protein crystals are mixed with substrate and the mixture is probed in an X-ray pulse.

Here, I will present some of the data obtained from the processing and refinement of the data of projects that I recently was part of. The first one is about the TR-SFX where we studied the sequence of conformational change of bacterial *Phytochromes* [1]. And the second one is about MISC where we studied the reaction of ß-lactamase from *M. tuberculosis* in microcrystals mixed with a ceftriaxone antibiotic solution [2]. I will also present some data and results of our recent beam time about enzymology.

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BioXFEL 4TH INTERNATIONAL CONFERENCE

THOMAS GRANT, SUNY BUFFALO

LIVE MONITORING OF TIME-RESOLVED SOLUTION SCATTERING DATA AT XFELS

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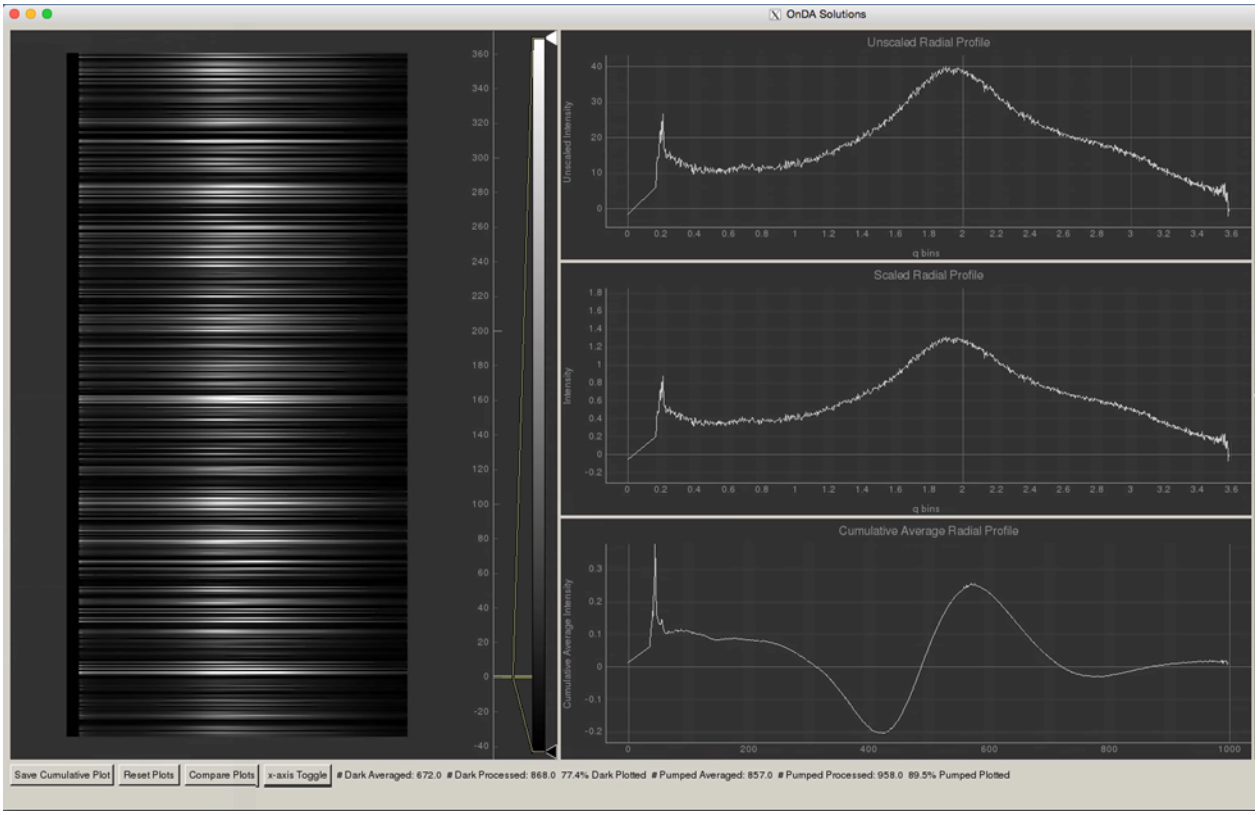
XFELs provide the opportunity to probe ultrafast processes with sub-picosecond time resolution. Time-resolved solution scattering measures global structural changes in a particle in solution as a function of time. LCLS produces 120 X-ray pulses per second, rapidly generating large amounts of data. Here we describe a tool for real-time monitoring of time-resolved solution scattering data at XFELs. The tool has been developed as a module of the OnDA online data analysis package, previously developed for serial crystallography experiments. OnDA Solution Scattering performs real-time radial averaging, normalization, and outlier rejection. Radial profiles from multiple images are averaged to increase signal to noise and plotted in real-time for live experimental feedback. Radial stacks are displayed to provide intuitive monitoring of the recent history of images. A minimum intensity threshold is applied to act as a "hitfinder" to verify that the X-ray beam is aligned with the sample, reducing the collection of invalid data. Buffer subtraction can be enabled by providing a buffer scattering profile, which enables the calculation of size parameters such as radius of gyration from small angle scattering data. Time-resolved experiments can be monitored for both pump-probe and mixing experiments, displaying difference scattering profiles rather than absolute scattering profiles. Combined, these tools provide live experimental feedback to quickly adjust experimental parameters to increase the collection of useful data, preserve valuable sample, and optimize the use of limited XFEL beam time.



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Timothy Stachowski, Hauptman-Woodward Institute

Initial Studies on the Dynamics of TGFβ-1 Latent Protein Dissociation

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Transforming growth factor beta 1 (TGFβ-1) is a homodimeric cytokine that influences various signal transduction pathways controlling tissue development, angiogenesis, wound healing, and hematopoiesis. Newly synthesized TGFβ-1 is complexed with a large prodomain called the latency associated peptide (LAP) that renders TGFβ-1 inactive. This restricts it from binding to its cognate receptors and activating signaling cascades. Recent studies suggest that secreted TGFβ-1 is activated through a coordinated force from integrin binding and cell contraction that mechanically unfolds LAP, subsequently releasing the activated TGFβ-1 dimer. Dysfunction of this process and overabundance of free TGFβ-1 is associated with tumorigenesis and inflammation.

The mechanical unfolding is a dynamic and large scale process. Studies show that in addition to the mechanical unfolding of LAP, release of TGFβ-1 can be induced by reactive oxygen species (ROS). We intend to image this process using complementary structural techniques. The initial steps will use x-ray irradiation and chemical methods to provide the ROS activation and x-ray free electron laser (XFEL) crystallography to reveal the initial and rapid changes on the residue level. Solution techniques will follow the rest of the process with our aim to develop fast SAX/WAX methods to reveal gross changes at a high degree of structural detail. The results from experimental studies will be used for computational modeling of the process. Conclusions from this work will contribute to our understanding of TGFβ-1 release and activation, which will provide insights for the development of novel therapeutics.

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Analysis of Protein Reaction Kinetics by Time-resolved Absorption Spectroscopy

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While unable to give detailed structural information, Time-Resolved Absorption Spectroscopy is a useful technique which complements traditional X-Ray Structure determination methods. Analysis of Time-Resolved Spectral data yields the rate constants of kinetic reactions in the protein as well as time points of interesting kinetic changes. This information is particularly useful when comparing differences between a protein in solution and a protein crystal. Time scales of kinetic reactions can vary between solution and crystal; thus Time-Resolved Absorption Spectroscopy provides a check on those times before X-ray Structure determination is carried out. Examples displayed are analyses from recent experiments including: An enzymatic mixing experiment involving Beta-Lactamase and a photo-reaction experiment involving phytochrome.

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X-RAY EMISSION SPECTROSCOPY WITHIN THE AXSIS PROJECT: ELECTRONIC DYNAMICS AND UNDAMAGED ELECTRONIC STRUCTURE STUDY OF PHOTOSTYSTEM II

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The AXSIS project, which is currently hosted by CFEL (DESY), is devoted to studying the water oxidation reaction in the native PSII complex by time-resolved attosecond X-ray nano-crystallography in combination with X-ray emission, X-ray absorption and 2D optical spectroscopies. The combination of these techniques with attosecond serial X-ray crystallography will give an unprecedented insight not only into the structure of PSII, but also into the dynamics that leads to the conversion from light to chemical energy. A high-resolution, multicrystal energy-dispersive X-ray emission spectrometer, based on the von Hamos geometry will be specially designed to collect Mn XES spectra of PSII.

Oxygenic photosynthesis sustains all higher life on earth by converting light energy from the sun into chemical energy. PSII is a nano solar-energy converter that captures the light from the sun to catalyse light-driven electron transport across the photosynthetic membrane. It provides the electrons for the photosynthetic electron transport chain by the water splitting process in the OEC, where it extracts in 4 sequential charge separation steps 4 electrons from water releasing 4 protons and oxygen. With each light flash, PSII transports one electron across the membrane, thereby "charging" the membrane like a “battery”. PSII is the only system in nature capable of forming molecular oxygen from water and sunlight. The catalytic center of the OEC consists of a Mn_4O_XCa cluster that couples the two-electron chemistry of water oxidation to the one-electron photochemistry of the reaction center by sequentially storing oxidization states in this series of the S-states S0 to S4. Each light-driven charge separation event in PSII extracts one electron from the OEC. X-ray structure analysis of PSII has made great progress in the last decade from the first X-ray structure of PSII at 3.8 Å resolution [1] to a 1.9 Å structure published in 2011, which provided the first atomic model of the metal cluster that catalyzes the water splitting reaction [2]. Our team has discovered large conformational changes at the Mn_4O_xCa core of the OEC and its protein environment, which even lead to large and reversible changes of the unit cell constant in the double excited state of PSII [3]. Very recently our team made a discovery that may extend X-ray structure determination towards scattering of individual molecules in “imperfect crystals” that allow a limited degree of displacement of the molecules around their crystallographic positions. We determined the S-state transition directly by detection of the 295 nm absorption change of the Mn cluster. By variation of the time delay between the flashes, we found that the S-state transition yield of a given excitation flash depends on the time delay between the flashes.

In spite of all the advances, water oxidation by the OEC is still not entirely understood and several models for the mechanism of oxygen evolution in PS II have been proposed to account for the detailed oxygen evolution by PS II. XES and optical spectroscopy measurements simultaneous to the crystallography experiment will help to reveal the oxidation state of the Mn atoms in the cluster as well as provide information about the dynamics of light absorption and excitation energy transfer within PSII. This contribution will highlight the implementation of the XES within the AXSIS project and show how they will contribute to the global understanding of how the OEC operates.

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William Bauer, Hauptman-Woodward Institute

Communicating X-ray Crystallography Concepts through Game-Based Learning

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Since the turn of the century, American students have been lagging behind other countries in math and science subjects. A recent study conducted by the Organization for Economic Cooperation and Development[1] found that American students rank 20th and 27th in science and math respectively out of 34 countries. The National Science Foundation, and by extension the BioXFEL Science and Technology Center, are committed to improving these statistics. One approach to engage students in scientific topics is to employ alternative methods that are inline with their current social tendencies. Teens and tweens spend an average of 4.5 - 6.5 hours in front of a screen everyday and ~20% of this time is spent playing games[2]. Therefore, game-based learning has the potential to engage a broad audience and deliver basic scientific content. Here, we apply this method to the field of X-Ray Crystallography through the use of a mobile game. Employing a simple, yet engaging, tap-based gameplay and a time-proven minimal 8-bit graphical art style, we deliver a game that introduces tweens and teens to XFELs, structure determination, and the structure and function of 3 different proteins.

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Yashas Rao, Stanford University

Visualizing Antibiotic Mechanisms Targeting the Ribosome at an XFEL

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The identity of Structural Biology has been redefined with the addition of X-ray Free Electron Lasers (XFEL) due to their unique ability to study biological systems at near-physiological temperatures using serial femtosecond X-ray crystallography (SFX). Our research utilizes XFELs to understand one of the most complex biological macromolecules, the ribosome. The ribosome is responsible for translating the genetic code, as mRNA, into functional proteins comprised of amino acids. The ribosome is made of two subunits: The decoding center which reads and translates a mRNA sequence (30S) and the peptidyl transferase center where the nucleotide sequence is converted into a chain of polypeptides, or protein (50S). Each of these subunits are complex, dynamic systems which undergo multiple conformational changes which signifies the importance of gathering structural data at near-physiological temperatures.

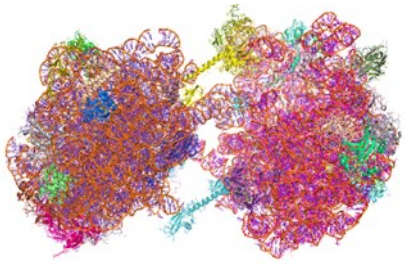

Figure 1: First ambient temperature structure of the 50S (dimer).

There has never been an ambient-temperature structure of the 50S ribosomal subunit. Using only 47 microliters of crystal sample and in just 47 minutes of data collection at LCLS, we obtained the first near-physiological temperature structure of the 50S ribosomal subunit. Although this structure is unbound to substrates of interest, we describe the simplicity and efficiency of sample preparation, data collection, and data analysis for this 3 MDa behemoth. The 50S crystallized as a dimer of 50S subunits, so 1.5 MDa each if separated. The 50S is a potential target for bacterial antibiotics, some examples including the disruption of the peptidyl transferase center by binding to the P-site or by clogging the peptide exit tunnel resulting in peptidyl congestion within the ribosome. Our research now seeks to understand mechanisms of novel antibiotics which target the 50S in order to understand the binding site mechanism and conformational changes which occur. We now have a proven method for collecting dynamic structural data of the 50S at a XFEL rather than relying on the static structures provided through synchrotron crystallography; we also have experience collecting XFEL ribosome data.

Aminoglycosides are the most commonly prescribed antibiotics when infection occurs and broad-spectrum coverage of bacteria is desired. They are commonly prescribed to newborns when an unspecified bacterial infection is detected, however they have severe side effects such as loss of hearing (Ototoxicity). Currently, there are no neutralizing agents effective against aminoglycoside-induced hearing loss and kidney dysfunction; there is a pressing need to develop a non-toxic version of aminoglycoside antibiotics. Our investigation studies novel derivatives of aminoglycosides, paromomycin and sisomicin, in order to improve drug design by studying the binding interactions at near-physiological temperatures which will result in alleviating the ototoxicity side effect. We have already seen that studying these derivatives at a XFEL has already demonstrated that antibiotics can have significantly altered binding modes compared to structures obtained at cryogenic temperatures (Figure 2). Our research utilizes XFELs to study relevant biological macromolecules in order to present science which can actually have an impact on the world.

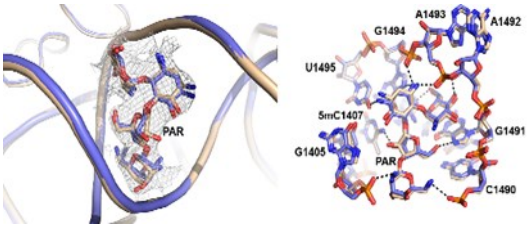

Figure 2: 30S-paramomycin binding pocket at ambient-temperatures from a XFEL

Zachary Dobson, Arizona State University

Discovery and isolation of a New, High-light Tolerant Cyanobacteria

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In nature, Photosystem II (PSII) has a half-life between 30 minutes to 1 hour in high light conditions. This is due to the photodamage and subsequent degradation to the D1 subunit. Because PSII is vital to photosynthetic organisms, there is a massive amount of energy used to repair this protein. If this damage, and thus repair process could be avoided, photosynthetic organisms would be able to focus more of the energy they capture from the sun on growth.

Recently, a new cyanobacterium was collected and isolated which naturally withstands the extremely high light conditions during much of the year. To determine how this organism is surviving in high light conditions, we want to investigate PSII specifically while still considering other aspects of the cells biology. Growth conditions have been established for large cultures needed for protein purification and subsequent structural studies of the photosynthetic membrane proteins. Currently there are three specific aims that are being investigated:

1) Structure Determination of PSII we plan to try to crystallize the PSII from this organism to determine if there is anything inherently different from either of the two known structures of PSII from Thermosynechococcus elongates or Thermosynechococcus vulcanus.
2) Genomic Studies to determine if the genes which code for PSII or any of the other large complexes of the photosynthetic machinery are different.
3) Protein Expression to determine which proteins and the relative amounts are being expressed under low and high light conditions.

An article published in Science regarding potential increases in crop yield by improving the xanthophyll-cycle in responding to natural light changes, such as temporary cloud cover through bioengineering. Kromdijk et al. demonstrated an increase of up to 20% in bio mass for crops with their modifications[1]. Food consumption will increase with increasing global population and more advances like this will be required to meet the demand. Increasing the half-life of PSII in photosynthetic organisms could lead to a much larger increase in yield as so much energy of photosynthetic organisms is used for the repair of PSII.

1. Kromdijk, J., Głowacka, K., Leonelli, L., Gabilly, S. T., Iwai, M., Niyogi, K. K., & Long, S. P. (2016). Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science*, *354*(6314). http://doi.org/10.1126/science.aai8878