San Juan, Puerto Rico

NSF

BioXFEL

3rd International Conference

A National Science Foundation Science and Technology Center

WELCOME

We would like to welcome you to the BioXFEL 3rd International Conference. It is an exciting time in the field of X-ray crystallography and the scientific community overall. The BioXFEL Science and Technology Center is keen to cultivate new research ideas and projects and provide educational support to current and future scientific minds in this growing field, and are thankful for your participation in expanding this dialogue with us. We are delighted to welcome our invited speakers who will highlight the advances of the XFEL, its application to various systems, and stimulate our conversations.

We call your attention to a few things within the conference agenda that expand beyond the panel discussion. Please join us if you can for any of the lunch sessions highlighting our education and professional development programs, our Keynote Speaker, the Boardwalk Reception, Poster Competition, and our graduate student and postdoctoral association's ABYS meeting. Also at the conclusion of the conference we will be announcing the winners of our Poster Competition.

Should you need any assistance while at the conference, please feel free to contact any of our administrative staff Jill Szczesek at 716.491.6151 or Erin Uppington 716.288.8194. They are here to answer any questions. Thank you again for attending and it is our sincere hope that you find the conference enjoyable and informative.

Sincerely,

Dr. Edward Snell
Collaborator
Conference Co-Chair

Dr. George Phillips
Co-Principal Investigator
Conference Co-Chair

P.S. Please ensure upon check-out that you have received the conference room rate of $189 per night.

AGENDA

TUESDAY, JANUARY 12, 2016

9:00 AM - 6:00 PM **ARRIVAL AND CHECK-IN INTERCONTINENTAL SAN JUAN—LOBBY FLEX AREA**

4:00 PM - 8:00 PM **POSTER SESSION SETUP—SALONS B & C**

3RD BIOXFEL INTERNATIONAL CONFERENCE

WEDNESDAY, JANUARY 13, 2016

7:30 AM - 8:30 AM **BREAKFAST—TRATTORIA ITALIANA**

8:35 AM – 8:45 AM **CONFERENCE WELCOME—SALON A**

8:45 AM – 12:45 PM **CRYSTALS, STRUCTURE, INFORMATION, & ACTION I —SALON A**
Session Chairs: Nadia Zatsepin (ASU) & Eaton Lattman (UB)

8:45 AM – 9:10 AM Henry Chapman (DESY)
New Methods in Structure Determination
9:10 AM – 9:20 AM Discussion

9:20 AM – 9:45 AM Helen Ginn (Oxford University)
Development of Methods in XFEL Analysis
9:45 AM – 9:55 AM Discussion

9:55 AM – 10:20 AM Rahel Woldeyes (UCSF)
Using XFELs to visualize solvent in the Flu M2 Proton Channel
10:20 AM – 10:30 AM Discussion

10:30 AM –11:00 AM **COFFEE BREAK—GRAND FOYER**

11:00 AM – 11:25 AM Vadim Cherezov (USC)
GPCR Crystallography with X-ray Lasers
11:25 AM – 11:35 AM Discussion

11:35 AM – 12:00 PM ! yŘNŜø ! Ijdžlfl· (SLAC)
Near-term Developments at LCLS and Science Opportunities and Plans for LCLS-II
12:00 PM – 12:10 PM Discussion

12:10 PM – 12:35 PM Ilme Schlichting (MPI)
Time-resolved SFX on Myoglobin-CO Dissociation: Challenges and Insights
12:35 PM – 12:45 PM Discussion

12:50 PM **GROUP PHOTO—OUTDOOR STAIRWAY**

1:00 PM – 2:00 PM **LUNCH GENERAL GROUP—CARIBBEAN BALLROOM**

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1:00 PM – 2:00 PM **LUNCH PRESENTATION ON BIOXFEL OPPORTUNITIES FOR UNIVERSITY OF PUERTO RICO STUDENTS—ATLANTIC BALLROOM**

2:00 PM – 2:20 PM **ASSESSMENT MEETING FOR UNIVERSITY OF PUERTO RICO STUDENTS ATLANTIC BALLROOM**

2:20 PM – 2:40 PM **ASSESSMENT MEETING FOR NEW GRADUATE STUDENTS & POSTDOCS ATLANTIC BALLROOM**

2:45 PM – 3:15 PM **POSTER BLITZ—SALON A**
Chairs: Thomas Grant (UB) and Edward Snell (HWI/UB)

3:15 PM – 4:00 PM **KEYNOTE SPEAKER: CATHY DRENNAN (MIT) —SALON A**
BioXFEL Education & Diversity Keynote Speaker Cathy Drennan (MIT)
Does Diversity Training Work?

4:00 PM – 6:00 PM **CRYSTALS, STRUCTURE, INFORMATION, & ACTION II —SALON A**
Session Chair: John Spence (ASU)

4:00 pm – 4:25 pm Martin Weik (IBS)
Time-resolved Serial Femtosecond Crystallography on a Photoswitchable Fluorescent Protein
4:25 pm – 4:35 pm Discussion

4:35 pm – 5:00 pm Petra Fromme (ASU)
Time-resolved Femtosecond Crystallography: Towards Molecular Movies of Molecules "In Action"
5:00 pm – 5:10 pm Discussion

5:10 pm – 5:30 pm Arjen Jakobi (EMBL)
In Cellulo Serial Femtosecond X-ray Diffraction of Alcohol Oxidase Crystals Inside Yeast Cells
5:30 pm – 5:35 pm Discussion

5:35 pm – 5:55pm Henrike Mueller-Werkmeister (U of Toronto)
Capturing Functionally Relevant Protein Motions at the Atomic Level: Femtosecond Time-resolved Crystallography of Ligand Dissociation in MbCO
5:55 pm – 6:00 pm Discussion

6:00 PM – 8:00PM **CONFERENCE WELCOME RECEPTION DINNER—BOARDWALK**

THURSDAY, JANUARY 14, 2016

7:30 AM - 8:30 AM **BREAKFAST—TRATTORIA ITALIANA**

8:45 AM – 12:45 PM **BEYOND CRYSTALS I —SALON A**
Session Chairs: George Phillips (Rice U.) & Lois Pollack (Cornell)

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8:45 AM – 9:10 AM	Andrew Aquila (SLAC) Recent Developments in Single Particle Imaging at LCLS and Future Plans for LCLS-II
9:10 AM – 9:20 AM	Discussion
9:20 AM – 9:45 AM	Heinz Graafsma (DESY) New Detectors for New Photon Science
9:45 AM – 9:55 AM	Discussion
9:55 AM – 10:20 AM	Abbas Ourmazd (UWM) The Machine-learning Route to Biology with X-ray Lasers
10:20 AM – 10:30 AM	Discussion
10:30 AM – 11:00 AM	**COFFEE BREAK—GRAND FOYER**
11:00 AM – 11:25 AM	Jeffrey Donatelli (LBL) Recent Developments in Fluctuation X-ray Scattering
11:25 AM – 11:35 AM	Discussion
11:35 AM – 12:00 PM	Jan Kern (LBL) Toward Molecular Movies of Metalloenzyme Catalysis Using fs X-ray Pulses - Studies on Photosystem II at LCLS
12:00 PM – 12:10 PM	Discussion
12:10 PM – 12:35 PM	Richard Kirian (ASU) Strategies for injecting aerosolized particles into an XFEL beam
12:35 PM – 12:45 PM	Discussion
1:00 PM – 2:00 PM	**LUNCH GENERAL GROUP—CARIBBEAN BALLROOM**
1:00 PM – 1:30 PM	**LUNCH ASSESSMENT MEETING FOR FEMALE STUDENTS** **ATLANTIC BALLROOM**
2:00 PM – 3:00 PM	**PROFESSIONAL DEVELOPMENT SEMINAR FOR ALL GRADUATE STUDENTS & POSTDOCS** **ATLANTIC BALLROOM** Warner Ithier Guzman (UPR-Rio Piedras) Professional Networking and Self Promotion
3:00 PM – 5:00 PM	**BEYOND CRYSTALS II —SALON A** Session Chair: Thomas Grant (UB)
3:00 PM – 3:25 PM	Marco Cammarata (University of Rennes) Myoglobin Femtosecond Dynamics in Solution
3:25 PM – 3:35 PM	Discussion
3:35 PM – 4:00 PM	Klaus Giewekemeyer (European XFEL) Towards 3D Single Particle Imaging Using a Model, Non-crystalline System with Weak 3D Diffraction Data
4:00 PM – 4:10 PM	Discussion

4:10 PM – 4:30 PM	Osamu Miyashita Hybrid Approach for X-ray Free Electron Laser Single Particle Analysis of Biomolecular Systems
4:30 PM – 4:35 PM	Discussion
4:35 PM – 4:55 PM	Haiguang Liu (Beijing CSRC) X-ray Free Electron Laser Diffraction from Inorganic Nanoparticles
4:55 PM – 5:00 PM	Discussion
5:00 PM – 6:30 PM	**CONFERENCE DINNER—CARIBBEAN BALLROOM**
6:30 PM – 8:30 PM	**POSTER SESSION—SALONS B & C**
8:30 PM – 9:30 PM	**ABYS (ASSOCIATION OF BIOXFEL YOUNG SCIENTISTS) MEETING—OFFSITE TBA**

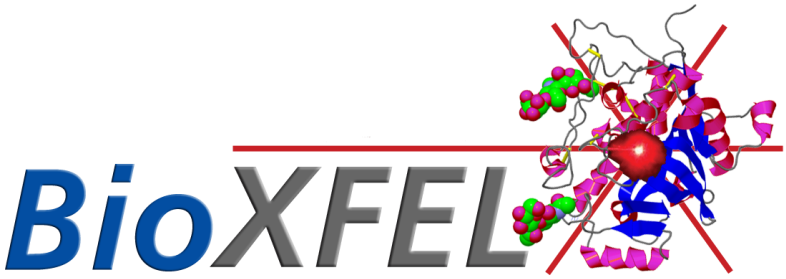
FRIDAY, JANUARY 15, 2016

7:30 AM – 8:30 AM	**BREAKFAST—TRATTORIA ITALIANA**
8:00 AM—8:30 AM	**POSTER SESSION CLEAN UP**
8:45 AM – 12:50 PM	**HYBRID XFEL AND SYNCHROTRON TECHNIQUES —SALON A** Session Chair: Martin Weik (IBS) & Marc Messerschmidt (HWI/DESY)
8:45 AM – 9:10 AM	Guillermo Calero (University of Pittsburgh) Efficient Strategies for Fixed Target Data Collection at the X-ray Proton Pump
9:10 AM – 9:20AM	Discussion
9:20 AM – 9:45 AM	Jörg Standfuss (PSI) Serial Crystallography of Retinal Binding Proteins Using Synchrotron Radiation and Free Electron Lasers
9:45 AM – 9:55 AM	Discussion
9:55 AM – 10:10 AM	Jonathan Clinger Preliminary Results from Time-Resolved Experiments on Phytochromes at LCLS/XPP
10:10 AM – 10:15 AM	Discussion
10:15 AM – 10:30 AM	Hasan DeMirci (Stanford PULSE Institute) Serial Femtosecond X-ray Crystallography of 30S Ribosomal Subunit Microcrystals in Liquid Suspension at Ambient Temperature
10:30 AM – 10:35 AM	Discussion
10:35 AM – 11:00 AM	**COFFEE BREAK—GRAND FOYER**
11:00 AM – 11:25 AM	Aina Cohen (SSRL) Macromolecular Femtosecond Crystallography at LCLS
11:25 AM – 11:35 AM	Discussion

11:35 AM – 12:00 PM	Robert Fischetti (Argonne National Lab) The Synergy between storage ring and XFEL based crystallography
12:00 PM – 12:10 PM	Discussion
12:15 PM – 1:00 PM	**LUNCH GENERAL GROUP—CARIBBEAN BALLROOM**
1:00 PM—4:00 PM	**BIOXFEL STC MEETING—FOR CENTER MEMBERS ONLY** **SALON A**
1:00 PM—1:30 PM	John Spence and Eaton Lattman Introduction and Update on Grant Renewal Process
1:30 PM—2:00 PM	Marc Messerschmidt DESY Opportunities
2:00 PM—2:10 PM	Discussion
2:00 PM—2:10 PM	John Spence Future Beamtime Applications & Collaborations

Wednesday

January 13th



3rd International Conference

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BioXFEL Education & Diversity Keynote Speaker

BioXFEL 3rd International Conference

CATHY DRENNAN, MIT

Does Diversity Training work?

Drennan, C.L.

Howard Hughes Medical Institute, Department of Chemistry and Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

My aha moment concerning diversity-training occurred when I asked an underrepresented minority (URM) undergraduate student why he left his STEM major, and he said, “I never had a TA who believed in me.” I was stunned by the response, since my experience with our graduate student TAs was so positive. I asked him to recall what these graduate student TAs had said, and, after careful thought, he replied, “it is not what my TAs said, it is what they didn’t say.” In that moment, I realized the importance of diversity-training to teacher training and also to mentor training. When a TA or mentor says nothing, different students take away very different messages; with one student interpreting silence as an indication that everything is perfect and another student interpreting that silence as an indication that failure is assured. In this talk, I will describe the development and evaluation of diversity-training material that can be used to train both researchers and educators on issues of unconscious bias and stereotype threat. The goal is this training is to help educators and mentors create the type of environment in their classrooms and/or laboratories in which all young scientists can reach their full potential. The training program that will be described is freely available to anyone who would like to adopt it at his or her home institution. http://drennan.mit.edu/education/education-interests/teacher-and-mentor-training/#Diversity-Training

BioXFEL Education & Diversity Keynote Speaker

BioXFEL 3rd International Conference

Biography

CATHERINE L. DRENNAN is a Professor and Investigator with the Howard Hughes Medical Institute and a Professor of Biology and Chemistry at the Massachusetts Institute of Technology.

Cathy grew up in New York Metropolitan area and received an A.B. in Chemistry from Vassar College in Poughkeepsie, New York. Working in the laboratory of Professor Miriam Rossi at Vassar, she discovered her passion for X-ray crystallography, but did not head straight to graduate school. Instead, after Vassar, she moved to West Branch, Iowa, where she worked as a high school teacher at a Quaker boarding school for three years. By day, Cathy taught high school science (Chemistry, Biology, Physics), and by night, she staged high school plays, such as The Importance of Being Earnest. Realizing that teaching science and scientific research go hand-in-hand, Cathy enrolled in a Ph.D. program at the University of Michigan to more fully experience the world of research. Carrying out her graduate studies in Biological Chemistry in the laboratory of the late Professor Martha L. Ludwig, Cathy used X-ray crystallography to show for the first time how vitamin B_{12} binds to a protein.

Following her postdoctoral studies with Professor Douglas C. Rees at the California Institute of Technology, Cathy joined the faculty at the Massachusetts Institute of Technology, where she has risen through the ranks to full Professor. Dedicated to both research and teaching, Cathy's educational initiatives include creating free resources for educators that help students recognize the underlying chemical principles in biology and medicine, and that help train scientists to be effective mentors. Her research laboratory at MIT uses the technique of X-ray crystallography to its fullest potential to study metalloenzymes of medical and environmental importance. Although crystallography has provided much of the structural data on biological systems, too often, this wealth of structural data is boiled down to static, two-dimensional representations. The Drennan laboratory has moved beyond simplified analyses to understand biology on a more dynamic level, emphasizing large, morphological changes, and events beyond those revealed in a single crystal structure.

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HENRY CHAPMAN, DESY

NEW METHODS IN STRUCTURE DETERMINATION

Henry N. Chapman

Center for Free-Electron Laser Science, DESY, Hamburg, Germany

The resolution of a macromolecular electron density map is crucial for proper biological interpretation of structure and function. In crystallography, the resolution is set by the highest scattering angles recorded in the diffraction pattern. The biggest limitation and frustration associated with X-ray crystallography is the fact that more often than not, macromolecular crystals do not yield diffraction patterns with Bragg peaks extending to high angles. Such Bragg peaks are the result of the coherent addition of scattering from the many regularly arranged molecules in the crystal, and their absence at high scattering angles is due to a loss of periodicity at a particular length scale—either due to a variability in the molecular structures in the crystal, or due to a variability in the rigid-body positions of those molecules. In the case of large macromolecular complexes, one may expect that the loss of high-angle Bragg peaks is not the fault of the molecules, given that cryo-EM can now often achieve resolutions of 3Å and that shrinking crystals (e.g. by dehydration) often improves the diffraction resolution. In this case, random translational disorder suppresses the coherent addition in Bragg peaks, as can be described by the familiar Debye Waller factor. But the molecules themselves still diffract as strongly as they would if they were perfectly periodically arranged. This scattering accumulates in an incoherent sum of the continuous diffraction of single molecules. That is, the disordered crystal provides the means to deliver a large number of aligned molecules into an X-ray beam. The continuous diffraction should allow de novo phasing, using iterative phasing algorithms of coherent diffractive imaging, enabled by the large increase in information recorded as compared with the Bragg peaks alone. We demonstrate these ideas on diffraction recorded at the LCLS from a large macromolecular complex, improving resolution from 4.5 Å to 3.5 Å and obtaining a model-free electron density image.

HELEN GINN, OXFORD UNIVERSITY

DEVELOPMENT OF METHODS IN XFEL ANALYSIS

Indexing methods used for serial femtosecond crystallography were originally developed for synchrotron experiments where crystals are rotated in the X-ray beam. This provides information about the reciprocal lattice in all three dimensions. The shots from both X-ray free electron lasers and serial synchrotron crystallography experiments are still images, which means what little three-dimensional data are available arise only from the curvature of the Ewald sphere. Indexing methods from synchrotron crystallography are thus less well-suited to still image data processing. We have developed a procedure for generating a one-dimensional powder pattern from ~20 images or more, which can be used to correct for poorly determined experimental parameters and help initial indexing of the powder pattern. We have also developed a new indexing method which aims to use as much information as possible from a single image given known unit cell dimensions and space group. This has been applied to a challenging data set with a cubic space group to generate a high quality electron density map with a 96% indexing rate, and provides 75-90% indexing rates for hexagonal and orthorhombic space groups. This method indexes an independent subset of all images compared to other methods, and is therefore a complementary technique which should significantly improve the indexing rates for still image data collection.

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RAHEL WOLDEYES, UC-SAN FRANCISCO

USING XFELS TO VISUALIZE SOLVENT IN THE FLU M2 PROTON CHANNEL

Rahel A. Woldeyes[1], Jessica L. Thomaston[2], Takanori Nakane[3], Aaron S. Brewster[4], Ayumi Yamashita[5], Tomoyuki Tanaka[5], Toshi Arima[5], Jun Kobayashi[5], Tetsuya Masuda[6], Mamoru Suzuki[7], Fumiaki Yumoto[8], Michihiro Sugahara[5], Nicholas K. Sauter[4], Rie Tanaka[5], Eriko Nango[5], So Iwata[5], William F. DeGrado[2], James S. Fraser[1]

[1]Department of Bioengineering and Therapeutic Sciences, [2]Department of Pharmaceutical Chemistry, University of California- San Francisco; [3]The University of Tokyo; [4]Physical Biosciences Division, Lawrence Berkeley National Laboratory; [5]RIKEN SPring-8 Center; [6]Kyoto University; [7]Osaka University; [8]High Energy Accelerator Research Organization, KEK

The influenza A virus must acidify its interior to release its RNA. This important step in the viral life cycle relies on matrix protein 2 (M2), a pH-regulated proton channel embedded in the lipid envelope of the virus. M2 has been a subject of intense study both because it the target of adamantane compounds that can be used to treat flu and because iit is an ideal model system to study proton conduction across membranes. The structural location of ordered water within the M2 channel plays a key role in both of these areas. Additionally, there is significant debate about the existence “water wires” of ordered solvent molecules that allow proton conduction by connecting the viral exterior and interior. It is therefore critical to determine the distribution of water molecules within the channel. Our previous high resolution X-ray data of the M2 channel crystallized in the lipid sponge phase revealed interesting differences in the distributions of water as a function of pH. However, preliminary room temperature data called into question whether data collected from cryo-cooled crystals was informative for biologically relevant mechanisms of proton transport. Unfortunately, damage to the small M2 crystals from the radiation dose at room temperature precluded mechanistic interpretations of the solvent density. To circumvent these issues and to observe the functionally important structural dynamics of M2, we used X-ray Free Electron Lasers to collect radiation-damage free, 1.4 Å datasets of M2 at both low and high pH. By avoiding the artifacts associated with cryo-cooling and eliminating the effects of radiation damage, the resulting room temperature data have allowed us to draw new conclusions about the solvent conformational heterogeneity. We will build on this work and use XFEL to monitor changes in the solvation of the pore of M2 as a function of both pH and time. Furthermore, these structures will help in the design of next-generation of antivirals address continuing problems with drug resistance.

Grant Acknowledgement: R.A.W. is supported by a NSF Graduate Research Fellowship. J.L.T and W.F.D are supported by NIH Grant R01-GM05642. J.S.F. is a Searle Scholar, Pew Scholar, and Packard Fellow, and is supported by NIH OD009180, NIH GM110580, and NSF STC-1231306. We also acknowledge support by X-ray Free Electron Laser Priority Strategy Program (MEXT) and Japan Science and Technology Agency (JST).

Vadim Cherezov, University of Southern California

GPCR Crystallography with X-ray Lasers

Cherezov V.

Bridge Institute, Department of Chemistry, University of Southern California, Los Angeles, CA 90089, USA

Structural studies of G protein-coupled receptors (GPCRs), and other biomedically relevant membrane proteins and complexes, are hampered by challenges related to growing sufficiently large crystals capable of withstanding radiation damage and yielding high-resolution data. We have developed a new approach of using a membrane mimetic gel-like matrix known as lipidic cubic phase (LCP) for growth and delivery of membrane protein microcrystals for data collection by serial femtosecond crystallography (SFX) at X-ray free electron lasers (XFEL). Microcrystals are delivered to the intersection point with an XFEL beam using a specially designed LCP injector, allowing to adjust LCP flow-rate and minimize crystal consumption. LCP-SFX uses highly intense 40-fs XFEL pulses to minimize radiation damage and collect room temperature high-resolution data from sub-10 μm crystals. Protein consumption is reduced by 2-3 orders of magnitude compared to a liquid injector, making the LCP-SFX method attractive for structural studies of challenging membrane and soluble proteins, and their complexes. Recent applications of this method led to solving difficult structures of the human δ-opioid receptor in complex with a bi-functional peptide ligand, a blood pressure regulator - angiotensin receptor, and a major GPCR signaling complex between rhodopsin and arrestin. Updates on experimental phasing of LCP-SFX data as well as adapting this method for Structure-Based Drug Design (SBDD) studies of GPCR targets will be presented.

Robert Schoenlein, SLAC

Near-term Developments at LCLS and Science Opportunities & Plans for LCLS-II

Robert Schoenlein[1]

1. Linac Coherent Light Source, SLAC National Accelerator Laboratory, Menlo Park, CA 94025, USA.

The unique capabilities of LCLS, the world's first hard X-ray FEL, have had significant impact on advancing our understanding of biology at the molecular level. Key applications include single-particle imaging, serial femtosecond nanocrystallography, time-resolved crystallography, time-resolved small-angle X-ray scattering, and time-resolved X-ray spectroscopy. This talk will outline some of the ongoing developments at LCLS that will further advance the impact of this facility on biological science.

Simultaneous with the near-term development of LCLS capabilities, a major upgrade of the facility (LCLS□II) is now underway. LCLS-II is being developed as a high-repetition rate X-ray laser with two simultaneously operating, independently tunable FELs. The baseline design features a 4GeV superconducting linac operating with continuous wave RF that is capable of accelerating 10^6 electron bunches per second to produce evenly spaced ultrafast X-ray laser pulses at a repetition rate up to ~1 MHz spanning the energy range from 0.25 to 5 keV. The superconducting linac will be installed in the first third of the SLAC linac tunnel. The final third of the SLAC linac will continue to operate as a warm copper accelerator at energies up to 15 GeV, providing tunable X-rays with photon energy up to 25 keV at 120 Hz.

This talk will present some of the important science opportunities and associated instrumentation planning for LCSL-II. The growth potential of this facility beyond LCLS-II will be outlined, focusing on future capabilities that will be particularly relevant for advancing our understanding of biology at the molecular level.

Ilme Schlichting, Max Planck Institute

Time-resolved SFX on Myoglobin-CO Dissociation: Challenges and Insights

Ilme Schlichting

Max Planck Institute for Medical Research, Jahnstr. 29, 69120 Heidelberg, Germany

Myoglobin is a well-established model system to study the structural dynamics involved in hemoprotein ligand binding. Photodissociation of the ligand carbon monoxide from the heme cofactor upon absorption of a visible photon initiates a hierarchical reaction involving several intermediates that has been followed by time-resolved Laue crystallography to 150 ps time-resolution , the lower limit accessible by synchrotron X-ray sources. However, this time resolution is not sufficient to capture the earliest events happening upon photodissociation, which include the motion of the ligand to a binding site above the heme plane, movement of the distal histidine, the heme iron
atom recoiling out of the heme plane and a “protein quake”, which is a series of rapid structural changes emanating from the breaking ligand bond towards the surface of the protein. In the earliest snapshots available at synchrotron sources, all these things have already occurred and the protein quake has already spread throughout the protein.

The recent advent of X-ray free electron lasers has extended the time resolution attainable with time-resolved crystallography into the chemical time scale of femtoseconds, which allows analysis of these early events upon breaking of the heme iron—carbon monoxide bond at high temporal and spatial resolution. We describe our recent time-resolved serial femtosecond crystallography experiments performed at the Linac Coherent Light Source (LCLS) in Stanford, which show the first snapshots of the effects of ligand dissociation in myoglobin at (sub) picosecond time resolution.

Martin Weik, Institut de Biologie Structurale

Time-resolved Serial Femtosecond Crystallography on Photoswitchable Fluorescent Proteins

Weik, M.

Institut de Biologie Structurale, Grenoble, France

Reversibly photoswitchable fluorescent proteins (RSFP) are essential tools in advanced fluorescence nanoscopy of live cells (such as PALM and RESOLFT). They can be repeatedly toggled back and forth between a fluorescent (*on*) and a non-fluorescent (*off*) state by irradiation with light at two different wavelengths. Mechanistic details of photoswitching, in particular on the ultra-fast photochemical time scale, remain largely unknown.

Our consortium combines time-resolved serial femtosecond crystallography (TR-SFX), ultrafast absorption spectroscopy in solution and simulation methods to study *off*-to-*on* photoswitching intermediates in two RSFP on the picosecond to microsecond time scale.

Two major bottlenecks had to be passed before TR-SFX could be conducted, *i.e*. the production of well-diffracting microcrystals in large quantities and efficient inline pre-illumination to photoswitch RSFP microcrystals from the *on* to the *off* state prior to injection.

First pump-probe TR-SFX experiments were conducted at both the LCLS and SACLA that, together with time-resolved absorption spectroscopy, provide first insight into a possible sequence of events involved in photoswitching.

This research project is conducted by the following scientists: Adam, Aquila, Barends, Bourgeois, Boutet, Byrdin, Cammarata, Colletier, Coquelle, de la Mora, Demachy, Doak, Feliks, Fieschi, Field, Foucar, Gotthard, Guillon, Hilpert, Iwata, Jakobs, Joti, Koglin, Kovacsova, Lévy, Macheboeuf, Mizohata, Motomura, Nango, Nass, Nury, Owada, Ridard, Robinson, Roome, Roux, Royant, Ruckebusch, Schiro, Schlichting, Shoeman, Sliwa, Sugahara, Tanaka, Thepaut, Togashi, Tono, Uriarte, Weik, Woodhouse, Yabashi.

PETRA FROMME, ARIZONA STATE UNIVERSITY

TIME-RESOLVED FEMTOSECOND CRYSTALLOGRAPHY: TOWARDS MOLECULAR MOVIES OF MOLECULES "IN ACTION"

Petra Fromme

Department of Chemistry and Biochemistry and the Center for Applied Structural Discovery at the Biodesign Institute, Arizona State University, Tempe, Arizona 85287-1604 USA

Biomolecules are highly dynamic, however most structures determined so far only provide a static picture of the molecule. Serial Femtosecond Crystallography (SFX) provides a novel concept for structure determination, where X-ray diffraction "snapshots" are collected from a fully hydrated stream of nanocrystals, using femtosecond pulses from the world's first high energy X-ray free-electron laser, the Linac Coherent Light Source. The first proof of concept of serial femtosecond crystallography was achieved using Photosystem I, a larger membrane protein complex involved in Photosynthesis as a model system [1],[2]. By using femtosecond pulses briefer than the time-scale of most damage processes, femtosecond nanocrystallography overcomes the problem of X-ray damage in crystallography [3] and extends to atomic resolution [4],[5]. It is also applied to membrane proteins crystallized in lipidic environments [6],[7], [8], [9] and crystals grown in living insect cells.

Femtosecond crystallography also opens a new avenue for determination of protein dynamics. First experiments on the proof of principle for time resolved serial femtosecond nanocrystallography have been performed on Photosystem I-ferredoxin [9] and Photosystem II nano-crystals [10] and conformational changes of the Mn_4Ca cluster and its protein environment were observed for the first time in the transition from the dark to the double excited state [11]. Very recently we were able to show that TR-SFX studies extend to atomic resolution using the photoactive yellow protein as a model system [12]. This pioneering work paves the way for the determination of molecular movies of the dynamics of membrane proteins "at work" in the future including the determination of molecular movies of water splitting.

References: [1] Chapman,HN, Fromme,P, Barty, A. et al Nature 2011, 470, 73-77 ; [2] Fromme P., Spence JC. Curr Opin Struct Biol 2011, 21: 509-516; [3] Barty,A, Caleman,C, Aquila,A et al. Nature References: [1] Chapman,HN, Fromme,P, Spence JC. Curr Opin Struct Biol 2011, 21: 509-516; [3] Barty,A, Caleman,C, Aquila,A et al. Nature Photonics 2012, 6, 35 –40; [4] Boutet S, Lomb L, Williams GJ, et al Science 2012, 337: 362-364; [6] Liu W, Wacker D, Gati C et al Science 2013, 342: 1521-1524.

Arjen Jakobi, European Molecular Biology Laboratory

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In Cellulo Serial Femtosecond X-ray Diffraction of Alcohol Oxidase Crystals Inside Yeast Cells

Arjen J. Jakobi[1, 2] , Daniel M. Passon[1] , Francesco Stellato[3] , Mengning Liang[3] , Kevin Knoops[4] , Thomas A. White[3] , Thomas Seine[1] , Marc Messerschmidt[5] , Henry N. Chapman[3, 7] , Matthias Wilmanns[1, 8]

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We have explored the possibility of using femtosecond pulses from an X-ray Free Electron Laser to collect diffraction data from protein crystals formed in their native cellular organelles. Peroxisomes are eukaryotic cell organelles capable of carrying an unusually high protein load. In peroxisomes of the methylotrophic yeast H. polymorpha, alcohol/methanol oxidase (AO) oligomerizes into 600kDA ocatmeric assemblies that spontaneously form 300nm to 2µm-sized crystals inside the organelle. The small size of these crystals and their weak diffraction properties has so far precluded structure solution by classical X-ray techniques. We show that size and number of crystals per cell can be modulated by genetic modification of critical components involved in the peroxisomal fission process and how this affects *in cellulo* diffraction properties. We exposed H. polymorpha cell suspensions to femtosecond X-ray pulses at the Coherent X-ray Imaging (CXI) beamline at the Linac Coherent Light Source and characterized Bragg-sampled diffraction obtained from the AO crystals in intact cells to a maximum resolution of 6Å. Our SFX results are supported by complementary X-ray powder diffraction and electron microscopy data. We expect that the lessons learned from the present study will help to address experimental challenges lying ahead for intracellular crystal formation and its exploitation for structure solution of biological macromolecules.

Henrike Mueller-Werkmeister, University of Toronto

Capturing Functionally Relevant Protein Motions at the Atomic Level: Femtosecond Time-Resolved Serial Crystallography of Ligand Dissociation in MbCO

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X-Ray FEL sources allow the direct observation of the "choreography of life" on time scales relevant to barrier crossing, i.e. the femtosecond motions governing chemistry driving biological function [Miller, R. J. D., Science, 2014, 343, 1108-1116.] A fundamental question becomes accessible experimentally: The investigation of protein dynamics with all atomic resolution on the shortest relevant timescale around 100 fs. Here is where bond-breaking events occur, which translate into larger structural changes and cause a protein to fulfill its function over a wide range of timescales. How these inherent fluctuations of the protein transduce chemical energy into function, i.e. what is the structure-function correlation with respect to understanding these evolutionarily optimized systems is the quest of our research.

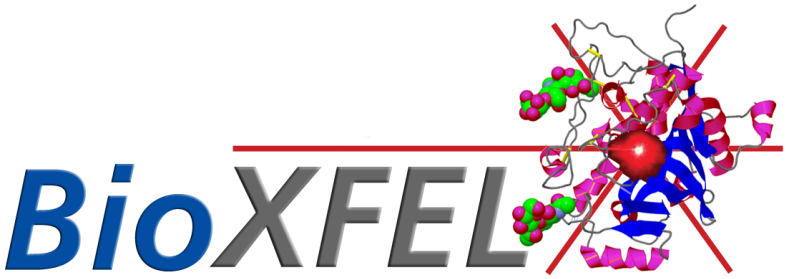
We have performed time-resolved serial femtosecond crystallography experiments (TR-SFX) using a fixed target for sample delivery at XPP/LCLS to study the photodissociation of carboxymyoglobin, which is a well studied model system for ultrafast protein dynamics. Previous time-resolved crystallography results using third-generation light sources are on the timescale of 150 ps and slower [Schotte, F. et al., 2003, Science 300, 1945] and gave insight into side chain motion after photodissociation. The actual bond-breaking event is expected faster than 50 fs [Armstrong, M. R. et al, Proc. Natl. Acad. Sci. USA, 2003, 100, 4990]. Our experiment accesses the dynamics in the time window between 0 and 2 ps with 100 fs steps to resolve both the ligand dissociation and the "talking" coordinate mediated through the proximal histidine motion involved in allosteric communication of the ligation state. The heme moiety doming and the overall protein motion coupled to the bond breaking process are resolved and the preliminary data demonstrate coupling to collective coordinates on exceptionally fast time scales. The diffraction data were processed using the approach by Ginn et al. (Acta Crystallogr. D Biol. Crystallogr. 71, 1400-1410 (2015)) and results are being analyzed further to determine the actual correlated motions in carboxymyoglobin. As it is important to control the laser excitation processes, we performed additional laser titration studies to determine the accurate photodissociation level of our crystals.

An important prerequisite for all variants of time-resolved experiments is efficient and precise sample delivery. We

used a fixed target matrix, referred to as photo-crystallography chip (PCC, Mueller et al., Structural Dynamics 2, 054302, 2015, Zarrine-Afsar et al., Anal Chem 83, 767, 2011), which is a nanofabricated silicon based chip with >10000 individual features for trapping protein crystals in random orientation. The design of the chip allows background free detection and low sample consumption. The feature size can be engineered to match the crystal size. In combination with a spectroscopy based mapping approach to identify the occupied positions on the PCC, we were able to reach high hit rates with an overall rate of >50% indexable diffraction patterns per PCC, allowing collection of a complete data set in about 10 min. We have also developed a portable, compact endstation for the PCC [Sherrell et al., *J. Synchrotron Radiat.* 22, 1372-1378 (2015)], as well as an imaging and spectroscopy setup for mapping the PCC and *in-situ* sample characterization.

THURSDAY

JANUARY 14TH



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Andrew Aquila, SLAC

Recent Developments in Single Particle Imaging at LCLS and Future Plans for LCLS-II

The Single Particle Imaging (SPI) Initiative is a community-involved initiative tasked with the goal of overcoming the technical challenges for reaching better and ultimately atomic resolution in single-particle imaging experiments at LCLS. It has more than 100 participating members from 21 institutions spanning 8 countries. Since its inception, one year ago, the SPI Initiative has completed four LCLS beamtimes; this talk will review the results/technical discoveries from each of the SPI beamtimes and discuss how the field has progressed. In addition, the experiences of SPI have gone into shaping the instruments proposed for LCLS-II. An introduction to the Tender X-ray Imaging (TXI) instrument for LCLS-II will also be presented.

Heinz Graafsma, DESY

New Detectors for New Photon Science

Heinz Graafsma [1,2]

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Solid-state hybrid pixel detector technology has given an enormous boost to the performance of the detectors used at synchrotron sources, and with the X-ray Free-Electron Lasers this has even been accelerated further. New integrating detectors use adaptive gain architectures, where each pixel individually and fully automatically adapts its gain to the incoming signal strength. This provides at the same time low-noise performance for weak signals, allowing distinguishing between single photons, and high dynamic range for strong signals. One example of such a system is the Adaptive Gain Integrating Pixel Detector (AGIPD), under development for the European XFEL. In addition to the large dynamic range the AGIPD system also provides high speed imaging up to 6.5 MHz. A short introduction of the operational principle will be given, followed by a few examples of performance testing. The current status and development time line will also be presented.

Figure 1 shows a single tile of the AGIPD detector. The systems under development for the Eu.XFEL will have 16 such tiles arranged in four independent quadrants.

Another challenge is presented by the low-energy FELs like FLASH. In order to be able to reliably detect photons down to 250 eV a system based on back-illuminated CMOS imagers is being developed. This system, PERCIVAL, has a different adaptive gain structure providing single photon sensitivity as well as a large dynamic range. PERCIVAL will have 27 micron pixel sizes, and run up to 120 Hz frame rates. Both a 2-million pixel monolithic and a 13-million pixel monolithic sensors are being developed. The performance parameters of the system will be detailed and first experimental results shown.

Figure 1. A single 128 x 512 pixel tile of the AGIPD system

Abbas Ourmazd, University of Wisconsin-Milwaukee

The Machine-Learning Route to Biology with X-ray Lasers

Abbas Ourmazd*

University of Wisconsin-Milwaukee

Machine learning is guided by the intrinsic properties of the data, rather than user-directed notions. It is thus possible to determine the structure, conformations, energy landscapes, and dynamics of biological machines in equilibrium, or in the course of time-driven processes. Examples will include structural snapshots from heterogeneous ensembles, and spectra containing a single photon.

*In collaboration with A. Dashti, R. Fung, A. Hosseinizadeh, P. Schwander, J. Frank et al., P. Fromme et al., R. Santra et al., and M. Schmidt et al.

Jeffrey Donatelli, Lawrence Berkeley National Lab

Multi-tiered Iterative Phasing for Fluctuation X-ray Scattering Reconstruction

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Fluctuation X-ray scattering (FXS) is an emerging imaging technique in which one collects a series of diffraction patterns from particles in solution with X-ray exposures taken below rotational diffusion times. The resulting images contain angularly varying information from which angular correlations can be computed, yielding several orders of magnitude more information than traditional solution scattering methods. However, determining molecular structure from FXS data introduces several challenges. In particular, in addition to the classical phase problem, one must also solve a hyper -phase problem to determine intensity information from the correlation data. In this talk, we present the multi-tiered iterative phasing (M-TIP) algorithm, which is able to simultaneously determine intensities, complex phases, and molecular structure from FXS data. Furthermore, we describe recent work on experimental FXS data, including challenges associated with extracting accurate correlations and successful reconstructions using M-TIP.

Jan Kern, Lawrence Berkeley National Lab

Toward Molecular Movies of Metalloenzyme Catalysis Using FS X-ray Pulses—Studies on Photosystem II at LCLS

Kern, J.1,2

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Biological catalysis of many important products involves the participation of metallo-enzymes. One important example is the large dimeric membrane protein photosystem II (PSII) that catalyzes the light driven oxidation of water into molecular oxygen and protons in all plants, algea and cyanobacteria. The exact reaction mechanism of this thermodynamically challenging transformation is still not known and a detailed knowledge of it could yield important clues to improve artificial catalysts for water oxidation. The recent advances in X-ray sources provided by the development of X-ray lasers (for example the LCLS at Stanford) bring the ultimate goal of recording molecular movies of such molecular machines in action closer to reality. By combining X-ray diffraction and X-ray spectroscopic methods with ultra bright and short (fs) pulses of such an X-ray laser it is possible to extract information from very dilute systems at room temperature before the manifestation of radiation induced changes. We have applied this approach using fs X-ray pulses at the LCLS to study the electronic and geometric structure of the active site of photosystem II, a Mn4CaO5 cluster and measured simultaneously oxidation state sensitive X-ray emission and X-ray diffraction data.1,2 The results of these studies for several different illumination states of PSII will be presented. In addition we have developed a setup to record transition metal L-edge spectra3 from solutions of metalloenzymes under physiological conditions and applied this to PSII. This allowed us to collect the first partial Mn L-edge spectrum of PSII.

References:

1 *Science* **340**, 491,(2013)
2 *Nature Comm* **5**, 4371,(2014)
3 *J Phys Chem Lett* **4**, 3641(2013)

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Richard Kirian, Arizona State University

Strategies for Injecting Aerosolized Particles into an XFEL Beam

Richard A Kirian [1,2], Salah Awel [2,3], Max Weidorn [2,4], Niko Eckerskorn [5], Daniel Horke [2,3], Nils Roth [2,4], Andrei V Rode [5], Jochen Küpper [2,3,4], Henry N Chapman [2,3,4]

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The development of single-particle imaging with x-ray free-electron lasers (XFELs) aims to circumvent the challenge of growing well-diffracting crystals in order to solve biomolecular structures. If successful, it will complement the powerful methods of cryogenic electron microscopy by adding the potential to study fast time-resolved processes while removing the need for cryogenic cooling. Much progress toward this end has been made since 2006 by various groups and most recently by the SLAC-led Single-Particle Initiative project.

The Single-Particle Initiative efforts have shown that further development of aerosol particle injectors is needed in order to achieve high-resolution structures at the present 120Hz XFEL pulse repetition rate. For example, at a focus of about 150 nm diameter, approximately 0.03% of the XFEL pulses intercept a particle using the current state-of-the art aerosol injector. At this rate, approximately 30 days of continuous data collection would be required in order to obtain 100,000 diffraction patterns, which may or may not be sufficient for determining a high-resolution molecular structure. While tremendous progress is being made with larger focal spots and lower photon energy, and much is being learned about how to optimize data collection with a nano-focused beam and hard x-rays, it appears that the sample delivery efficiency problem must eventually be addressed. Higher repetition rate machines such as the European XFEL and LCLSII will partly alleviate this problem, but does not fully address the sample wastage problem.

In this presentation, I will discuss sample injection for single-particle imaging. In particular, I will describe our experiments with a simple convergent nozzle that produces a tight particle beam focus down to about 3.8 µm along with our efforts to develop an optical manipulation technique for guiding particles with micrometer precision to the XFEL focus. This “optical funnel” that we are developing utilizes radiation pressure and photophoretic forces in order to guide particles to a desired location. These efforts are under heavy development, but thus far indicate that there is much potential for dramatic improvements in data collection rates and sample delivery efficiency.

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MARCO CAMMARATA, UNIVERSITY OF RENNES

MYOGLOBIN FEMTOSECOND DYNAMICS IN SOLUTION

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Myoglobin is an oxygen storage abundantly present in many mammals. Together with its robustness and the readily achievable laser excitation (bond breaking between protein and ligand), Myoglobin is a perfect model system for an in depth understanding of protein dynamic. X-ray scattering and absorption experiments at XPP/LCLS have been performed to study the global and the active site dynamics. At the active site the biphasic (~70 and ~500 fs) change are interpreted as change in the Iron position (relative to the heme), the second component of which it is supposed to be connected to the displacement of the F helix [1]. The low angle part of the X-ray scattering data have been analyzed to retrieve the change of radius of gyration and protein volume and show oscillations typical of viscoelastic systems [2]. In this presentation the main findings and the current work in progress will be discussed.

[1] M. Levantino, H. T. Lemke, G. Schirò, M. Glownia, A. Cupane and M. Cammarata, Observing heme doming in myoglobin with femtosecond X-ray absorption spectroscopy, Struct. Dyn. 2, 041713 (2015); doi: 10.1063/1.4921907

[2] M. Levantino, G. Schirò, H.T. Lemke, G. Cottone, J.M. Glownia, D. Zhu, M. Chollet, H. Ihee, A. Cupane , M. Cammarata, *Ultrafast myoglobin structural dynamics observed with an X-ray free-electron laser*, Nature Communications 6, 6772 (2015) doi:10.1038/ncomms7772

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KLAUS GIEWEKEMEYER, EUROPEAN XFEL

TOWARDS 3D SINGLE PARTICLE IMAGING USING A MODEL, NON-CRYSTALLINE SYSTEM WITH WEAK 3D DIFFRACTION DATA

Giewekemeyer, K.,[1] and Aquila, A.,[1, 2] and Loh, N.D.,[2,3] and Shanks, K.S.,[4] and Weiss, J.,[4] and Tate, M.W.,[4] and Philipp, H.T.,[4] and Stern S.,[5] and Vagovic, P.,[5] and Yoon, C.H.,[1,5,2] and Mehrjoo, M.,[1] and Chushkin, Y.,[6] and Zontone, F.,[6] and Vine, D.J.,[7] and Harder, R.,[7] and Chang, C.,[2] and Tiberio, R.,[8] and Sakdinawat, A.,[7] and Gruner, S.M.,[4,9] and Williams, G.J.,[2] and Mancuso, A.P.[1]

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The prospect of structure determination without crystallization is the main motivation for current efforts to develop Single Particle Imaging (SPI) at X-ray Free Electron Lasers. Utilizing the so-called Diffract-and-Destroy principle, FEL-based SPI is carried out by injecting a continuous stream of reproducible particles into the focused X-ray beam, so that particles in random 3D orientations interact statistically with the highly intense photon pulses of the FEL. The resulting diffraction patterns then have to be categorized, in the most simple case as "hits" and "blanks", and further processed to obtain a 3D diffraction intensity of the sample in Fourier space (intensity reconstruction). This diffraction volume can then be used for iterative phasing to produce the real-space density distribution of the particle (density reconstruction).

In order to advance FEL-based SPI onto the resolution level of biological macromolecules, a great variety of experimental and algorithmic challenges have to be overcome, such as sample injection, identification of hits, intensity reconstruction, minimizing experimental background, and density reconstruction.
We concentrate here on the aspect of the experimental intensity reconstruction, i.e. orientation determination of each diffraction pattern in 3D diffraction space. An experimental demonstration for orientation determination of SPI data in the very relevant weak scattering limit (<~100 scattered photons per pattern), is still outstanding.

We report on an analogue experiment at a synchrotron source by illuminating a small (<1 µm) lithographically produced particle with a coherent synchrotron beam and collecting hundreds of thousands of diffraction patterns in various particle orientations. We will show how this data, without explicit knowledge about the frame orientations and with less than 100 photons per pattern, can be used to reconstruct a 3D diffraction volume in Fourier space.
We will also discuss the phasing step (density reconstruction) as well as conclusions that can be drawn from this experiment for FEL-based SPI.

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OSAMU MIYASHITA, RIKEN

HYBRID APPROACH FOR X-RAY FREE ELECTRON LASER SINGLE PARTICLE ANALYSIS OF BIOMOLECULAR SYSTEMS

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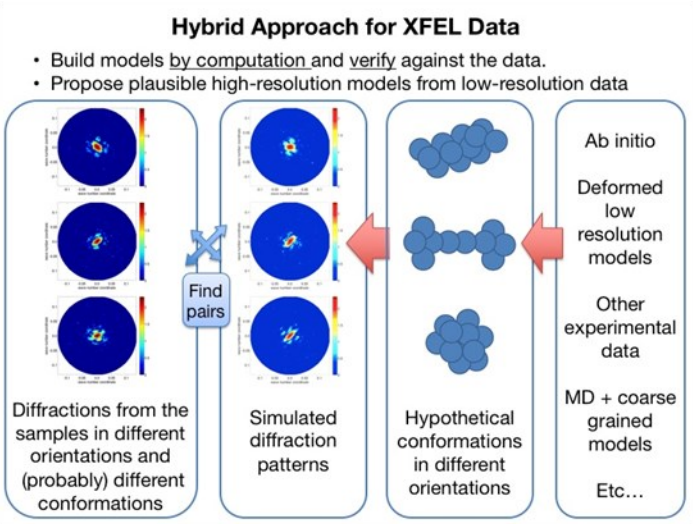
X-ray free electron laser (XFEL) is an exciting new technology that could significantly extend our structural knowledge of biological systems. Strong laser light from XFEL enables the measurement of single molecular complex, without necessity of crystallization. Since crystallization is not necessary, it could be applied to a wider variety of systems under various physiological conditions.

For XFEL single particle analysis, reconstruction of 3D structure requires a large number of diffraction patterns, however, in the current XFEL experiments on biological systems, such measurements are still difficult and the analysis often relies on a few 2D diffraction patterns. In addition, the current standard approach is to obtain 2D real -images from diffraction patterns via phase recovery procedure. However, for biological systems, due to their low diffraction power, the procedure is not straightforward, and it often fails to provide an interpretation of the data. If the procedure is not successful, the real image cannot be obtained or the results are unreliable. Thus, there is not enough data to reconstruct the model of the target systems without additional information.



Therefore, we have been developing hybrid algorithms that combine molecular mechanics and image data processing algorithms. Instead of directly reconstructing the model from the diffraction pattern, we first construct hypothetical models using molecular mechanics simulations, computational modeling techniques and/or other experimental data

such X-ray crystallography. Then, the models are matched against available XFEL diffraction patterns to identify the model that is most likely to be represented in the experimental data. This approach could be especially useful to study functional dynamics of biological systems.

As the first step toward such hybrid modeling, we established a protocol to assess the agreement between the model structure and the target XFEL diffraction pattern and show that XFEL data can be used to study the conformational transitions of biological molecules. We tested our algorithms for biological molecular complexes of different size, elongation factor 2, CCM virus, and ribosome, and examined the experimental condition, in particular the XFEL beam intensity, that is required to perform such study. The proposed algorithm can be combined with molecular dynamics simulations or normal mode analysis to perform more automated structure modeling.

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HAIGUANG LIU, BEIJING CSRC

X-RAY FREE ELECTRON LASER DIFFRACTION FROM INORGANIC NANOPARTICLES

Xuanxuan Li[1], Chun-Ya Chiu[7], Hsiang-Ju Wang[7], Robert Lawrence[2,3], Sabine Botha[4], Christopher Kupitz[5], Stephan Kassemeyer[4], Richard Kirian[6], Daniel James[6], Dingjie Wang[6], Garrett Nelson[6], Marc Messerschmidt[8], Sebastien Boutet[8], Garth Williams[8], Thomas R. M. Barends[4], Elisabeth Hartmann[4], Aliakbar Jafarpour[4], Lutz M. Foucar[4], Robert L. Shoeman[4], Anton Barty[9], Henry Chapman[9], Mengning Liang[9], Fenglin Wang[9], Nadia A. Zatsepin[6], Shibom Basu[3,5], Raymond Fromme[3,5], R. Bruce Doak[4], Petra Fromme[3,5], John. C. H. Spence[3,6], Uwe Weierstall[3,6], Michael H. Huang[7], Ilme Schlichting [4], Brenda G. Hogue[2,3,10]*, Haiguang Liu[1]*

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XFELs provide new opportunities for structure determination of biomolecules, viruses, and nanomaterials. Inorganic core-shell nanocrystals, consisting of a palladium (Pd) shell and gold (Au) core, were used as a model system to investigate feasibility and problems of single particle image reconstruction from XFEL CDI data. The priori information obtained from electron microscopy was used to guide the analysis and as a reference to cross-validate the results obtained from the X-ray scattering data. The approach allowed us to analyze scattering data and infer measurement limits of the XFEL pulses for the nanoparticles under the given LCLS instrument setup. From the analysis of 54,405 scattering patterns, the orientations of 2,573 patterns were recovered to a reasonable confidence level. The recovered size distribution is consistent with the data from electron microscopy measurements (Figure 1). The orientation distribution indicates a bias toward the orientations that produce stronger scattering features (Figure 2), which could be attributed to either orientation preference or an inherent defect of the data analysis method.

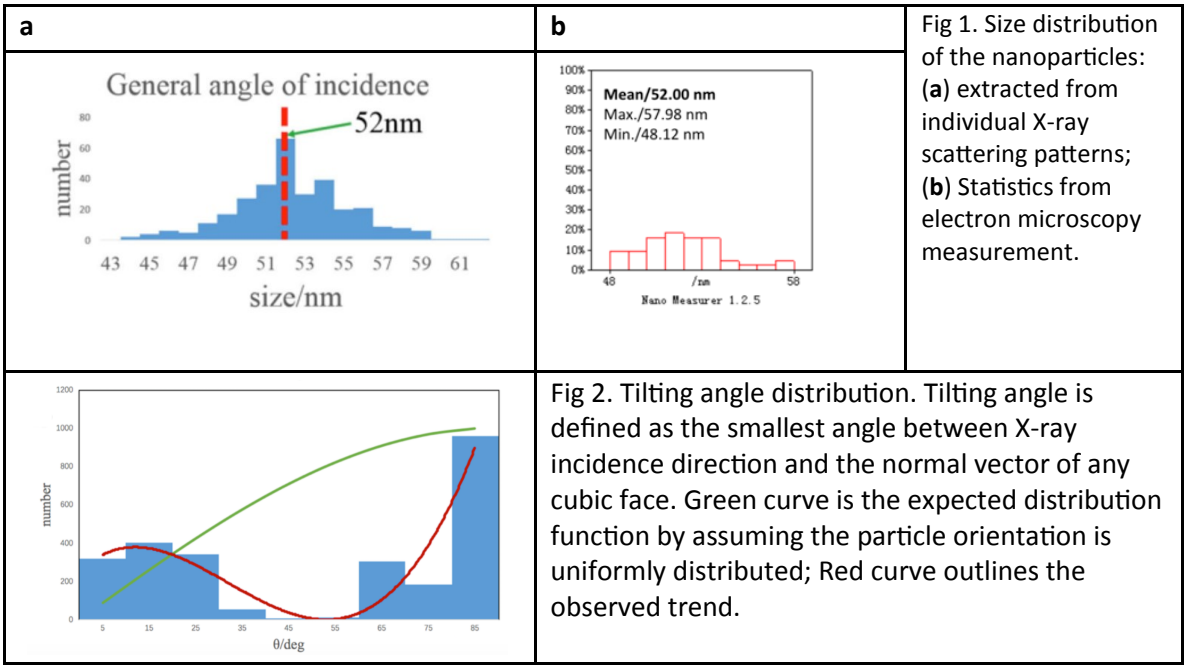
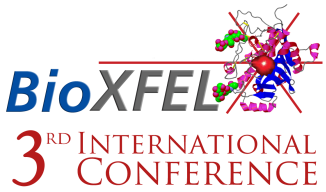


Fig 1. Size distribution of the nanoparticles: (**a**) extracted from individual X-ray scattering patterns; (**b**) Statistics from electron microscopy measurement.

Fig 2. Tilting angle distribution. Tilting angle is defined as the smallest angle between X-ray incidence direction and the normal vector of any cubic face. Green curve is the expected distribution function by assuming the particle orientation is uniformly distributed; Red curve outlines the observed trend.



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Friday

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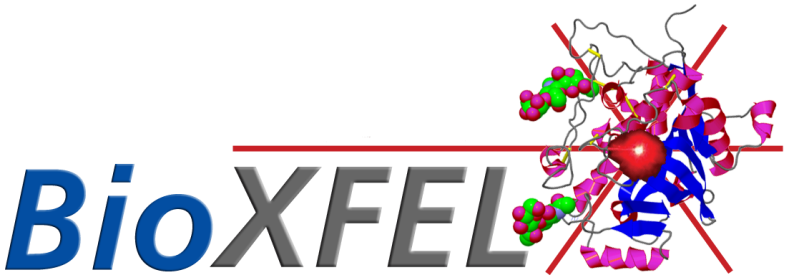

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GUILLERMO CALERO, UNIVERSITY OF PITTSBURGH

EFFICIENT STRATEGIES FOR FIXED TARGET DATA COLLECTION AT THE X-RAY PROTON PUMP

Guillermo Calero[1], Christopher Barnes[1], Jinhu Song[2], Ying Wu[1], Guouwu Lin[1], Angela Gronenborn[1], Jinwoo Ahn[1] and Aina Cohen[2]

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The field of X-ray crystallography is evolving. Development of X-ray free electron lasers, which provides X-rays ten times more brilliant than modern day synchrotrons, opens the possibility of solving three-dimensional structures of samples that can only crystallize as nano-crystals (NCs) or exhibit dose dependent- diffraction or -radiation damage. Data collection of NCs relies on use of a gas dynamic virtual nozzle (GDVD) liquid jet that injects NCs slurry into the path of a synchronized X-ray pulse under vacuum. With each pulse a single diffraction image is collected before the intensity of the beam destroys the crystal. Data collection of larger-size crystals using X-FELs pulses occurs at cryogenic temperatures using conventional or specially designed crystal mounting devices (henceforth referred as X-PP setup). Notwithstanding the proven success of the injector setups, two possible drawbacks of this methodology commonly arise. First, injector setups often require large amounts of protein to produce several milliliters of the slurry (containing 20-30% crystals). For some proteins this is a viable option, however for some challenging proteins that require special conditions for expression and purification, this is not practical. Second, there is potential damage that crystals could sustain during the injection process. Goniometer based fixed-target approaches at the XFEL are advantageous for data collection using delicate crystals, crystals in limited supply, large crystals or for crystal quality screening to prepare for injector-based experiments. The goniometer-based setup used at LCLS XPP, developed by the Cohen group, positions individual crystals into the beam path. However, since the intensity of the beam can create a damaged area around the target spot (20 to 30 µm in diameter depending on the beam diameter), the number of useful diffraction images per crystals is limited. As a consequence, data collection can be time consuming as many crystals need to be positioned and exposed.

We have developed an automated approach to efficiently expose low micron sized crystals that conserves crystals and beam time by mapping crystals in random orientations in relation to known reference points on multiple crystal holders (MCHs). To test our automated system of data collection, diffraction experiments were performed at XPP station using crystals mounted on MCHs of a novel structure of E. coli dGTPase. A total of 335 images were collected from 7 MHCs. Data was indexed and processed (using 211 images) to 3.3 Å resolution using the program cctbx.xfel, however data diffracting to 2.6 Å was observed for some images. The structure was solved using molecular replacement with a model previously generated using experimental phases from lower resolution synchrotron data. The structure was refined using manual building and Buster and reveals the position of the catalytic metal.

Jörg Standfuss, Paul Scherrer Institute

Serial Crystallography of Retinal Binding Proteins Using Synchrotron Radiation and Free Electron Lasers

Jörg Standfuss

Group Leader, Paul Scherrer Institute, Villigen PSI, Switzerland

In 2017 the Swiss Free Electron Laser (SwissFEL) will start its operation at the Paul Scherrer Institute. Serial femtosecond crystallography (SFX) using such X-ray free-electron lasers (XFELs) is a powerful method to determine high-resolution structures of pharmaceutically relevant membrane proteins. Another major promise of XFELs is to study light-triggered dynamics of biomolecules.

One of the current bottlenecks in XFEL science is that most facilities are still under construction and, even when they will be finished, access will likely remain scarce. In this presentation I will describe how we have adapted high viscosity injector systems to carry out serial millisecond crystallography (SMX) at synchrotron sources, where beamtime is more abundant. Based on these results we improved density and homogeneity of crystal preparations for efficient time-resolved data collection at XFEL sources. Structural intermediates of the light-driven proton pump bacteriorhodopsin (bR) obtained with pump probe delays in the picosecond to millisecond range clearly demonstrate the feasibility of using sample efficient high viscosity injectors to characterize the molecular dynamics of membrane proteins in a native like environment.

JONATHAN CLINGER, RICE UNIVERSITY

PRELIMINARY RESULTS FROM TIME-RESOLVED EXPERIMENTS ON PHYTOCHROMES AT LCLS/XPP

Jonathan Clinger,[1] Mitchell Miller,[1] George Phillips, Jr.,[1] Sethe Burgie,[2] Richard Vierstra,[2] Franklin Fuller,[3] Sheraz Gul,[3] Jan Kern,[3] Junko Yano,[3] Vittal Yachandra,[3] Marc Allaire,[3] Aaron Brewster,[3] Tara Michels-Clark,[3] Artem Lyubimov,[3] Nicholas Sauter,[3] Roberto Alonso Mori,[4] Diling Zhu,[4] Silke Nelson,[4] James Glownia,[4] Claudiu Stan,[4] Babak Andi,[5] and Allen Orville[5,6]

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Time-resolved serial femtosecond crystallography experiments were conducted at the XPP beamline at the LCLS for two constructs of *D. radiodurans* bacteriophytochrome (BphP) to better than 2.0 Å resolution. We used the on-demand acoustic drop injector and tape drive sample delivery methods developed by the collaboration between Lawrence Berkeley and Brookhaven National Labs. Crystal plates of the chromophore-binding domain, with 50 µm in the largest dimension, were grown in batches. Two dark-adapted data sets were collected, as well as 12 time-resolved data sets after laser illumination with different time delays, laser powers, and excitation wavelengths. A dark-adapted data set from the chromophore binding domain plus the photo-sensory module was also collected with the acoustic injector method. An updated *CCTBX.XFEL* package was implemented to provide approximately real-time feedback for indexing/integrating/merging as a function of overall resolution and in the highest resolution bin. This improved real time decision making and beam time efficiency. Data processing is underway.

This research was funded 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. Portions of this research were carried out at the Linac Coherent Light Source (LCLS) at the SLAC National Accelerator Laboratory. LCLS is an Office of Science User Facility operated for the U.S. Department of Energy Office of Science by Stanford University. This material is based upon work supported by the STC Program of the National Science Foundation through BioXFEL under Agreement No. 1231306.

HASAN DEMIRCI, STANFORD PULSE INSTITUTE

SERIAL FEMTOSECOND X-RAY CRYSTALLOGRAPHY OF 30S RIBOSOMAL SUBUNIT MICROCRYSTALS IN LIQUID SUSPENSION AT AMBIENT TEMPERATURE

DeMirci, H.[1,2], Sierra, R.G.[1], Stan, C.A.[1], Laksmono, H.[1]

1. Non-Periodic Imaging Group, Stanford PULSE Institute, Menlo Park, CA. 2. Stanford Synchrotron Radiation Lightsource (SSRL), Menlo Park, CA.

High-resolution ribosome structures determined by X-ray crystallography have provided important insights into the mechanism of translation. Such studies have thus far relied on large ribosome crystals kept at cryogenic temperatures to reduce radiation damage. Here we describe the application of serial femtosecond X-ray crystallography (SFX) using an X-ray free-electron laser (XFEL) to obtain diffraction data from ribosome microcrystals in liquid suspension at ambient temperature. 30S ribosomal subunit microcrystals diffracted to beyond 3.4 Å resolution, demonstrating the feasibility of using SFX for ribosome structural studies. The ability to collect diffraction data at near-physiological temperatures promises to provide fundamental insights into the structural dynamics of the ribosome and its functional complexes.

AINA COHEN, SSRL

MACROMOLECULAR FEMTOSECOND CRYSTALLOGRAPHY AT LCLS

A.E. Cohen representing the SSRL SMB and LCLS XPP/MFX team.

A new experimental station, the Macromolecular Femtosecond Crystallography (MFX) instrument is under development at LCLS with first experiments planned for July 2106. MFX will provide a permanent location for a stable and flexible endstation supporting X-ray diffraction experiments in atmosphere. MFX will allow for the optimization of the operations of LCLS in general but also specifically as it relates to structural biology by relocating many of the experiments from the XPP and XCS instruments. Goniometer-based diffraction experiments will be supported at MFX using robotically mounted samples at cryogenic temperatures, amenable to longer term storage and mounting at a moment's notice. The experimental front-end is based on developments at SSRL and LCLS XPP to provide an efficient flexible 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. The MFX project and recent results using radiation sensitive crystals in limited supply at LCLS-XPP will be described. The MFX endstation will be very flexible and support a variety of experimental setups including crystal injectors. Also supported will be a new sample delivery device that uses a mesh to mount crystals directly from a vial of mother liquor. Initial experiments demonstrate the efficiency of this device for structure determination using delicate crystals at physiological temperatures.

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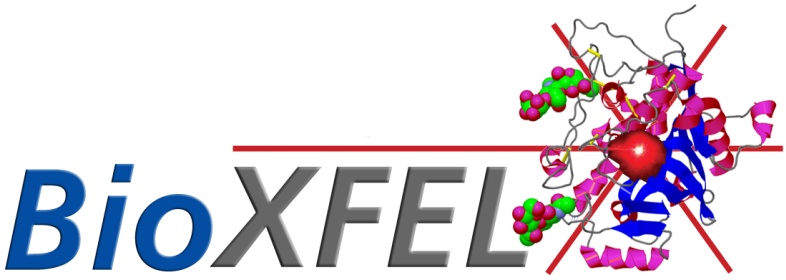
ROBERT FISCHETTI, ARGONNE NATIONAL LABORATORY

THE SYNERGY BETWEEN STORAGE RING AND XFEL BASED CRYSTALLOGRAPHY

Robert F. Fischetti, GM/CA and APS, Argonne National Laboratory, Lemont IL, USA

3rd generation storage-ring-sources coupled with newly developed microcrystallography capabilities have been used to determine the atomic resolution structure of many important biological problems. Some of the recent developments that have improved data quality include user-selectable micro-beams, raster mapping, vector data collection, fine-phi-slicing enabled by shutter-less data collection, multiple crystal strategy and SONICC alignment of crystals. The Linac Coherent Light Source, has driven new technology developments, such as "serial crystallography", which in some cases has achieved higher resolution structures. These new technologies such as viscous jet sample injectors are now being implemented on storage-ring-source beamlines. Another very exciting but controversial topic is the potential for outrunning secondary radiation damage at room temperature. In the near future, 4th generation storage-ring sources based on multibend achromatic (MBA) lattices will increase brightness by at least two orders of magnitude. In this talk, I will present the current state of microcrystallography, recent results using viscous jet sample delivery on storage-ring beamlines and the potential game-changing future for structural biology enabled by the APS-MBA source.

Poster Competition Abstracts



3RD International Conference

A National Science Foundation Science and Technology Center

BioXFEL 3rd International Conference

Poster Blitz

Wednesday 2:45—3:15 Salon A

Chairs: Thomas Grant (UB) & Edward Snell (HWI/UB)

2016 BioXFEL Poster Blitz			
#	First	Last	Title
1	Bahige	Abdallah	Protein Crystallization Screening using an Actuated Microfluidic Gradient Generator
2	Christopher	Barnes	Towards Highly Efficient Serial Femtosecond Crystallography Data Collection
3	Sarah	Bowman	Leveraging the Unique Features of Transition Metals in Metalloprotein Crystallography
4	Jesse	Coe	Investigating the Photosystem I-Ferredoxin Complex Using Time-Resolved Serial Femtosecond Crystallography and Continuous Diffraction Phasing
5	Stefan	Kolek	Experimental Design for Microbatch-under-oil Optimization to Establish Batch Crystallization Conditions that are Suitable for XFEL Data Collection
6	Lars	Redecke	InCellCryst – An Automated Pipeline for Serial Crystallography in Living Insect Cells
7	Rob	Lawrence	Implications of Diffuse X-ray Scattering Measured from Virus Crystals
8	Isabelle	Martiel	Fixed-Target Femtosecond Protein Crystallography at SwissFEL
9	Jose	Olmos	Time-Resolved X-ray Crystallography of Enzymes with an X-ray Free Electron laser
10	Manoj	Saxena	Breaking the Mold: Challenging the Paradigm of Transferrin Metal Binding and Activity Regulation
11	Raymond	Sierra	Concentric-flow Electrokinetic Injector Enables Serial Crystallography of Ribosome and Photosystem II
12	Ganesh	Subramanian	Characterizing the Intermediate State Electronic and Molecular Structure during the Photolysis of Methylcobalamin, using Ultrafast Pump-probe X-ray Absorption Spectroscopy
13	Jessica	Thomaston	High-resolution Crystal Structures of the Influenza AM2 Proton Channel: Insights into Water Networks
14	Michael	Thompson	Infrared Laser-Induced Temperature-Jump: A General Perturbation Method for Time-Resolved Crystallographic Studies of Protein Dynamics
15	Chelsie	Conrad	Reduction of Sample Consumption for Serial Crystallography by Viscous Sample Delivery

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POSTER SESSION

THURSDAY 6:30—8:30 SALONS B & C

JUDGING COMMITTEE: ANNE STONE (MOLECULAR DIMENSIONS), THOMAS GRANT (UB), & LOIS POLLACK (CORNELL)

2016 BioXFEL STC Conference Poster Competition			
#	First Name	Last Name	Title
1	Bahige	Abdallah	Protein Crystallization Screening using an Actuated Microfluidic Gradient Generator
2	Salah	Awel	Optical Focusing and Visualization of Isolated Particles for Diffractive Imaging Experiments
3	Christopher	Barnes	Towards Highly Efficient Serial Femtosecond Crystallography Data Collection
4	William	Bauer	BioXFEL Education and Diversity Programs
5	Sabine	Botha	Distinguishing Protein Nanocrystals from Amorphous Precipitate by Depolarized Dynamic Light Scattering
6	Sarah	Bowman	Leveraging the Unique Features of Transition Metals in Metalloprotein Crystallography
7	Martin	Caffrey	In Meso In Situ Serial X-ray Crystallography of Soluble and Membrane Proteins at Cryogenic Temperatures
8	George	Calvey	Mixing Injector for Time-Resolved Serial Femtosecond Crystallography
9	Jose	Carmona-Negron	Crystallographic Studies of Ferrocene-Estrogen Derivatives Complexed with Human Serum Albumin and Estrogen Receptor alpha as Potential Drug Delivery Targets
10	Gabriela	Casanova	Conformational Analysis of Cold Shock Protein at Different Temperatures Using SAXS and Crystallography
11	Joe	Chen	Phase Retrieval from Randomly Terminated Finite Crystals
12	Jonathan	Clinger	Preliminary Results from Time-Resolved Experiments on Phytochromes at LCLS/XPP
13	Jesse	Coe	Investigating the Photosystem I-Ferredoxin Complex Using Time-Resolved Serial Femtosecond Crystallography and Continuous Diffraction Phasing
14	Chelsie	Conrad	Reduction of Sample Consumption for Serial Crystallography by Viscous Sample Delivery
15	Eugene	Dao	Microcrystallization for XFEL-based Serial Femtosecond Crystallography
16	Ali	Dashti	Mapping the Conformations and Energy Landscape of Molecular Machines
17	Hasan	DeMirci	Serial Femtosecond X-ray Crystallography of 30S Ribosomal Subunit Microcrystals in Liquid Suspension at Ambient Temperature
18	Adalberto	Diaz	Molecular Biophysical Studies and Crystallization Screening of Centrin-Prp40Ap Complex

19	Ximeng	Dow	Determination of Protein Nanocrystals Size Distribution Using Second Harmonic Generation Correlation Spectroscopy (SHG-CS)
20	Helen	Duyvesteyn	PhiX-174: Making in vivo Virus XFELs de facto
21	Austin	Echelmeier	Protein Crystal Delivery for Serial Femtosecond X-ray Crystallography Utilizing Microfluidic Droplets
22	Rita	Graceffa	Delivery Methods for Biological Samples at European XFEL
23	Thomas	Grant	Ab Initio Modeling of Time-resolved Difference Profiles from XFEL Solution Scattering
24	Reinaldo	Hernandez	Optimization of Phospholipid Bilayer Nanodiscs for Use in Single Particle Imaging Studies
25	Arjen	Jakobi	In Cellulo Serial Femtosecond X-ray Diffraction of Alcohol Oxidase Crystals Inside Yeast Cells
26	Stefan	Kolek	Experimental Design for Microbatch-under-oil Optimization to Establish Batch Crystallization Conditions that are Suitable for XFEL Data Collection
27	Krishna	Rajan	Application of Manifold Learning Methods for Analysis of Structural Data from the BioXFEL
28	Robert	Lawrence	Implications of Diffuse X-ray Scattering Measured from Virus Crystals
29	Chufeng	Li	Exploring to Improve Accuracy in Crystal Orientation Determination in SFX Data Analysis
30	Haiguang	Liu	X-ray Free Electron Laser Diffraction from Inorganic Nanoparticles
31	Brian	Mahon	Human Carbonic Anhydrase as a Model System for Time-Resolved Serial-Femtosecond Crystallography
32	Darya	Marchany-Rivera	Oxy-Hemoglobin III Structural Model and Crystal Structure Determination
33	Isabelle	Martiel	Fixed-Target Femtosecond Protein Crystallography at SwissFEL
34	Victoria	Mazalova	2D Optical and X-ray Emission Spectroscopies within the AXSIS project: Electronic Dynamics and Undamaged Electronic Structure Study of Photosystem II
35	Mitch	Miller	Testing the Direct Phasing of Protein Structures with High Solvent Contents
36	Osamu	Miyashita	Hybrid Approach for X-ray Free Electron Laser Single Particle Analysis of Biomolecular Systems
37	Henrike	Mueller-Werkmeister	Capturing Functionally Relevant Protein Motions at the Atomic Level: Femtosecond Time-resolved Serial Crystallography of Ligand Dissociation in MbCO
38	Garrett	Nelson	3D Printing for Nozzle Fabrication
39	Prakash	Nepal	Extraction of Structural Changes from Difference SAXS/WAXS Alone
40	Jose	Olmos	Time-Resolved X-ray Crystallography of Enzymes with an X-ray Free Electron laser

41	Nadia	Opara	Fixed Target Approach for Protein Crystal Delivery at X-ray Free Electron Lasers
42	George	Phillips	XRayView 5.0, an Updated Educational Crystallography Program Incorporating Visualization of Serial Femtosecond Crystallography
43	Hin Cheuk	Poon	A Symmetrized Phasing Algorithm Applied to the Experimental XFEL Data from Rice Dwarf Virus (RDV)
44	Lars	Redecke	InCellCryst – An Automated Pipeline for Serial Crystallography in Living Insect Cells
45	Josiris	Rodriguez	Crystallization and X-ray Diffraction Data for CRP-hemoglobin I Complex
46	Philip	Roedig	Serial Femtosecond Crystallography on a Chip with a Data Acquisition Rate of Up to 120 Hz
47	Manoj	Saxena	Breaking the Mold: Challenging the Paradigm of Transferrin Metal Binding and Activity Regulation
48	Nicole	Scarborough	Dye Methods for Enhanced Contrast in Second Harmonic Generation Imaging of Protein Crystals
49	Reyhaneh	Sepehr	Structural Dynamics beyond Pump-probe Timing Jitter
50	Raymond	Sierra	Concentric-flow Electrokinetic Injector Enables Serial Crystallography of Ribosome and Photosystem II
51	Ganesh	Subramanian	Characterizing the Intermediate State Electronic and Molecular Structure during the Photolysis of Methylcobalamin, using Ultrafast Pump-probe X-ray Absorption Spectroscopy
52	Jessica	Thomaston	High-resolution Crystal Structures of the Influenza AM2 Proton Channel: Insights into Water Networks
53	Michael	Thompson	Infrared Laser-Induced Temperature-Jump: A General Perturbation Method for Time-Resolved Crystallographic Studies of Protein Dynamics
54	Sandi	Wibowo	The Singular Value Decomposition of Pair Correlations Matrices from an XFEL Experiment
55	Max	Wiedorn	Novel Sample Delivery System for Serial Crystallography at XFELs

Bahige Abdallah, Arizona State University

Protein Crystallization Screening using an Actuated Microfluidic Gradient Generator

Abdallah, B.G., Roy-Chowdhury, S., Fromme, R., Fromme, P., and Ros, A.

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Center for Applied Structural Discovery, The Biodesign Institute, Arizona State University, Tempe, AZ 85287, USA

Protein crystallization is the major bottleneck of structure determination by X-ray crystallography, hampering the process by years in some cases. Additionally, sample is wasted due to numerous screening runs using significant amounts of protein. Here, we demonstrate a microfluidic method implementing crystallization screening using nanoscale volumes of protein per trial. The device is made with cost effective materials and is completely automated for efficient and economical experimentation. Over 150 trials can be implemented with unique concentrations of protein and precipitant established by gradient generation and isolated by elastomeric valves for crystallization incubation. The device was calibrated using a fluorescent dye and compared to a numerical model where concentrations of each trial can be quantified to establish crystallization phase diagrams. Additionally, we were able to model various crystallization precipitant types including typical salting-out conditions and viscous systems such as polyethylene glycol. Using this device, we successfully crystallized lysozyme and phycocyanin as visualized by compatible crystal imaging methods such as brightfield microscopy, UV fluorescence, and second order nonlinear imaging of chiral crystals. Concentrations heeding observed crystal formation were quantified and found to be in agreement with reported conditions for both proteins. Low sample consumption, efficient experimentation, and compatibility with a variety of proteins and imaging techniques allow this device to be a powerful crystallization screening tool for crystallography, specifically for optimizing crystal characteristics for serial femtosecond crystallography at X-ray free-electron lasers.

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SALAH AWEL, CENTER FOR FREE ELECTRON LASER SCIENCE

OPTICAL FOCUSING AND VISUALIZATION OF ISOLATED PARTICLES FOR DIFFRACTIVE IMAGING EXPERIMENTS

Awel, S.,[1,4] Kirian, R.A.,[1,3] Ekerskorn, N.,[2] Wiedorn, M.,[1,5] Horke, D.,[1,4] Rode, A.,[2] Küpper, J.,[1,4,5] and Chapman, H.N.,[1,4,5]

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The short, intense, and coherent x-ray pulses produced by x-ray free-electron lasers (XFELs) have lead to major advances in macromolecular structure d etermination. Efforts are underway to extend the successful "serial femtosecond crystallography" paradigm to include *isolated* proteins, viruses or cells, without the need for growing well -ordered crystals (often the principal bottleneck to structure determination). This "single-particle imaging" scheme consists of directing a stream of randomly oriented bioparticles across the focus of the XFEL beam so that high-resolution three-dimensional electron density maps can be constructed from multiple diffraction patterns of identical particles.

Presently, the difficulty of generating isolated bioparticles and efficiently delivering them to a sub-micrometer x-ray focus is a limiting factor in the development of single-particle imaging. For a 100 nm x-ray focus, current sample delivery efficiencies (fraction of particles that are intercepted by an x-ray pulse) are on the order of 10^{-7} on average, and hit fractions (fraction of x-ray pulses that intercept a particle) are below 0.1%. With such efficiencies, high-resolution experiments require samples prepared in large quantities and extended data collection times. In order to mitigate this problem, we are developing techniques for guiding aerosolized nanoparticles to the X-ray focus with specially shaped laser illumination [1, 2]. Our current experiments aim at transversely confining streams of aerosolized particles as they exit an aerosol injector [3] with a counter-propagating "hollow" quasi-Bessel beam. The experiment exploits radiation pressure and thermal (photophoretic) forces arising from the interaction of the particles with surrounding gas molecules [4].

References:

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CHRISTOPHER BARNES, UNIVERSITY OF PITTSBURGH

TOWARDS HIGHLY EFFICIENT SERIAL FEMTOSECOND CRYSTALLOGRAPHY DATA COLLECTION

Christopher O. Barnes[1], Elena G. Kovaleva[2], Xiafeng Fu[1], Jinhu Song[2], Guowu Lin[1], Simon C. Weiss[1], Aaron S. Brewster[3], Elizabeth L. Baxter[1], Aina E. Cohen[1*] and Guillermo Calero[2*]

[1]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA.
[2]Stanford Synchrotron Radiation Lightsource, Menlo Park, CA 94025, USA.
[3]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

Serial femtosecond crystallography (SFX) employing high-intensity X-ray free-electron laser (XFEL) sources have enabled structural studies on microcrystalline protein samples at non-cryogenic temperatures. However, the identification and optimization of conditions that produce well diffracting microcrystals remains an experimental challenge. Here, we report methods for improving the “hit-rate” and indexing efficiency during both liquid-jet and fixed -target experiments. Our parallel SFX and transmission electron microscopy (TEM) experiments of fragmented

microcrystals illustrate the utility of TEM analysis in evaluating sample monodispersity
and lattice quality, parameters we determined as critical to the efficiency of liquid-jet SFX experiments. In addition, we reveal methods to produce homogenously sized microcrystals for use with a novel multi-crystal holder designed in our laboratory. Preliminary results suggest that the random crystal orientations, which occur during crystal mounting, allow for X-ray data collection that optimizes resolution, while significantly decreasing the number of crystals needed to complete a SFX dataset using a fixed-goniometer setup.

William Bauer, Hauptman-Woodward Institute

BioXFEL Education and Diversity Programs

William Bauer [1] and the Education and Diversity Committee [1,2,3,4]

[1]Hauptman-Woodward Medical Research Institute, Buffalo NY. [2]Rice University, Houston Texas. [3]Arizona State University, Tempe AZ. [4]University of Wisconsin Milwaukee, Milwaukee WI.

Our newly developed BioXFEL Education and Diversity programming employs a customized trainee-centric approach. BioXFEL scholars have created a cohesive group, known as the Association of BioXFEL Young Scientists (ABYS) to direct and personalize their own educational experiences and professional development activities. ABYS informs the Education and Diversity Committee of their requests and priorities so that the Committee may develop specific programs and directly addresses the needs of the students and postdoctoral scholars. Our current education programming includes an online journal club, and BioXFEL lecture series, annual workshops, and cross-training scholarships. Our new diversity plan has been redesigned to focus on collaborations with minority-serving institutions by creating a customized set of programs. These programs are designed to fully integrate the members of the collaborating institutions into the scientific and educational objectives of the Center while also meeting the needs of the participants. Our collaboration with UPR will be used to develop programs and guidelines for incorporation of minorities that are underrepresented in science and can be applied to future collaborations. We are currently offering summer undergraduate internships, graduate exchange internships, online and customized classes to our UPR collaborators. Stop by the poster to learn more about educational opportunities within the BioXFEL Center.

Sabine Botha, University of Hamburg

Distinguishing Protein Nanocrystals from Amorphous Precipitate by Depolarized Dynamic Light Scattering

Sabine Botha1,2 and Robin Schubert1,2, Arne Meyer3, Karsten Dierks3, Svetlana Kapis2, Rudolph Reimer4, Markus Perbandt1,2 and Christian Betzel1,2

1. The Hamburg Centre for Ultrafast Imaging, Luruper Chaussee 149, Hamburg 22761, Germany

2. Institute of Biochemistry and Molecular Biology, Laboratory for Structural Biology of Infection and Inflammation, University of Hamburg, Notkestrasse 85, Hamburg 22603, Germany

3. XtalConcepts, Marlowring 19, Hamburg 22525, Germany

4. Heinrich-Pette-Institute, Leibniz-Institute for Experimental Virology, Martinistrasse 52, Hamburg 20251, Germany

Growth and preparation of high quality micro-sized protein crystals, optimal for data collection experiments at modern micro-focus synchrotron (SR) beamlines and growth of nanocrystals required for data collection at Free-Electron-Laser (FEL) radiation sources is a new and challenging task. We will present advanced methods for precisely monitoring crystal growth and optimizing the preparation of crystalline particles too small to be observed by light microscopy. The identification of the presence of a spatial repetitive orientation of macromolecules (crystal nuclei) in the early stages of the crystallization process is essential for detecting and identifying nanocrystals. The optical properties of a crystal lattice offer the potential to detect the transition from disordered to higher ordered particles. A unique experimental setup was designed and constructed to detect nanocrystal formation by analyzing depolarized, scattered laser light. The ability of a lattice to depolarize laser light depends on the different refractive indices along the different crystal lattice axes. The results obtained so far demonstrate that the successful detection of nano-sized protein crystals at early stages of crystal growth is possible by analyzing the signal intensity of the depolarized component of the scattered light. This method and approach allows an effective differentiation between protein-dense liquid cluster formation and ordered nanocrystals [1]. The data and results obtained so far were verified by complementary methods such as X-ray powder diffraction, second harmonic generation, ultraviolet two-photon excited fluorescence and scanning electron microscopy.

Further, this particular advanced laser light scattering technique can be combined with a state of the art protein crystallization robotic setup (Xtal-Controller [2]), allowing the controlled nanoliter increment addition of protein, precipitant and additive solution towards a crystallization solution sitting on a microbalance. By combining the described techniques, crystallization phenomena can be characterized in detail and methods can be optimized for the efficient production of nanocrystals. Details and examples will be presented.

[1] Schubert *et al.,* Journal of Applied Crystallography, Issue 48, 1476-1484, (2015)

[2] Meyer *et al.* Acta Crystallographica Section F68, 994-998, (2012)

Acknowlegements

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SARAH BOWMAN, MIT

LEVERAGING THE UNIQUE FEATURES OF TRANSITION METALS IN METALLOPROTEIN CRYSTALLOGRAPHY

Bowman, S.E.J.[1,2], Bridwell-Rabb, J.[1,2] and Drennan, C.L.[1,2,3]

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Metal ions and metallocofactors play important roles in a broad range of biochemical reactions. It has been estimated that as much as 25-30% of the proteome uses transition metal ions to carry out a variety of essential functions. The metals ions incorporated within metalloproteins fulfill functional roles based on chemical properties, the diversity of which arises as transition metals can adopt different redox states and geometries, dictated by the identity of the metal and its coordinating amino acid ligands. These properties allow transition metals to engage in a multitude of biochemical reactions, expanding the range of reactivity available to biomolecules.

Crystallography of metalloproteins presents challenges and unique advantages. Some of the challenges include incorporating the correct metal or metallocofactor, maintaining the proper environment for the protein to be purified and crystallized (including providing anaerobic, cold, and/or aphotic environments), and being mindful of the possibility of X-ray induced damage to the proteins. Some of the advantages include the ability to determine phases using anomalous dispersion and using spectroscopic techniques to investigate the metal sites within single crystals. Many metals have rich electronic properties that can provide intense and spectroscopically unique signatures, and recent advances in simultaneous or parallel spectroscopic techniques on single crystals has yielded exciting developments in metalloprotein structure determination. Here we will present results from the Drennan lab at MIT on the advantageous uses of metals in crystallography, including using metallocofactors to obtain phasing information, using K-edge X-ray absorption spectroscopy to identify metals coordinated in metalloprotein crystals, and using UV-vis spectroscopy on crystals to clarify large scale domain movements.

BioXFEL 3RD INTERNATIONAL CONFERENCE

MARTIN CAFFREY, TRINITY COLLEGE DUBLIN

IN MESO IN SITU SERIAL X-RAY CRYSTALLOGRAPHY OF SOLUBLE AND MEMBRANE PROTEINS AT CRYOGENIC TEMPERATURES

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A method for presenting crystals of soluble and membrane proteins growing in the lipid cubic or sponge phase for in situ diffraction data collection at cryogenic temperatures is described (Huang et al., Acta Cryst. D. 2016. In press). The method dispenses with the need for the technically demanding and inefficient crystal harvesting step that is an integral part of the lipid cubic phase or in meso method for growing crystals. Crystals are dispersed in a bolus of mesophase sandwiched between thin plastic windows. The bolus contains tens to hundreds of crystals, visible with an in-line microscope at macromolecular crystallography synchrotron beamlines and suitably disposed for conventional or serial crystallographic data collection. Wells containing the crystal-laden boluses are removed individually from hermetically sealed glass plates in which crystallization occurs, affixed to pins on goniometer bases and excess precipitant removed from around the mesophase. The wells are snap cooled in liquid nitrogen, stored and shipped in Dewars, and manually or robotically mounted on a goniometer in a cryo-stream for diffraction data collection at 100 K as is done routinely with standard, loop-harvested crystals. The method is a variant on the recently introduced in meso in situ serial crystallography (IMISX) method that enables crystallographic measurements at cryogenic temperatures where crystal lifetimes are enormously enhanced whilst reducing protein consumption dramatically. The new approach has been used to generate high-resolution crystal structures of a G protein-coupled receptor, α-helical and β-barrel transporters, and an enzyme as model integral membrane proteins. Insulin and lysozyme were used as test soluble proteins. The quality of data that can be generated by this method was attested to by performing sulfur and bromine SAD phasing with two of the test proteins.

Acknowledgements. The work was funded in part by grants from Science Foundation Ireland (12/IA/1255). PM was supported by a grant (WELBIO CR-2012S-04) to C. Govaerts (Université libre de Bruxelles) from the Belgian National Funds for Scientific Research. X-ray diffraction data were collected on beamlines PX I and PX II at the Swiss Light Source, Villigen, Switzerland.

George Calvey, Cornell University

Mixing Injector for Time-Resolved Serial Femtosecond Crystallography

George Calvey, Andrea M. Katz, Yujie Chen and Lois Pollack

Cornell University, Ithaca, NY 14853, USA

We developed a mixing injector for observing chemically induced structural changes in serial femtosecond crystallography experiments. The mixing injector is capable of introducing a reactive species to microcrystals, causing a structural change, shortly before the crystals enter the x-ray beam. Because the reactive species can diffuse into the microcrystals on a millisecond time scale, never before seen structural changes can be observed and time resolved. The mixing injector is capable of accessing time points from <1 to >100 milliseconds and has many aspects that make it extremely robust, including an integrated filter comparable to a microfluidic post array. The design was successfully demonstrated without mixing at beamtime LH96 and will be used in a mixing experiment at LK17 in December 2015.

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JOSE CARMONA-NEGRON, UNIVERSITY OF PUERTO RICO

CRYSTALLOGRAPHIC STUDIES OF FERROCENE-ESTROGEN DERIVATIVES COMPLEXED WITH HUMAN SERUM ALBUMIN AND ESTROGEN RECEPTOR ALPHA AS POTENTIAL DRUG DELIVERY TARGETS

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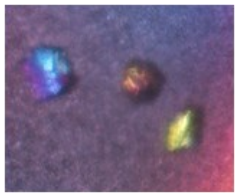
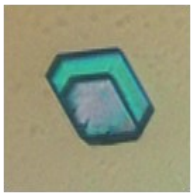
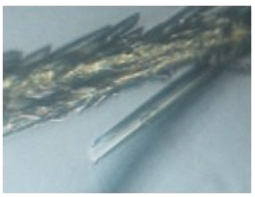
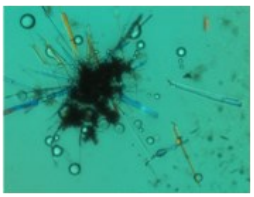
Unlike common drugs that consist of entirely organic groups, the incorporation of metal in organic frames provides a new range of not only structure and bonding properties but also reactivity. A new chapter in bioinorganic chemistry was open after cisplatin antineoplastic activity was discovered and 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, similar to that of conventional therapeutic drugs such as tamoxifen and cisplati. 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.

Enrique Meléndez is thankful for the financial support of NSF-BioXFEL program (grant # *1231306*) for the research assistantship of José A. Carmona-Negrón.

Gabriela Casanova, University of Puerto Rico

Conformational Analysis of Cold Shock Protein at Different Temperatures Using SAXS and Crystallography

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Cold Shock protein (Csp) has been found in more than 400 different types of bacteria from a wide range of habitat temperatures. Previous experiments have suggested that the cold shock protein from *Bacillus caldolyticus* undergoes a conformational change when exposed to cold temperatures (below 5° C). Samples ranging in concentration from 1 mg/mL to 3.5 mg/mL were studied with SAXS (Small Angle X-Ray Scattering). Our data demonstrates that the protein transitions from a monomer to a dimer as the temperature is decreased below 0° C. It is hypothesized that the transitions is accompanied by temperature domain swap. Cold shock protein has been crystallized in several conditions at both 4°C and -8° C in an attempt to trap the protein in the dimeric state. To our knowledge, this also represents the first time that a protein has been crystallized at -8°C. Preliminary results show that there is no apparent domain swap at 4°C. Initial analysis of the -8°C crystal structure suggests a domain swap model. Crystal structure refinement is still in process.

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Joe Chen, Arizona State University

Phase Retrieval from Randomly Terminated Finite Crystals

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In serial femtosecond crystallography with x-ray free-electron lasers, diffraction data from crystals that have a small number of unit cells can be obtained [1]. The fewer number of unit cells gives rise to measurable diffraction between the Bragg reflections, courtesy of a shape transform that is no longer composed of delta-like peaks. This inter-Bragg scattering provides enough extra information to allow the crystallographic phase problem to be solved in principle.

By separating the effect of the shape transform from the overall diffraction via the inherent periodicity of the shape transform about the reciprocal lattice, the averaged diffracted intensity from an ensemble of crystals can be directly converted into the diffracted intensity of the crystals' unit cell [2]. The problem then becomes that of reconstructing a single, non-periodic object (the contents of the unit cell) from the amplitude of its Fourier transform, which typically has a unique solution that can be found using iterative phase retrieval algorithms [3,4].

However, if there is more than one molecule per unit cell then the inter-Bragg diffraction from such crystals will depend on the particular configuration of the molecules on the edge of the crystal, as different unit cells can be defined for different surface terminations. The diffracted intensity recovered from the method described above can no longer be attributed to a single kind of unit cell but is instead, to a first approximation, equal to the incoherent average over a set of unit cells that derives from different arrangements of the molecule based on the space group at hand [5,6].

Following from the recent experimental verification of shape-transform phasing without edge effects [7], the applicability of this approach when multiple unit cells are present is explored. Results so-far indicate successful phase retrieval under this more realistic circumstance is still possible.

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JONATHAN CLINGER, RICE UNIVERSITY

PRELIMINARY RESULTS FROM TIME-RESOLVED EXPERIMENTS ON PHYTOCHROMES AT LCLS/XPP

Jonathan Clinger,[1] Mitchell Miller,[1] George Phillips, Jr.,[1] Sethe Burgie,[2] Richard Vierstra,[2] Franklin Fuller,[3] Sheraz Gul,[3] Jan Kern,[3] Junko Yano,[3] Vittal Yachandra,[3] Marc Allaire,[3] Aaron Brewster,[3] Tara Michels-Clark,[3] Artem Lyubimov,[3] Nicholas Sauter,[3] Roberto Alonso Mori,[4] Diling Zhu,[4] Silke Nelson,[4] James Glownia,[4] Claudiu Stan,[4] Babak Andi,[5] and Allen Orville[5,6]

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Time-resolved serial femtosecond crystallography experiments were conducted at the XPP beamline at the LCLS for two constructs of *D. radiodurans* bacteriophytochrome (BphP) to better than 2.0 Å resolution. We used the on-demand acoustic drop injector and tape drive sample delivery methods developed by the collaboration between Lawrence Berkeley and Brookhaven National Labs. Crystal plates of the chromophore-binding domain, with 50 µm in the largest dimension, were grown in batches. Two dark-adapted data sets were collected, as well as 12 time-resolved data sets after laser illumination with different time delays, laser powers, and excitation wavelengths. A dark-adapted data set from the chromophore binding domain plus the photo-sensory module was also collected with the acoustic injector method. An updated *CCTBX.XFEL* package was implemented to provide approximately real-time feedback for indexing/integrating/merging as a function of overall resolution and in the highest resolution bin. This improved real time decision making and beam time efficiency. Data processing is underway.

This research was funded 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. Portions of this research were carried out at the Linac Coherent Light Source (LCLS) at the SLAC National Accelerator Laboratory. LCLS is an Office of Science User Facility operated for the U.S. Department of Energy Office of Science by Stanford University. This material is based upon work supported by the STC Program of the National Science Foundation through BioXFEL under Agreement No. 1231306.

Jesse Coe, Arizona State University

Investigating the Photosystem I-Ferredoxin Complex Using Time-Resolved Serial Femtosecond Crystallography and Continuous Diffraction Phasing

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The use of serial femtosecond X-ray crystallography (SFX) is a recently developed technique in which a stream of nano-microcrystals are subjugated to an extremely brilliant femtosecond pulse of X-rays, generated by a free electron laser (FEL), that allow diffraction before the ensuing Coulomb explosion destroys the crystal (**Fig. 1**).[2] The use of unique crystals for each diffraction pattern allows time-resolved SFX (TR-SFX) of fast and irreversible processes. This can be achieved for photoactive proteins by introducing an optical photoexcitation to the protein stream at a specified time, τ, prior to interaction with the FEL, resulting in a populated intermediate state being probed.

PSI is a membrane protein complex that plays an integral role in photosynthesis, catalyzing the terminal step of photo-excited electron transport through the thylakoid membrane to its PSI's natural acceptor ferredoxin (Fd). This process results in the PSI bound protein Fd becoming reduced and undocking to participate in the reduction of $NADP^+$ to NADPH, which is crucial in the subsequent conversion of CO_2 into carbohydrates. Although spectroscopic studies and preliminary SFX work have provided evidence that the PSI-Fd interaction is a multistep process with transition states that occur on the orders of nano and microseconds[1,2], there is currently no model for any step of this process, including the ground state.

A primary difficulty in characterizing the structure of the PSI-Fd co-complex is that crystals to date have been heavily resolution limited in their Bragg diffraction. However, continuous diffuse scattering has been observed to occur well beyond Bragg resolution. Continuous diffraction predominantly caused by random translational deviations within the crystal have recently been shown to allow de novo phasing and extension of resolution within a diffraction model[3]. PSI -Fd crystals exhibit this type of diffuse scattering and current work is focused on exploiting this in order to obtain the first atomic resolution model of the complex. Revealing the electron transfer mechanism between PSI and Fd will be a significant step toward understanding of the full photosynthetic mechanism as well as improving our grasp of electron transfer within large membrane complexes.

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grant as well as the National Institute of Health Femtosecond nano-crystallography of membrane proteins (award 617095583), NSF (award 0919195), and NSF BioXFEL Science Technology Center (award 1231306).

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Time-resolved SFX experimental setup

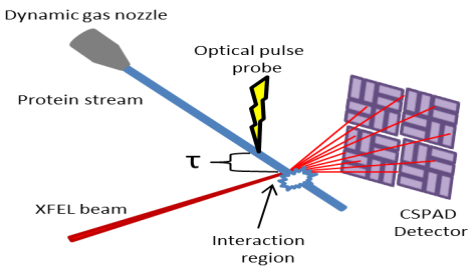

Figure 1: A fully hydrated jet of protein microcrystals in their mother liquor at ambient temperature is first photoexcited by an optical pump probe at a delay time τ to populate an excited state. This is followed by interaction with the highly brilliant XFEL beam, operating in femtosecond pulses, which results in diffraction of the undamaged crystal prior to its complete destruction, the basis of the "diffract before destroy" principle. Diffraction patterns are then recorded on the CSPAD detector for subsequent data analysis. [1,4]

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CHELSIE CONRAD, ARIZONA STATE UNIVERSITY

REDUCTION OF SAMPLE CONSUMPTION FOR SERIAL CRYSTALLOGRAPHY BY VISCOUS SAMPLE DELIVERY

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Most serial crystallography experiments have relied on a liquid jet, delivering sample in the mother liquor focused into a stream by compressed gas. However, this liquid stream moves at a fast rate, only allowing approximately every 1 out of 10,000 crystals to be probed by the X-ray beam, meaning that most of the valuable sample is wasted[1]. For this reason, the liquid jet can require 10-100 milligrams of sample for a complete data set and hours of data collection. Recently lipidic cubic phase (LCP) has been found to be a new sample delivery medium. It not only removes the need to harvest membrane protein crystals grown within LCP[2] but the viscous nature of LCP allows for a slower moving jet and thus less sample is wasted between X-ray shots. However, LCP is not suitable as a general delivery system for membrane proteins as mixing with LCP is suspected to remove the detergent micelles used to stabilize many membrane proteins and their crystals. Aiming for a universal viscous sample carrier, a medium that is compatible with both soluble and membrane proteins has been developed which allows crystals, grown via traditional methods (e.g. vapor diffusion, dialysis,) to be mixed post-crystallization prior to injection. The agarose jet provides low background, no Debye-Sherrer rings, and is compatible for sample delivery in vacuum environments[3]. The inert properties of agarose have been shown to be compatible with a wide variety of systems, including membrane proteins, soluble proteins, multi-protein complexes, and virus crystals[3,4]. Additionally, other viscous media, as well as one that is compatible at atmospheric pressure, will also be presented. Thus this work allows sample limited proteins of difficult to crystallize systems to be investigated by serial crystallography.

This work was supported by the National Institute of Health femtosecond nano-crystallography of membrane proteins (award 617095583) and National Science Foundation BioXFEL Science and Technology Center (award no. 1231306).

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EUGENE DAO, STANFORD UNIVERSITY

MICROCRYSTALLIZATION FOR XFEL-BASED SERIAL FEMTOSECOND CRYSTALLOGRAPHY

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Serial femtosecond crystallography at X-ray free electron lasers brings important advances with regard to the collection of data at ambient temperature and free of conventional radiation damage. Accordingly, sample preparation demands differ from the goals of crystallization for probing at synchrotron sources. The intensity of X-ray pulses at an XFEL means smaller crystal sizes and larger crystal quantities are preferred for SFX instead of large crystals.
We present a crystallization workflow that uses simple crystallization screening and optimization methods and is adapted to the sample demands of serial femtosecond crystallography. We applied this method for the micro-crystallization of streptavidin. The purified protein was screened against potential conditions. Promising candidates were identified and optimized to obtain highly-diffracting (1.1-1.5 Å) microcrystals.

The effort was then scaled up to produce a large quantity of microcrystals with the desired size distribution and uniformity for an SFX experiment.

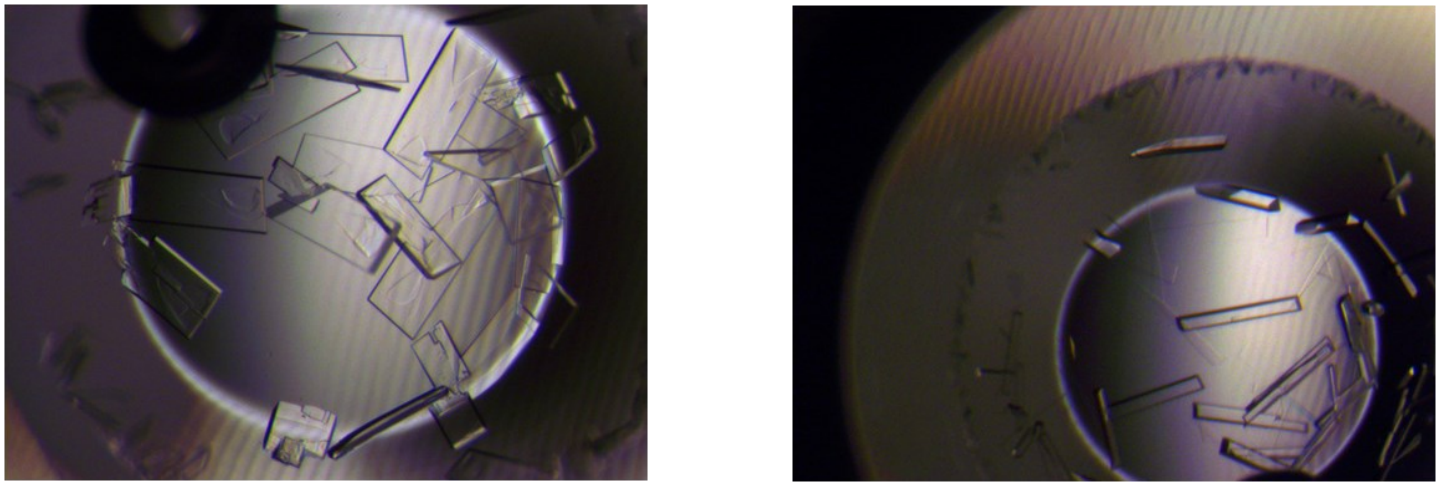

Fig. 1. Examples of streptavidin crystals grown in microbatch conditions during the screening step.

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ALI DASHTI, UNIVERSITY OF WISCONSIN-MILWAUKEE

MAPPING THE CONFORMATIONS AND ENERGY LANDSCAPE OF MOLECULAR MACHINES

Ali Dashti-[1], Peter Schwander[1], Russell Fung[1], Hstau Liao[2,3], Ahmad Hosseinizadeh[1], Amedee des Goerges[4], Wen Li[2,3], Joachim Frank[2,3]and Abbas Ourmazd[1]

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A primary goal of single-particle imaging is to study the structural changes underlying the function of molecular machines. Recent advances in cryogenic electron microscopy (cryo-EM) have made it possible to map single-particle structure at the 0.2-0.5nm level, where the secondary structure can be directly resolved. Using cryo-EM snapshots as an example, we describe a new algorithmic platform able to determine the energy landscape of molecular machines and map their continuous conformational evolution along any trajectory on the energy landscape. This approach does not require timing information, supervision, or templates, and can be applied to XFEL snapshots.

Hasan DeMirci, Stanford PULSE Institute

Serial Femtosecond X-ray Crystallography of 30S Ribosomal Subunit Microcrystals in Liquid Suspension at Ambient Temperature

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High-resolution ribosome structures determined by X-ray crystallography have provided important insights into the mechanism of translation. Such studies have thus far relied on large ribosome crystals kept at cryogenic temperatures to reduce radiation damage. Here we describe the application of serial femtosecond X-ray crystallography (SFX) using an X-ray free-electron laser (XFEL) to obtain diffraction data from ribosome microcrystals in liquid suspension at ambient temperature. 30S ribosomal subunit microcrystals diffracted to beyond 3.4 Å resolution, demonstrating the feasibility of using SFX for ribosome structural studies. The ability to collect diffraction data at near-physiological temperatures promises to provide fundamental insights into the structural dynamics of the ribosome and its functional complexes.

ADALBERTO DIAZ, UNIVERSITY OF PUERTO RICO

MOLECULAR BIOPHYSICAL STUDIES AND CRYSTALLIZATION SCREENING OF CENTRIN-PRP40AP COMPLEX

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Centrin is a ~20 KDa protein that belongs to the superfamily of Ca^{2+}-binding proteins. This multifunctional protein is one of the "eukaryotic signature proteins" and 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). These proteins share 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. 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, crystal screening has been performed in collaboration with the high-throughput crystallization screening lab (HTSlab) at HWI in order to find optimal conditions for protein crystal growth.

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Ximeng Dow, Purdue University

Determination of Protein Nanocrystals Size Distribution Using Second Harmonic Generation Correlation Spectroscopy (SHG-CS)

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The increasing trend of using smaller and smaller protein crystals in serial crystallography has introduced new challenges in the protein structure determination pipeline. One such challenge is the lack of pre-screening methods to reliably detect and distinguish nanocrystals and small microcrystals from solution or amorphous protein material prior to analysis at a synchrotron or XFEL facility. Given the high value of XFEL and synchrotron beam time, as well as the expense and time involved in crystallization, a reliable, non-destructive sample screening method for nanocrystal generation and size characterization is highly desirable.

Here we present second harmonic generation correlation spectroscopy (SHG-CS) as a rapid, non-destructive and crystal specific method for protein nanocrystal detection and recovery of the size distribution of protein nanocrystals. SHG-CS uses the Brownian motion of the nanocrystals in solution to retrieve the size distribution of the sample through autocorrelation function. As shown in **Fig. 1.**, diffusion of a crystal through the laser focal volume results in a fluctuation in detected SHG intensity. Multiplying the SHG signal by itself with a time shift yields an autocorrelogram, which offers a measure of the characteristic diffusion time of the particle. The characteristic diffusion time can be further related to the particle diameter. Unlike dynamic light scattering (DLS) which cannot distinguish between amorphous and crystalline materials, SHG is remarkably sensitive to crystalline order and has no contributions arising from amorphous aggregates.

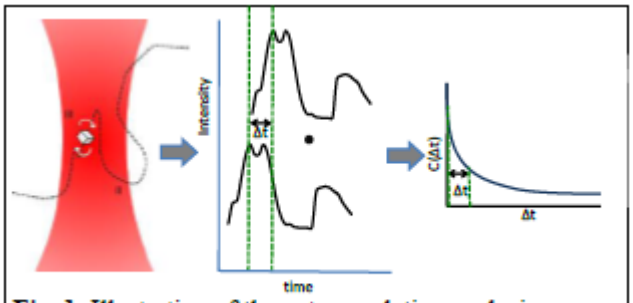

Fig. 1: Illustration of the autocorrelation analysis

Custom built instrumentation and software were developed to perform SHG-CS measurements. An analytical expression for the autocorrelogram of the translational diffusion of particles was developed, in which the inherent size distribution of the sample was represented by a probability density function. Corrections for the optical force introduced by the large electric field generated by the laser were also included, and can be expanded to other nonlinear correlation spectroscopy techniques where an ultrafast pulsed laser is focused in the sample. SHG-CS was applied to the analysis of $BaTiO_3$ nanoparticles ranging from 200nm to 500nm and photosystem I nanocrystals. Representative results for photosystem I nanocrystals are shown in **Fig. 2**. A size distribution was recovered for each sample and compared with the size distribution measured by scanning electron microscopy (SEM). Excellent agreement was observed between the two independent measurements.

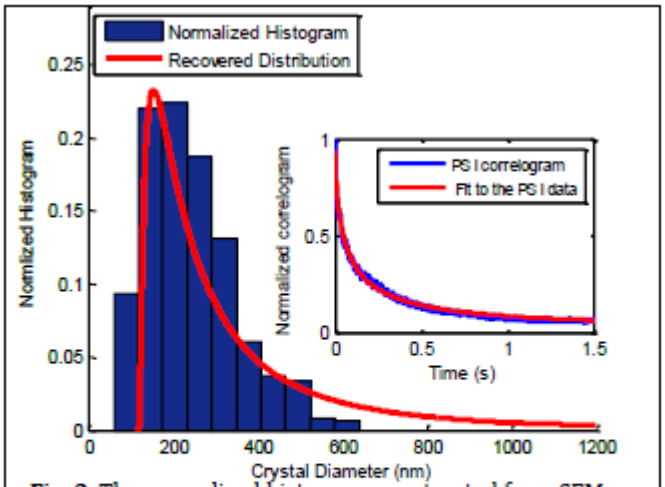

Fig. 2. The normalized histogram constructed from SEM images (blue) and the recovered size distribution (red) of the photosystem I nanocrystals. Inset figure shows the result of nonlinear fit to the experimental correlogram.

Helen Duyvesteyn, University of Oxford

PhiX-174: Making in vivo Virus XFELs de facto

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Despite their high abundance; 3186 different known species,[1] over 10^{31} virus particles in the biosphere,[2] and immense impact on cellular organisms,[3,4] few atomic-level virion structures have been solved. Such structural information is crucial for understanding the virosphere,[3,4] but hampered by the large size and complexity of viral structures, leading to crystal fragility, poor diffraction properties and low completeness.[5] We circumvented this through growing diffracting nanocrystals using viral condensates generated within lysis-defective prokaryotic cells by infection with phage phiX174. The live infected cells were then injected into the XFEL beam using a gas dynamic virtual nozzle. Given the size of the virus particles and the size of the bacterial cells viral condensates, if they accumulate in the infected cells, could only harbour a small number of particles in each dimension so that the signal amplification from the crystal lattice would be very limited. Despite this, we observed traces of Bragg diffraction from virus infected cells, but not uninfected cells.

Although *in vivo* XFELs is non-novel,[6] we report a unique instance of *viral* particle structures analysed within the cell. For the 1971 images generated, spots up to 40 Å resolution were manually selected from the back CSPAD detector. Powder patterns generated from pairs of manually picked spots were consistent with crystals occupying the face-centred cubic F23 space group, a hypothesis supported by preliminary indexing attempts.

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Austin Echelmeier, Arizona State University

Protein Crystal Delivery for Serial Femtosecond X-ray Crystallography Utilizing Microfluidic Droplets

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Determining a protein's structure can elucidate how it functions within a biological system. Traditionally, structure determination has been done with synchrotron X-ray crystallography. Certain proteins, such as biologically relevant membrane proteins, crystallize favorably as microcrystals, but not readily as large crystals required by traditional synchrotron X-ray crystallography. To characterize these microcrystals, the serial femtosecond X-ray crystallography (SFX) technique using an X-ray free electron laser (XFEL) has been developed. This method uses X-ray pulses fast enough to outrun X-ray damage, yielding diffraction patterns before the crystals are destroyed.

A current limiting factor for obtaining a full data set for structure determination is the large volume of crystal suspension needed to facilitate a continuous stream of sample being injected into the XFEL. This can be on the order of milliliters of concentrated crystal suspension, which is in large part wasted between X-ray pulses. Due to the significant time and amount of precious protein needed for crystallization, wasted sample needs to be minimized. To accomplish this, we propose a microfluidic device to generate aqueous droplets of protein crystal suspension in an oil carrier phase to reduce sample consumption whereby protein crystal-containing droplets are generated intermittently similar to how the X-ray beam pulses. During periods when the beam is off, oil will be present in the sample stream thus reducing waste.

A microfluidic droplet generator was interfaced with a gas dynamic virtual nozzle (GDVN) injector at the Linac Coherent Light Source (LCLS) Coherent X-ray Imaging (CXI) chamber. Droplets containing a suspension of granulo virus were jetted into the path of the laser at a frequency of 9 Hz and a sample flow rate of 5.5 µL min^{-1}. For comparison, 10-15 µL min^{-1} are typical flow rates when using a GDVN without droplets, so the water-in-oil droplets display a notable decrease in sample flow rate. The hit rate was ~1% with a resolution up to 2.6 Å. Additionally, we describe our continued optimization of the droplet generation as well as our efforts in combining the microfluidic droplet generator with an on-chip SFX sample delivery nozzle for streamlined droplet injection.

RITA GRACEFFA, EUROPEAN XFEL

DELIVERY METHODS FOR BIOLOGICAL SAMPLES AT EUROPEAN XFEL

Rita Graceffa,[1] Carsten Deiter,[1] Elisa Delmas,[1] Matthäus Kitel,[1] Kristina Lorenzen,[1] James Moore,[1] Charlotte Uetrecht[1,2] and Joachim Schulz[1]

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The European XFEL will deliver high intensity x-ray pulses at an average repetition rate of up to 27 kHz with bunch separation of 220 nanoseconds (4.5MHz repetition rate within a bunch train, 10Hz bunch train repetition rate). This extremely high repetition rate is a great chance for the scientific impact of the European XFEL and a challenge for the experiment design in order to make an efficient use of the facility. Sample delivery methods are a key point of the experiment set-up. The Sample Environment Group is responsible for developing and coordinating sample preparation and delivery techniques for the six scientific instruments of the facility and external users. In this poster, we illustrate the methods developed for biological samples, delivered in liquid jet, aerosols and making use of mass spectrometry based methods. Moreover we present a fast sample scanner with sub-micron positioning to scan across grids where samples are enclosed. The sample environment group will offer user support and sample preparation laboratories. Integrated biology infrastructure for crystallography and single particle imaging experiments will be provided by the XBI user consortium. The sample preparation group integrates this project into the overall biology support concept of the European XFEL.

BioXFEL 3RD INTERNATIONAL CONFERENCE

THOMAS GRANT, UNIVERSITY AT BUFFALO

Ab Initio Modeling of Time-resolved Difference Profiles from XFEL Solution Scattering

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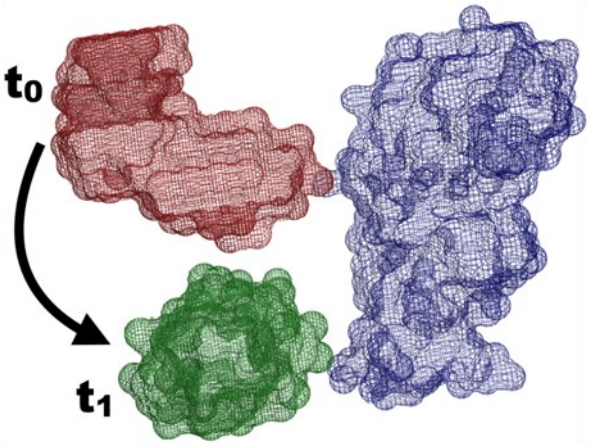
XFELs open up many possibilities for time-resolved structural studies of biomolecules. Time-resolved X-ray solution scattering (TR-XSS) attempts to probe the changes in the structures of biomolecules in solution. However, due to the small differences in scattering signal, experimental errors dominate the solution scattering signal for time-resolved experiments. To ameliorate these errors, difference scattering profiles comparing two successive time points are used rather than absolute scattering profiles for each time point. Currently, direct analysis of these difference scattering profiles can only be performed if the structure at one time is known and molecular dynamics simulations exist which can be directly compared to the difference scattering profile. While useful, limitations to this method of analysis exist, including the requirement that accurate molecular dynamics simulations calculated from known high-resolution structures exist and that the simulation trajectories capture the structural changes occurring.

In this study, ab initio bead modeling, widely used in SAXS, is applied to model the difference scattering profiles produced by TR-XSS. The resulting bead models have four possible categorizations: solvent (bead was solvent at time t_0 and t_1); immobile (bead was particle at time t_0 and t_1); negative (bead was particle at t_0 and solvent at t_1) or positive (bead was solvent at t_0 and particle at t_1). The bead model thus shows which volumes of space exist as particle at different time points, similar to difference electron density maps in crystallography. In this preliminary study, special cases are shown to be successful when knowledge about the structure at t_0 exists. Current improvements are ongoing requiring no prior knowledge of molecular structure.



This method enables real space structural modeling of difference scattering profiles from TR-XSS from XFELs as well as from synchrotrons, while potentially requiring no a priori information.

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Reinaldo Hernandez, University of Puerto Rico

Optimization of Phospholipid Bilayer Nanodiscs for Use in Single Particle Imaging Studies

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[1]University of Puerto Rico, Mayagüez. [2] BioXFEL. [3]Hauptman Woodward Medical Research Institute .

A great challenge in the research of membrane proteins is to acquire them in an active, water-soluble, and monodisperse form. This brings the need for the incorporation of the membrane proteins into a simulated or native-like membrane or detergent micelles that imitate the original characteristics of a biological membrane. However, solubilizing these types of proteins with detergents micelles or reconstituting them in liposomes usually suffers from loss of activity and lack of data due to aggregation and heterogeneity. Phospholipid nanodiscs seem promising to overpass the extensive problems of the detergent solubilizing methods. In this method, membrane proteins are solubilized with a detergent in the presence of phospholipids and a surrounding, genetically engineered membrane scaffold protein (MSP). As the detergent is removed by hydrophobic beads, the target protein assembles simultaneously with the phospholipids into a discoidal bilayer with a controlled diameter due to the size of the MSP. Recent interest in the studies of biological molecules with single particle imaging using a X-ray free electron laser (XFEL) brought up an important role for these bilayer nanodiscs, which eventually can become the main delivery mechanism for membrane proteins. Herein, we describe specific protocols for the optimize expression and purification conditions of four different size nanodiscs.

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Arjen Jakobi, European Molecular Biology Laboratory

In Cellulo Serial Femtosecond X-ray Diffraction of Alcohol Oxidase Crystals Inside Yeast Cells

Arjen J. Jakobi[1, 2] , Daniel M. Passon[1] , Francesco Stellato[3] , Mengning Liang[3] , Kevin Knoops[4] , Thomas A. White[3] , Thomas Seine[1] , Marc Messerschmidt[5] , Henry N. Chapman[3, 7] , Matthias Wilmanns[1, 8]

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We have explored the possibility of using femtosecond pulses from an X-ray Free Electron Laser to collect diffraction data from protein crystals formed in their native cellular organelles. Peroxisomes are eukaryotic cell organelles capable of carrying an unusually high protein load. In peroxisomes of the methylotrophic yeast H. polymorpha, alcohol/methanol oxidase (AO) oligomerizes into 600kDA ocatmeric assemblies that spontaneously form 300nm to 2µm-sized crystals inside the organelle. The small size of these crystals and their weak diffraction properties has so far precluded structure solution by classical X-ray techniques. We show that size and number of crystals per cell can be modulated by genetic modification of critical components involved in the peroxisomal fission process and how this affects *in cellulo* diffraction properties. We exposed H. polymorpha cell suspensions to femtosecond X-ray pulses at the Coherent X-ray Imaging (CXI) beamline at the Linac Coherent Light Source and characterized Bragg-sampled diffraction obtained from the AO crystals in intact cells to a maximum resolution of 6Å. Our SFX results are supported by complementary X-ray powder diffraction and electron microscopy data. We expect that the lessons learned from the present study will help to address experimental challenges lying ahead for intracellular crystal formation and its exploitation for structure solution of biological macromolecules.

Stefan Kolek, Douglas Instruments Ltd.

Experimental Design for Microbatch-under-oil Optimization to Establish Batch Crystallization Conditions that are Suitable for XFEL Data Collection

Stefan A. Kolek,[1] Patrick D. Shaw Stewart, [1] and Peter F. M. Baldock.[1]

1. Douglas Instruments Ltd. Douglas House, Westfield Farm, East Garston, Berkshire, UK, RG17 7HD.

The determination of the structures of soluble macromolecules by XFEL data collection currently requires large samples of suspensions of nano or microcrystals, often of the order of 0.1 ml per sample. Since vapor diffusion crystallization experiments cannot easily be scaled up, this points to "batch" crystallization where the protein and crystallization solution are dispensed at the final concentration. Microbatch-under-oil is a convenient and well-established crystallization method, and it is a true batch method when drops are covered with paraffin oil.

This presentation will focus on rational experimental design for optimizing protein crystallization that is compatible with microbatch. Multivariate designs, including the well-known Central Composite and Box-Behnken designs, will be discussed, with an example. These designs aim to avoid confusion that may arise when variables interact - that is to say, changes in one variable affect the optimum settings of one or more other variables.

KRISHNA RAJAN, BIOXFEL

APPLICATION OF MANIFOLD LEARNING METHODS FOR ANALYSIS OF STRUCTURAL DATA FROM THE BIOXFEL

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Correlative data analysis methods can help to accelerate the identification of dynamical changes in diffraction data by enhancing signal / noise ratio using manifold learning and machine learning methods. Since there are many experimental factors that influence the recorded data, (such as crystal quality, jet hit rate, sample concentration, and fixed-sample scan time), it is a major challenge to process and interpret the data. In this contribution we describe the application of manifold learning methods to explore correlations and structure within the large and complex data sets generated from BioXFEL based experiments. The potential value of using “Big Data / Deep Data” analytics in its ability to extract information that would otherwise be difficult to model and find ways to significantly accelerate data analysis using machine learning methods is discussed.

BioXFEL 3RD INTERNATIONAL CONFERENCE

ROBERT LAWRENCE, ARIZONA STATE UNIVERSITY

IMPLICATIONS OF DIFFUSE X-RAY SCATTERING MEASURED FROM VIRUS CRYSTALS

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Diffuse X-ray scattering was measured concurrently with ~40 Å X-ray diffraction from crystals of Sindbis virus at the SLAC XFEL. Sindbis virus is a ~70 nm diameter icosahedrally structured virus with a lipid membrane envelope and a single-stranded 11.7 kb RNA genome. The diffuse scattering produced from Sindbis crystals was shown to be highly similar to small-angle X-ray scattering (SAXS) measured from Sindbis in solution. However, analysis of the data reveals that the diffuse scattering measured at the XFEL originates from only the crystals, and not from any background scattering from virus particles. This may be an indication of particle-to-particle heterogeneity with respect to the envelope, capsid, or orientation of RNA within the capsid. Or, it may be caused by polycrystalline lattice arrangements within the crystals. Further investigation into the nature of this diffuse scattering may determine how it can contribute additional structural information.

Chufeng Li, Arizona State University

Exploring to Improve Accuracy in Crystal Orientation Determination in SFX Data Analysis

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The superior brilliance, coherence and femto-second level pulse duration of X-ray free electron Lasers (XFEL) have enabled studies of bio-molecular structures and conformational dynamics and opened up a new field termed Serial Femto-second Crystallography (SFX). Because of the nature of the light source, diffraction snapshots of bio-molecular crystals in random orientations are recorded in series and merged to obtain the amplitude of structure factors. Each snapshot pattern corresponds to one slice of reciprocal space made by the Ewald sphere, as defined by the diffraction geometry. Therefore, partial reflection intensities are recorded in diffraction patterns, in contrast to the case of crystallography using synchrotron light source where full reflection intensities can be recorded using a rotation series. A Monte-Carlo integration approach was first used to merge all patterns to obtain the structure factor list in SFX. This has proven successful in averaging out the stochastic factors including, partiality crystal size variation and the shot-to-shot intensity variation in the beam, and is the most common method for SFX data analysis. However, millions of patterns are needed to converge to a reliable structure solution using Monte-Carlo integration due to its stochastic nature. Several recent papers report enhanced data quality and efficiency when applying post-refinements and partiality analysis before merging the data. Since partiality varies rapidly with crystal orientation, accurate orientation determination is essential for effective partiality analysis. In addition, data efficiency can be increased by improving the indexing rate, especially for those patterns with low resolution and few Bragg spots that can be recognized. This work explores new methods to improve the accuracy in orientation determination for individual diffraction patterns using not only the geometrical information but also reflection intensities. The Genetic Algorithm is implemented in search process for orientation reconstruction from simulated patterns of mosaic photosystem 1 (PS1) nanocrystals. The crystal orientation was successfully reconstructed with an accuracy of about 0.5 degrees in the Euler angles and within an average computation time of about 1000 s. A random background and Poisson noise were added to the diffraction signal to test the robustness of the algorithm and its capability for handling patterns with low resolution. It was observed that the algorithm worked for patterns containing less than 15 Bragg spots, that can be recognized. Potential synergy may be expected between this accurate orientation determination and more sophisticated partiality modeling. Furthermore, two-color diffraction scheme might also help better in determining the orientation and therefore improving the partiality correction [1], hence the overall data quality and efficiency. Future work is under way including optimization of the search algorithm to reduce the computational time, and the ability to determine crystal orientation from an experimental data set.

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BioXFEL 3RD INTERNATIONAL CONFERENCE

HAIGUANG LIU, BEIJING CSRC

X-RAY FREE ELECTRON LASER DIFFRACTION FROM INORGANIC NANOPARTICLES

Xuanxuan Li[1], Chun-Ya Chiu[7], Hsiang-Ju Wang[7], Robert Lawrence[2,3], Sabine Botha[4], Christopher Kupitz[5], Stephan Kassemeyer[4], Richard Kirian[6], Daniel James[6], Dingjie Wang[6], Garrett Nelson[6], Marc Messerschmidt[8], Sebastien Boutet[8], Garth Williams[8], Thomas R. M. Barends[4], Elisabeth Hartmann[4], Aliakbar Jafarpour[4], Lutz M. Foucar[4], Robert L. Shoeman[4], Anton Barty[9], Henry Chapman[9], Mengning Liang[9], Fenglin Wang[9], Nadia A. Zatsepin[6], Shibom Basu[3,5], Raymond Fromme[3,5], R. Bruce Doak[4], Petra Fromme[3,5], John. C. H. Spence[3,6], Uwe Weierstall[3,6], Michael H. Huang[7], Ilme Schlichting [4], Brenda G. Hogue[2,3,10]*, Haiguang Liu[1]*

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XFELs provide new opportunities for structure determination of biomolecules, viruses, and nanomaterials. Inorganic core-shell nanocrystals, consisting of a palladium (Pd) shell and gold (Au) core, were used as a model system to investigate feasibility and problems of single particle image reconstruction from XFEL CDI data. The priori information obtained from electron microscopy was used to guide the analysis and as a reference to cross-validate the results obtained from the X-ray scattering data. The approach allowed us to analyze scattering data and infer measurement limits of the XFEL pulses for the nanoparticles under the given LCLS instrument setup. From the analysis of 54,405 scattering patterns, the orientations of 2,573 patterns were recovered to a reasonable confidence level. The recovered size distribution is consistent with the data from electron microscopy measurements (Figure 1). The orientation distribution indicates a bias toward the orientations that produce stronger scattering features (Figure 2), which could be attributed to either orientation preference or an inherent defect of the data analysis method.

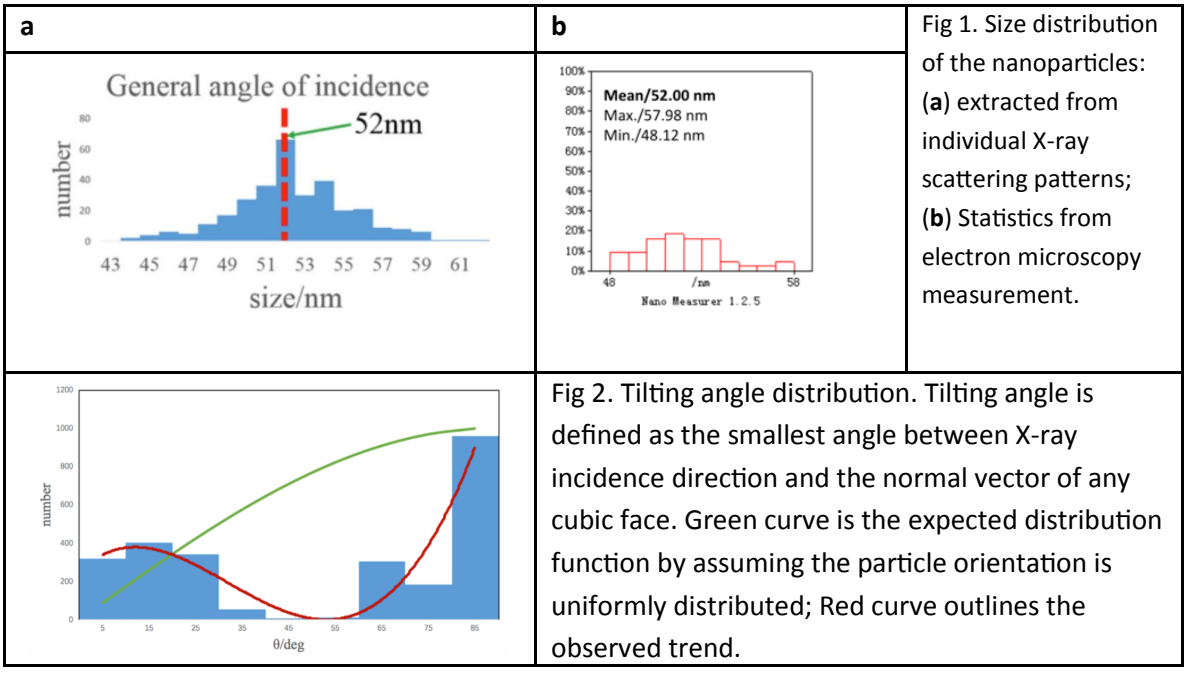

Fig 1. Size distribution of the nanoparticles: (**a**) extracted from individual X-ray scattering patterns; (**b**) Statistics from electron microscopy measurement.

Fig 2. Tilting angle distribution. Tilting angle is defined as the smallest angle between X-ray incidence direction and the normal vector of any cubic face. Green curve is the expected distribution function by assuming the particle orientation is uniformly distributed; Red curve outlines the observed trend.

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Brian Mahon, University of florida

Human Carbonic Anhydrase as a Model System for Time-Resolved Serial-Femtosecond Crystallography

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Structurally mapping the substrate/product and drug trajectory pathways in the active site of enzymes is central to deciphering the mechanism of a reaction or effectiveness of an inhibitor. Until recently, information of these entry/exit pathways has been limited to computational derived mapping from static structural observations using molecular dynamics (MD) simulations. With the advancement of time-resolved serial femtosecond crystallography (TR-SFX) techniques and 4th generation X-ray sources (X-ray free electron lasers; XFELs), it is now becoming plausible to capture enzymatic reaction intermediates and trajectory pathways in the form of high-resolution structural information[1,2]. To date, this has been applied to study intermediates of myoglobin[3] and photosystems I and II[4]. However, to advance the frontiers of TR-SFX further, new model systems need to be implemented. Here, we suggest the use of one of the fastest known enzymes in human carbonic anhydrase II (CA II) as a candidate model enzymatic system for TR-SFX. CA II is zinc metalloenzymes that catalyzes the reversible hydration of CO_2 and HCO_3^-. Since its discovery in 1933, CA II has been a hallmark enzyme for the advancement of our knowledge of structural biology, enzymology, molecular dynamics, drug discovery, and clinical medicine[5]. CA II is highly stable and easily expressed, purified and crystallized. This has translated to many X-ray and neutron structural studies that have led to the development of clinically used inhibitors and an understanding of its catalysis[5]. Despite the ~80 years that CA II has been studied, there is still a lack of structural understanding of the intermediate steps of entry/exit pathways of CO_2/HCO_3^- and drugs to the active site. Here we present a method to produce CA II microcrystals that diffract to a high resolution of <3.0 Å at a synchrotron source that are suitable for TR-SFX at an XFEL, and suggests how this can be implemented to perform TR-SFX experiments. Use of CA II as a model system for TR-SFX will not only provide novel information on the enzyme's catalysis but will also shows the capabilities of an XFEL to capture intermediates of nature's fastest enzymatic reaction, but also maybe further our understanding of structural guided drug design and the role solvent molecules play in these mechanisms.

Grant Acknowledgement: This work was supported in part by NIH grant GM25154

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Darya Marchany-Rivera, University of Puerto Rico

BioXFEL 3rd International Conference

Oxy-Hemoglobin III Structural Model and Crystal Structure Determination

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Hemoglobin III (HbIII) is one of the two oxygen reactive hemeprotein in the clam Lucina pectinata. This clam inhabits in a sulfur rich environment, which has made it a subject of study of hydrogen sulfide chemistry with hemeproteins. HbIII is the only hemeprotein of the clam that has not been structurally describe with crystallographic data. It is known that it exist as a dimer with HbII inside the clam and that it might be present as a homodimer. The actual mechanism of the oxygen reactivity of HbIII, homodimer or as the heterodimer, is partially known, were the distal amino acids tyrosine and glutamine are responsible for the high oxygen affinity. To determine a structural model the solution state of the protein was evaluated by SAXS experiments and suitable crystallization conditions were determined. Suitable crystals were cryocooled with different solutions and cryo techniques, and screened at an in house X-ray source. Trial experiments at different pH and temperatures were assessed. SAXS data confirms that at physiological concentrations in the clam HbIII is a dimer. High concentrations of sodium formate are suitable for oxy-HbIII crystal growth, obtaining the bigger crystals in a 4.5M sodium formate concentration. In house experiments resulted in lack of diffraction at cryotemperature and low quality data at room temperature suggesting high susceptibility to radiation damage. Crystals did not grow at low temperatures but did at different pH. A partial data set was collected at SSRL with low resolution on a crystal cryoprotected with a $LiSO_4$ saturated solution buffered with 0.1M Tris-HCl pH 7.5. Further optimization of crystal growth is necessary and a complete data set is required to accurately assign a space group and solve the crystal structure.

This work was funded in part by the National Science Foundation STC award number 1231306 and the SLOAN Foundation Minority Fellowship.

Isabelle Martiel, Paul Scherrer Institute

Fixed-Target Femtosecond Protein Crystallography at SwissFEL

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The Swiss free electron laser (SwissFEL) is currently under construction at the Paul Scherrer Institute, Switzerland. From the start of operation, foreseen at the end of 2017, SwissFEL will offer state-of-the-art possibilities for femtosecond protein crystallography. While the historically best-established methods for the delivery of protein crystals are based on jets, fixed-target methods are quickly developing as important alternatives with complementary advantages. Fixed target methods include synchrotron-like measurements on a relatively small set of large crystals [1, 2], as well as serial crystallography on microcrystals deposited on a solid support [3]. These methods will be actively used and further developed at SwissFEL.

The purpose of the present poster is to update the XFEL user community on these developments. The SwissFEL fixed target setup is designed to be installed at Experimental Station B during dedicated measurement campaigns. It will support both synchrotron-like rotation experiments and fast scanning data acquisition, in a photon energy range of 5.0 -12.7 keV. SwissFEL will deliver 2-20 fs pulses (FWHM) with a total energy of up to 1.4 mJ, which can be focused down to a spot of 2-3 µm size at the sample position. A fast scanning sample stage will be designed to exploit optimally the 100 Hz repetition rate of SwissFEL. An automatic sample changer will ensure high-throughput use of the XFEL beam. The users will have the choice between air and helium sample environments, as well as between cryogenic and room-temperature conditions.

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Victoria Mazalova, CFEL / DESY

2D Optical and X-ray Emission Spectroscopies within the AXSIS project: Electronic Dynamics and Undamaged Electronic Structure Study of Photosystem II

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Photosynthesis provides oxygen to maintain life on earth by converting light energy from the sun into chemical energy. Photosystem II (PSII) is the only system in nature capable of forming molecular oxygen from water and sunlight. PSII acts as the nano solar-energy converter that captures light from the sun and catalyzes 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 oxygen-evolving complex (OEC), where it extracts 4 electrons from water releasing 4 protons and oxygen during 4 sequential charge separation steps. The catalytic center of the OEC consists of a Mn4OXCa cluster which is formed by four Mn atoms and one Ca atom in the so-called cubane geometry. The Mn cluster of the OEC couples the two-electron chemistry of water oxidation to the one-electron photochemistry of the reaction center by sequentially storing oxidation states passing through a four electron and/or proton removing steps – from S0 to S4. Each light-driven charge separation event in PSII extracts one electron from the OEC before releasing dioxygen during S3→S0 transition.

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 [1] to a 1.9 Å structure resolution published in 2011, which provided the first atomic model of the metal cluster that catalyzes the water splitting reaction [2]. The new method for collecting diffraction data from sub-micron protein crystals with femtosecond pulses from X-ray Free Electron Laser (FEL) source was recently developed [3]. The method allows extracting 3D structural information before the proteins are destroyed by the intensive short X-ray pulse. While ultrashort X-ray pulses could “outrun” crystal structure damage, the electronic structure is still perturbed on the femtosecond time scale. As shown by electron spectroscopy of X-ray FEL irradiation of
gas molecules, as X-ray intensity and pulse duration increases (or as absorption cross section increases, e.g. by reducing wavelength), spectra will be perturbed by repeating steps of photoemission followed by Auger decay [4], losing two valence electrons in each step. Collisional ionization from free electrons emitted from elsewhere in the sample will cause the loss of many other valence electrons [5]. The loss of these electrons will influence the signal used to characterize the redox state. This issue can be almost completely eliminated by using X-ray pulses significantly shorter than the Auger decay rate, that is, attosecond pulses.

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 XES, XAS and 2D optical spectroscopy. The combination of these techniques 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. XAS/XES and optical spectroscopy measurements simultaneous to the crystallography experiment will indeed 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/XAS and 2D optical spectroscopy within the AXSIS project and show how they will contribute to the global understanding of how the OEC operates.

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MITCH MILLER, RICE UNIVERSITY

TESTING THE DIRECT PHASING OF PROTEIN STRUCTURES WITH HIGH SOLVENT CONTENTS

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The phase problem in X-ray crystallography is a fundamental problem where the phases required to obtain an image of the electron density in the crystal are not directly recorded with the diffraction intensities and must be deduced by other means. Traditionally, in protein crystallography, the phases have come from MIR, MAD/SAD (where differences in intensity due to a subset of added or natively present heavier or anomalously scattering atoms are exploited) or molecular replacement (which requires a similar known structure be placed in the cell to obtain starting phases). While these techniques have been very successful, the phasing of new structures without sufficiently close homologs and for cases where it can be difficult to obtain heavy atom or anomalous scattering substitutions remains a problem. In the case of serial femtosecond crystallography, where there are machine limitations on the accessible wavelengths for MAD/SAD phasing and very scarce beamtime availability for traditional heavy atom techniques, there is a demand for additional methods for the *de novo* phasing of new structures.

In the case of crystals with high solvent content, there has been progress using iterative transform phasing algorithms that have been developed in the fields of coherent diffraction imaging and transmission electron microscopy. Several groups have had success starting from a low resolution protein masks. Recently, He & Su (2015, *Acta Crystallogr*. **A71**:92) reported the successful *de novo* phasing of a couple of structures with high solvent content using a hybrid input-output algorithm combined with a dynamically adjusted protein mask. Here we report on some of our on-going trials to better understand the general applicability of the current algorithm for the direct phasing of high solvent structures using a set of structures from the Protein Data Bank.

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BioXFEL 3RD INTERNATIONAL CONFERENCE

OSAMU MIYASHITA, RIKEN

HYBRID APPROACH FOR X-RAY FREE ELECTRON LASER SINGLE PARTICLE ANALYSIS OF BIOMOLECULAR SYSTEMS

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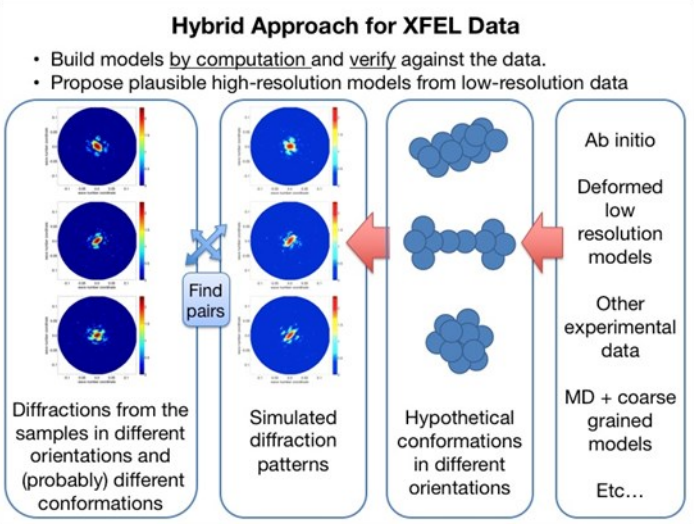
X-ray free electron laser (XFEL) is an exciting new technology that could significantly extend our structural knowledge of biological systems. Strong laser light from XFEL enables the measurement of single molecular complex, without necessity of crystallization. Since crystallization is not necessary, it could be applied to a wider variety of systems under various physiological conditions.

For XFEL single particle analysis, reconstruction of 3D structure requires a large number of diffraction patterns, however, in the current XFEL experiments on biological systems, such measurements are still difficult and the analysis often relies on a few 2D diffraction patterns. In addition, the current standard approach is to obtain 2D real -images from diffraction patterns via phase recovery procedure. However, for biological systems, due to their low diffraction power, the procedure is not straightforward, and it often fails to provide an interpretation of the data. If the procedure is not successful, the real image cannot be obtained or the results are unreliable. Thus, there is not enough data to reconstruct the model of the target systems without additional information.



Therefore, we have been developing hybrid algorithms that combine molecular mechanics and image data processing algorithms. Instead of directly reconstructing the model from the diffraction pattern, we first construct hypothetical models using molecular mechanics simulations, computational modeling techniques and/or other experimental data such X-ray crystallography. Then, the models are matched against available XFEL
diffraction patterns to identify the model that is most likely to be represented in the experimental data. This approach could be especially useful to study functional dynamics of biological systems.

As the first step toward such hybrid modeling, we established a protocol to assess the agreement between the model structure and the target XFEL diffraction pattern and show that XFEL data can be used to study the conformational transitions of biological molecules. We tested our algorithms for biological molecular complexes of different size, elongation factor 2, CCM virus, and ribosome, and examined the experimental condition, in particular the XFEL beam intensity, that is required to perform such study. The proposed algorithm can be combined with molecular dynamics simulations or normal mode analysis to perform more automated structure modeling.

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Henrike Mueller-Werkmeister, University of Toronto

Capturing Functionally Relevant Protein Motions at the Atomic Level: Femtosecond Time-Resolved Serial Crystallography of Ligand Dissociation in MbCO

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X-Ray FEL sources allow the direct observation of the "choreography of life" on time scales relevant to barrier crossing, i.e. the femtosecond motions governing chemistry driving biological function [Miller, R. J. D., Science, 2014, 343, 1108-1116.] A fundamental question becomes accessible experimentally: The investigation of protein dynamics with all atomic resolution on the shortest relevant timescale around 100 fs. Here is where bond-breaking events occur, which translate into larger structural changes and cause a protein to fulfill its function over a wide range of timescales. How these inherent fluctuations of the protein transduce chemical energy into function, i.e. what is the structure-function correlation with respect to understanding these evolutionarily optimized systems is the quest of our research.

We have performed time-resolved serial femtosecond crystallography experiments (TR-SFX) using a fixed target for sample delivery at XPP/LCLS to study the photodissociation of carboxymyoglobin, which is a well studied model system for ultrafast protein dynamics. Previous time-resolved crystallography results using third-generation light sources are on the timescale of 150 ps and slower [Schotte, F. et al., 2003, Science 300, 1945] and gave insight into side chain motion after photodissociation. The actual bond-breaking event is expected faster than 50 fs [Armstrong, M. R. et al, Proc. Natl. Acad. Sci. USA, 2003, 100, 4990]. Our experiment accesses the dynamics in the time window between 0 and 2 ps with 100 fs steps to resolve both the ligand dissociation and the "talking" coordinate mediated through the proximal histidine motion involved in allosteric communication of the ligation state. The heme moiety doming and the overall protein motion coupled to the bond breaking process are resolved and the preliminary data demonstrate coupling to collective coordinates on exceptionally fast time scales. The diffraction data were processed using the approach by Ginn et al. (Acta Crystallogr. D Biol. Crystallogr. 71, 1400-1410 (2015)) and results are being analyzed further to determine the actual correlated motions in carboxymyoglobin. As it is important to control the laser excitation processes, we performed additional laser titration studies to determine the accurate photodissociation level of our crystals.

An important prerequisite for all variants of time-resolved experiments is efficient and precise sample delivery. We used a fixed target matrix, referred to as photo-crystallography chip (PCC, Mueller et al., Structural Dynamics 2, 054302, 2015, Zarrine-Afsar et al., Anal Chem 83, 767, 2011), which is a nanofabricated silicon based chip with >10000 individual features for trapping protein crystals in random orientation. The design of the chip allows background free detection and low sample consumption. The feature size can be engineered to match the crystal size. In combination with a spectroscopy based mapping approach to identify the occupied positions on the PCC, we were able to reach high hit rates with an overall rate of >50% indexable diffraction patterns per PCC, allowing collection of a complete data set in about 10 min. We have also developed a portable, compact endstation for the PCC [Sherrell et al., *J. Synchrotron Radiat.* 22, 1372-1378 (2015)], as well as an imaging and spectroscopy setup for mapping the PCC and *in-situ* sample characterization.

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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 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 report the application of 2-photon polymerization (2PP) in producing a 3D-printed GDVN with submicron printing resolution. Essential details of the specific 2PP 3D-printing technique are laid out, and testing results both in laboratory and crystallography experimentation are provided. This work is supported by NSF BioXFEL STC Program award 1231306.

PRAKASH NEPAL, UNIVERSITY OF WISCONSIN-MILWAUKEE

EXTRACTION OF STRUCTURAL CHANGES FROM DIFFERENCE SAXS/WAXS ALONE

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SAXS/WAXS has traditionally been used to determine only molecular shapes but not the internal structure of molecules as it has been assumed that a SAXS/WAXS curve has not enough information to do so. However in some ways the extraction of information from SAXS/WAXS is akin to the extraction of information from powder diffraction. In both cases one tries to recover the full 3D structure of a molecule from a single line profile representing the radial variation of a powder pattern. In powder diffraction this line profile consists of sharp lines, which are projections of Bragg spots onto the line profiles. The main difference in SAXS/WAXS is that one is not dealing with crystallites, so there are no sharp Bragg spots. However since we know that, in a crystal, the Bragg spots are just samples of the underlying reciprocal space of the particle, powder diffraction methods are able to recover the structure of a unit cell. The fact that there is a different finite sampling of the unit cell in different directions is of little consequence as the line profile consists of the sum of all the different finite samplings. Consequently the total line profile consists of information about the complete Fourier space in coded form. We show in this paper that it is possible to extract at least the information about small changes in a structure from a difference SAXS/WAXS spectrum 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 demonstrate this method on experimental SAXS/WAXS data from PYP in addition to Blue/Green Light-Absorbing Cyanobacteriochrome and even SAXS/WAXS data that was the subject of a high-profile recent paper by Neutze et al. [1] 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 [2] 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.

This work was supported by the National Science Foundation grant number 1231306.

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Jose Olmos, Rice University

Time-Resolved X-ray Crystallography of Enzymes with an X-ray Free Electron laser

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A major limitation of time-resolved crystallography is the uniform initiation of enzymatic reactions across protein crystals for the collection of structural intermediates. I am involved in sample preparation for a proof-of-principle experiment designed to show that reaction initiation can be reasonably achieved in micron-sized crystals by mixing crystals and substrate in an injector jet as the jet stream is probed with an X-ray Free Electron Laser (XFEL).

To this end and in collaboration with various groups of the BioXFEL consortia, I have worked extensively on the enzyme: Beta-lactamase. Beta-lactamase confers antibiotic resistance to *Mycobacterium tuberculosis* by hydrolysis of a Beta-lactam ring in various antibiotics. The information gained from these studies will be very insightful and increase the understanding of enzymatic dynamics. Further, the successful completion of these experiments opens the door of possibility for enzymology studies using an XFEL in the approach of reaction initiation by substrate diffusion.

The work I have carried out on Beta-lactamase has involved molecular cloning of the DNA constructs, expression of recombinant protein, protein purification, protein crystallization screens, and characterization of various crystalline samples through microscopy and powder diffraction. Further, sample preparation was carried out at SLAC National Lab for data collection in the Coherent X-ray Imaging (CXI) experimental hall using a rapid mixing jet and the LCLS, X-ray Free Electron Laser.

An additional interest of my research, that would be interesting to actively purse upon the success of the enzymology experiment of Beta-lactamase, involves the use of adenylate kinase as a model protein for the study of protein dynamics. Current forms of AK crystals comprise crystal lattice packing contacts that restrict the active site of the molecule and that do not accommodate the full range of motions of the enzyme. A new crystal form will be engineered by means of recombinant protein fusion partners to accommodate the full cycle the enzyme undergoes during its catalytic reaction. My hypothesis is that by fusing a protein, AK, onto a rigid scaffold, CalS11, the scaffold can form the lattice while leaving the AK to be dynamic. To this end, I have cloned a DNA construct, expressed and purified the construct, and carried out crystallization screens of the AK-CalS11 enzyme-scaffold.

This material is based upon work supported by the STC Program of the National Science Foundation through BioXFEL under Agreement No. 1231306, the National Science Foundation Graduate Research Fellowship Program (GRFP), and the National Science Foundation Interdisciplinary Research in Science and Engineering (IRISE).

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NADIA OPARA, PAUL SCHERRER INSTITUTE

TIME-RESOLVED X-RAY CRYSTALLOGRAPHY OF ENZYMES WITH AN X-RAY FREE ELECTRON LASER

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Serial femtosecond protein crystallography in the "diffract-before-destruction" data collection regime emerged as a new approach to structure determination, enabled by the development of Free Electron Lasers (FELs). It is a promising approach for protein structure determination of sub-micron sized protein crystals [1, 2]. However, the capability of fast probing of the sample with the high hit rates requires an efficient delivery system of large amounts of tiny crystals, since every shot leads to their complete destruction.

In serial crystallography experiments at FELs, the use of fixed targets is a powerful alternative to flow jet-based solutions, namely liquid jet and lipidic cubic phase injectors. It provides well-positioned samples on ultrathin solid supports, cryo-cooled or preserved from evaporation [3]. It is especially advantageous when the amount of the available crystalline protein material is limited. Highly X-ray permeable, low Z-number material packaging of the fragile crystals is essential to keep the background and diffuse scattering to a minimum [4]. Several experiments employing fixed targets showed the effectivity of the method [e.g. 5, 6, 7].

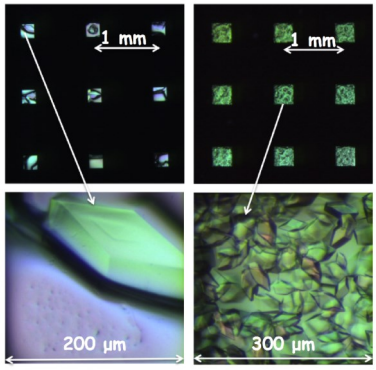

Fig. 1. *In situ* grown large protein crystals on silicon/silicon nitride chips. Here, the size and density of the sample have been optimized for a time-resolved measure-ment.

Preserved from evaporation in the "sandwich" chamber, lysozyme crystals provided high-quality diffraction data collected at room temperature.

Here we show a further development of the fixed target approach, where the protein was directly crystallized on ultrathin, X-ray transparent silicon nitride membranes in silicon chips [Fig. 1.]. Thin, double-sided adhesion tape gave sufficient sealing to prevent drying out in an assembly of two silicon chips in a sandwich fashion with crystals grown on one of the membranes. Subsequent optimizations go towards automated filling of nanoliter volume cavities with protein solutions suitable for *in situ* crystallization or automated deposition of protein crystal suspensions with nanoliter precision. This way of material deposition requires optimized conditions to prevent sample from evaporation, which can be provided by a humidified atmosphere or by cooling the substrate to the dew point.

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George Phillips, Rice University

XRayView 5.0, an Updated Educational Crystallography Program Incorporating Visualization of Serial Femtosecond Crystallography

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The educational crystallography program XRayView uses interactive computer graphics to introduce basic concepts of X-ray diffraction by crystals, including the reciprocal lattice, the Ewald sphere construction, Laue cones, the wavelength dependence of the reciprocal lattice, primitive and centered lattices and systematic extinctions, rotation photography, Laue photography, space group determination and Laue group symmetry, and the alignment of crystals by examination of reciprocal space (Phillips, 1995, *Biophys. J*. **69**:1281). Serial femtosecond crystallography (SFX) enables characterization of micron-sized crystals that are too small to generate useful data via a synchrotron. The 100-fs X-ray pulses enable capturing molecular dynamics on timescales previously accessible only via molecular dynamics simulations. In addition, short-lived conformations that are critical for catalysis and other functions can be observed. Here, we describe an updated XRayView that incorporates a visualization of the SFX process as a series of randomly oriented crystals being injected into an XFEL beam path to generate hundreds to thousands of individual scattering patterns that can be summed to produce a three-dimensional powder pattern. The injector and the beam are slowed down by about 100 relative to reality to simulate injection velocities using a focused stream injector. A new exercise has been developed in which the user can tune such parameters as injection velocity, XFEL pulse frequency, and crystal concentration to optimize the hit rate to balance between sample conservation and minimization of multiple hits by the same beam. The updated program enables students to become familiar with the most advanced capabilities of molecular crystallography in addition to the traditional approach of using large crystals on a goniometer in a synchrotron experiment.

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Hin Cheuk Poon, University of Wisconsin-Milwaukee

A Symmetrized Phasing Algorithm Applied to the Experimental XFEL Data from Rice Dwarf Virus (RDV)

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The 3D diffraction volume of randomly oriented RDV in 3D was determined from the average pair and triple angular correlations on measured diffraction patterns. As a first approximation, the virus is taken to be icosahedral and the spherical harmonic expansion coefficients of the 3D diffraction volume can be easily extracted from the simplified correlation coefficients. [1] The symmetrized diffraction volume thus obtained was phased with the Shrinkwrap algorithm [2]. It was found that in order to obtain an icosahedral charge distribution, symmetry constraint has to be imposed. The results will be compared with those from X-ray crystallography.

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LARS REDECKE, LÜBECK UNIVERSITY

INCELLCRYST – AN AUTOMATED PIPELINE FOR SERIAL CRYSTALLOGRAPHY IN LIVING INSECT CELLS

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X-ray crystallography requires the growth of well-ordered, sufficiently sized protein crystals to obtain structural insights at atomic resolution, routinely performed by parameter screening *in vitro*. However, spontaneous protein crystallization can also occur within living cells, as known for more than a century. But most of these *in vivo* crystals were not accessible for x-ray structure determination so far, due to their small size that coincides with low diffraction capabilities and sensitivity to radiation damage. This picture has changed with the realization of serial crystallography approaches at X-ray free-electron lasers (XFEL) and third-generation synchrotron sources characterized by extreme energy and brilliance. We recently demonstrated successful high-resolution diffraction data collection from micron-sized protein crystals of *Trypanosoma brucei* cathepsin B (TbCatB) grown within baculovirus-infected, living insect cells (1-3). Even if these results already indicate that *in vivo* grown protein crystals could act as targets for structural biology, a more general application of this approach requires a detailed understanding of the natural protein crystallization process within a cell.

So far, we provided first real-time insights into the crystal growth of nine entirely different proteins in Sf9 insect cells applying live-cell imaging techniques. Moreover, we identified the cellular compartments of crystal origin as a first parameter for systematic screening to improve the success rate of intracellular protein crystallization (4). Recent successful diffraction data collection applying serial crystallography techniques qualified the obtained *in vivo* crystals as suitable targets for structure elucidation.

Inspired by these results we currently establish an automated easy-to-use pipeline to exploit living cells as native bioreactors for the production of micro- and nano-crystals of recombinant proteins, which will be presented. After cloning and cell transfection, successful protein crystallization is screened by variation of the intracellular compartment using specific translocation signals that are attached to the termini of the proteins. Second-harmonic generation (SHG) techniques will be combined with depolarised dynamic light scattering (dDLS) and small-angle light scattering (SALS) techniques for efficient crystal detection, followed by automated cell sorting and application of the crystal-containing cells on innovative chip or tape sample holders for diffraction data collection directly within the living cell by application of serial approaches at XFELs or highly brilliant synchrotron sources. Microfluidics will allow high-throughput operation and therefore a direct implementation of the setup at SFX or SSX beamlines.
Extending the well-established techniques of x-ray crystallography, this pipeline will not only abolish the need for time-consuming optimization of recombinant protein production and conventional crystallization, but also provide new possibilities to elucidate the structure of proteins that did not crystallize so far applying conventional approaches.

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Josiris Rodriguez, University of Puerto Rico

Crystallization and X-ray Diffraction Data for CRP-hemoglobin I Complex

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Lucina pectinata (*L. pectinata*) belongs to a large family of bivalves that harbor an uncultivable symbiont along with a high concentration of an unnamed cysteine rich protein (CRP) and a high concentration of hemeproteins. These proteins are believed to play a role in the symbiosis of *L. pectinata* with the thioautotrophic bacteria. CRP may facilitate hydrogen sulfide (H_2S) transport *in vivo*, interacting specifically with hemoglobin I (HbI). This project aims to study CRP in order to determine a structural model of CRP/HbI to investigate function of CRP and the mechanism of processing H_2S. Proteins were isolated from the native source using size exclusion and ion exchange chromatography. Protein purity was monitored using SDS page electrophoresis. Concentrations were determined using UV-Vis characteristics bands with previously published extinction coefficients. Samples were submitted for screening at HTS laboratory. Crystal hits were optimized using hanging drop vapor diffusion and batch under oil. CRP/HbI complex were crystallized using previous crystallization conditions. Best crystals were obtained at 23°C using lithium sulfate 1.8M pH 7.5 and ammonium sulfate 2.0M pH 7.0. A total of 48 crystals were sent for diffraction at the SLAC in California. The diffraction resolution is approximately 2.5 Å. Diffraction data were processed for indexing using imosflm software. Phasing of the data was done in Phenix phaser software. The known structure of HbI monomer and the cDNA sequence were used for molecular replacement. Preliminary structure model shows four molecules in the asymmetric unit: 2 HbI dimers. Xtriage tool in Phenix suggested enough volume content for 4-6 HbI molecules or 4 HbI with 2 CRP. Structure refinement is in process with an R work: 0.30 and an R free: 0.33.

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PHILIP ROEDIG, DESY

SERIAL FEMTOSECOND CRYSTALLOGRAPHY ON A CHIP WITH A DATA ACQUISITION RATE OF UP TO 120 HZ

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Serial femtosecond crystallography (SFX) has opened new opportunities in the field of macromolecular crystallography[1]. It allows structure determinations from micrometer and sub-micrometer sized crystals which is not possible at conventional synchrotron sources. Benefiting from the diffraction-before-destruction approach a diffraction image is recorded from every individual crystal before being destroyed by the X-ray beam. A major challenge of current SFX experiments using a liquid-jet for sample delivery is the large sample consumption. SFX structure determinations using a conventional liquid jet typically require tens to hundreds of milligrams of macromolecular crystals and suffer from low hit rates[2]. Many attempts have been tried to reduce the amount of sample required. A promising approach is the use of fixed substrates[3,4].

Following this approach we have developed a micro-patterned silicon chip as a sample holder for thousands of macromolecular crystals which can be used both at synchrotron sources and X-ray free electron lasers[5]. The chip has a membrane part which has a thickness of about 10 µm and consists of more than 20,000 holes with sizes down to 1 µm. For sample loading a drop of ~2 µl of crystal suspension is pipetted onto the chip. By attaching a wedge of filter paper at the lower side of the membrane part the mother liquor is soaked through the holes while crystals larger than the pore size are retained and align themselves according to the pore pattern (see Figure 1).

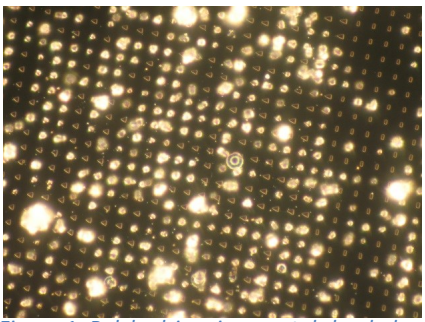

Figure 1: Polyhedrin microcrystals loaded on our micro-patterned silicon chip.

Since the intensity diffracted into Bragg reflections is proportional to the illuminated crystal volume, reduction of the generated background signal is important in cases of very small crystals and/or large-unit-cell system such a viruses or large molecular complexes. This means especially for smaller crystals that the Bragg intensity is often buried in the background. By removing the surrounding mother liquor and since single-crystalline silicon is used as substrate material, the background signal generated by our chip is highly reduced and limited to a few silicon Bragg reflections, which only occur at certain orientations and thus can be avoided.

Using our chip SFX experiments have been performed on macromolecular samples at beamline XPP at LCLS. During data collection the hole membrane part of a single chip was raster scanned through the X-ray beam within a few minutes. The transverse translation speed of the chip was matched to the repetition rate of the laser in the way that neighboring pores were hit by two successive X-ray pulses, resulting in highly increased hit rates of more than 70 %. The diffraction experiments were performed both at cryogenic and ambient temperatures. The obtained diffraction data was analyzed with appropriate software and structural differences of measurements made at cryogenic and room temperature are studied.

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Manoj Saxena, University of Puerto Rico-rio Piedras

Breaking the Mold: Challenging the Paradigm of Transferrin Metal Binding and Activity Regulation

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Human serum transferrin (hsTf) is a protein that mediates the transport of iron from blood to cells. Assisted by the synergistic anion (carbonate) hsTf transports Fe(III) by binding Ti in a closed conformation. Previous studies suggest its role as a potential transporter of other metals, like titanium. Ti is a widely used metal in cosmetics, foods and implants. A substantial amount of Ti is leached into the blood from these implants. However, the fate of the leached Ti in blood and its transport into the cells is not known. Understanding the Ti interaction with hsTf assumes a greater significance with our ever increasing exposure to Ti in form to titanium implants.

Based on *in vitro* studies it was speculated that Ti(IV) can bind transferrin assisted by a synergistic anion. However, the role and identity of the synergistic anion(s) and the conformational state in which sTf binds Ti(IV) are not known. Here we have solved the first X-ray crystal structure of a Ti(IV)-bound hsTf. We found that hsTf binds Ti(IV) in an open conformation with carbonate and citrate synergistic anions at its metal binding sites. Our studies with cell lines further suggest that Ti(IV)-sTf could be transported into cells and that hsTf and citrate regulate the metal's blood speciation and attenuate its cytotoxic property. Our results provide the first glimpse into the possible mechanism by which Ti leached from titanium implants could be transported in the body.

Nicole Scarborough, Purdue University

Dye Methods for Enhanced Contrast in Second Harmonic Generation Imaging of Protein Crystals

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Recently, second harmonic generation (SHG) microscopy has become a useful tool in the field of structural biology for the detection of protein crystals due to its specificity to non-centrosymmetric crystals. SHG, or the frequency doubling of light, is a process specific to crystalline media lacking inversion centers. However, SHG response varies depending upon the crystal symmetries. Through theoretical models and experimental data, it is estimated that ~84% of the known protein crystal structures give detectable SHG signal using current SHG microscopy instrumentation.[1] Extending this coverage could be extremely useful to structural biologists who must routinely screen entire 96 well plates, with hundreds of crystallization conditions, in search of diffraction-quality protein crystals. The method can also be extended to use for screening of the formation of nanocrystalline showers before introduction into an X-ray Free Electron Laser for serial femtosecond crystallography. The identification of crystallization conditions that can lead to diffraction quality crystals can reduce the time and money spent on the initial crystallization process.

A series of SHG active were investigated to assess their ability to enhance the nonlinear optical (NLO) response across a range of protein crystals with varying degrees of inherent SHG activity. Unlike the intercalation of fluorophores, the SHG-phores produce no significant background SHG from solvated dye or from dye intercalated into amorphous aggregates.[2] These dyes were shown to enhance the SHG activity of protein crystals such as tetragonal ($P4_32_12$) lysozyme crystals, which typically generate little to no SHG signal. This result which can drastically decrease the number of false negatives during screening. Dyes can either be introduced to the protein after crystallization or included in the initial crystallization process. The kinetics of the dye intercalation indicate that an increase in SHG activity becomes easily noticeable within minutes of exposure to the dyes. Both the symmetry of the crystal lattice and molecular structure of the SHG-phore dictate the degree of anticipated enhancement, allowing for further optimization. The variability in the enhancement likely arises from a combination of multiple effects, including but not limited to differences in the initial SHG-activity of the protein crystal, the degree of SHG-phore incorporation, the degree of ordering of the incorporated SHG-phores, and the inherent hyperpolarizability of the SHG-phore. Further studies are being conducted to determine the use of SHG detection on fluorescently tagged proteins. SHG measurements on dyed crystals allow for the characterization of not only the size distribution of crystals in solution but their crystalline quality as well, unlike dynamic light scattering. These results provide a means to increase the overall diversity of protein crystals and crystal sizes amenable to detection by SHG microscopy to aid in high-throughput analysis.

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REYHANEH SEPEHR, UNIVERSITY OF WISCONSIN-MILWAUKEE

STRUCTURAL DYNAMICS BEYOND PUMP-PROBE TIMING JITTER

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A proof of concept study recently showed that it is possible to extract dynamics two orders of magnitude below the timing uncertainty between the optical pump and x-ray probe pulses in X-ray Free Electron Lasers (XFELs).

The approach recognizes that concatenating the experimental snapshots ordered according to their uncertain timestamps results in a series of frames each containing a weak arrow of time. Non-linear Singular Value Decomposition SVD can then be used to reconstruct the data with time resolution better than determined by the single-shot timing jitter.

Raymond Sierra, Stanford PULSE Institute

Concentric-flow Electrokinetic Injector Enables Serial Crystallography of Ribosome and Photosystem II

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We describe a concentric-flow electrokinetic injector for efficiently delivering microcrystals for serial femtosecond X-ray crystallography analysis that enables studies of challenging biological systems in their unadulterated mother liquor. We used the injector to analyze microcrystals of Geobacillus stearothermophilus thermolysin (2.2-Å structure), Thermosynechococcus elongatus photosystem II (<3-Å diffraction) and Thermus thermophilus small ribosomal subunit bound to the antibiotic paromomycin at ambient temperature (3.4-Å structure).

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BioXFEL 3RD INTERNATIONAL CONFERENCE

GANESH SUBRAMANIAN, ARIZONA STATE UNIVERSITY

CHARACTERIZING THE INTERMEDIATE STATE ELECTRONIC AND MOLECULAR STRUCTURE DURING THE PHOTOLYSIS OF METHYLCOBALAMIN, USING ULTRAFAST PUMP-PROBE X-RAY ABSORPTION SPECTROSCOPY

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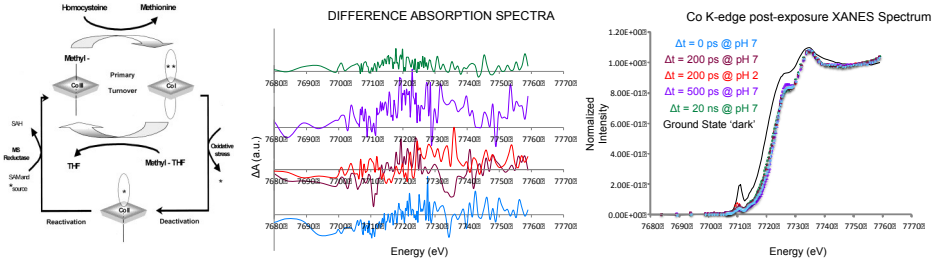
The cleavage of the Cobalt-Carbon (Co-C) bond is the most crucial step in the catalytic processes involving Vitamin B12 cofactors. In mammals, Methylcobalamin (MeCbl) plays a crucial role as a coenzyme of methionine synthase, which is required for the formation of methionine from homocysteine in the methylation cycle (Fig. a) that involves DNA or proteins. Insufficient amounts of MeCbl could result in the development of megaloblastic anemia and eventually sub-acute combined degeneration of the spinal cord [1]. Consequently, cobalamins are rigorously tested as potential therapeutic drugs: promoting the regeneration of injured nerves, treating/slowing cognitive decline arising from Alzheimer's disease [2,3] etc.

In understanding the catalytic activity of the MeCbls, a variety of experiments have been performed [4]. Under non-enzymatic conditions, the Co-C bond cleavage is generally achieved by using optical photons as with transient optical absorption [5]. Ab-initio simulations of the MeCbl electronic structure [6] have also been performed, in conjunction with these experiments. Yet, due to the lack of atomic resolution site-specific measurements, there is no conclusive evidence on the intermediate structures and reaction pathway.

In the current work, the first ever time-resolved pump-probe X-ray absorption measurements have been performed on the MeCbl-water (pH 7 and pH 2) system at the Advanced Photon Source (APS) 11-ID-D using an excitation wavelength of 400 nm. From our experiments, we observe that the bond cleavage: (a) predominantly occurs by reducing the oxidation state and coordination of Cobalt (b) is achieved in the few 100 ps timescales. Recombination of the separated species occurs in the few ns timescales (Fig. b). Accumulation of irreversible product to form a 4-coordinate species, leads to a substantial but similar change (pH 7 and pH 2) in the absorption spectra (Fig. c). DFT calculations are in progress to quantitatively assess the excited state electronic and molecular structures, through the catalytic cycle.



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JESSICA THOMASTON, UC-SAN FRANCISCO

HIGH-RESOLUTION CRYSTAL STRUCTURES OF THE INFLUENZA AM2 PROTON CHANNEL: INSIGHTS INTO WATER NETWORKS

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The M2 proton channel of influenza A is essential for the reproduction of the flu virus and is also a model system for the study of selective, unidirectional proton transport across a membrane. Water wires inside the channel pore have been thought to play a role in both the conduction of protons to the gating His 37 residues and the stabilization of positive charges within the channel, but until now these waters have not been seen at atomic resolution. Here we used lipidic cubic phase crystallization techniques to solve high resolution (1.10 Å) structures of M2 under cryogenic data collection conditions. We observed a continuous path of water wires over 16.8 Å of the channel pore, from the N-terminus of the channel to the gating His37 residues. Room temperature data collection techniques were used to examine the water networks at high and low pH at room temperature, which revealed a water network that appears to be more mobile, especially at low pH. However, it was not clear if this loss of solvent ordering at room temperature was a result of the increased amount of radiation damage during data collection. XFEL data was collected for both high and low pH conditions to a resolution of 1.4 Å to provide room temperature structures free of radiation damage.

Michael Thompson, UC-San Francisco

Infrared Laser-Induced Temperature-Jump: A General Perturbation Method for Time-Resolved Crystallographic Studies of Protein Dynamics

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Time-resolved X-ray crystallography is among the most information-rich experimental techniques in structural biology, and it represents one of the most promising applications of free electron laser technology in the life sciences. To date, systems that have been successfully studied are those in which a protein conformational change is coupled to excitation of a photoactive ligand molecule, because the conformational change can be initiated with an ultrafast laser pulse. Unfortunately, the number of proteins that undergo photochemistry as part of their functional cycle is small, and there is a fundamental need to develop generalized methods that can be used to synchronously excite conformational transitions in *any* protein molecule, even in the absence of specific photochemistry. In contrast, our recent "multi-temperature" crystallographic study of a model enzyme, cyclophilin A (CypA) demonstrated that temperature perturbation is an effective way to experimentally manipulate the conformational ensemble of a crystalline protein. Notably, increasing the temperature allowed us to increase the population of physiologically-relevant minor conformational states so that they became increasingly apparent in the electron density maps derived from those crystals at higher temperatures. Our goal is to develop time-resolved crystallographic experiments that utilize infrared (IR) laser-induced temperature-jump (T-jump) excitation methods for synchronous initiation of a conformational change. Laser T-jump methods should be applicable to all protein molecules, because they take advantage of vibrational excitation of the solvent (water), rather than excitation of the protein itself (or its ligands). Excitation of the solvent rapidly introduces thermal energy into the system, which is subsequently transferred to the protein molecules and used to synchronously populate high-energy conformational states. Currently, our efforts are directed toward designing a time-resolved, T-jump crystallographic study of CypA. To this end, we are developing serial femtosecond crystallography (SFX) data collection protocols for CypA. Additionally, we are performing T-jump SAXS/WAXS experiments to develop a kinetic model for how the X-ray scattering signal changes following the IR laser pulse, which will guide the development of our crystallographic experiment. Ultimately, the experiment we are designing will demonstrate the utility of the T-jump method in time-resolved crystallography, and will help answer outstanding questions about CypA dynamics by differentiating between two alternative models for coupled atomic motions.

Sandi Wibowo, University of Wisconsin-Milwaukee

The Singular Value Decomposition of Pair Correlations Matrices from an XFEL Experiment

S. Wibowo and D. K. Saldin

Department of Physics, University of Wisconsin-Milwaukee

The problem of determining the structure of a biomolecule, when all the evidence from experiment consists of individual diffraction patterns from random particle orientations, is the central theoretical problem with an XFEL. One of the methods proposed is a calculation over all measured diffraction patterns of the average angular correlations between pairs of points on the diffraction patterns. It is possible to construct from these a matrix B characterized by angular momentum quantum number *l,* and whose elements are characterized by radii *q* and *q'* of the resolution shells. If it is possible to find the spherical harmonic expansion coefficients, which depend also on the extra magnetic quantum numbers *m*, from these matrices, it will be possible to reconstruct an oversampled version from which the real-space structure of the particle may be found by an iterative phasing algorithm. What we show in this paper is that the identity of the magnetic quantum numbers *m* may be revealed by singular value decomposition of the B matrices. At the very least this determines information about the particle symmetry from the experimental data, and it may provide a means of reconstructing the diffraction volume of a general particle, by this method, independent of any assumed symmetry.

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MAX WIERDORN, CENTER FOR FREE ELECTRON LASER SCIENCE

NOVEL SAMPLE DELIVERY SYSTEM FOR SERIAL CRYSTALLOGRAPHY AT XFELS

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The use of gas dynamic virtual nozzles (GDVN) enabled serial femtosecond crystallography (SFX) to be performed at x-ray free-electron lasers (XFEL). A suspension of micron-sized crystals is injected into the x-ray beam. To get sufficient scattered signal from these small crystals, high intensity x-ray pulses above the Henderson limit are used to probe the crystals. The advantages of SFX include that the crystalline sample can be probed in its native buffer at room temperature.

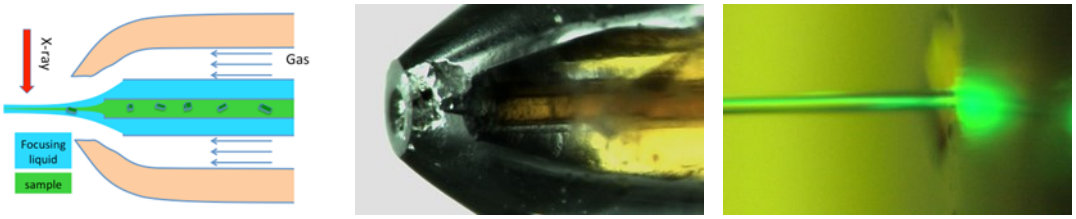


FIG 1: (a) Schematic of and (b) an assembled a double-flow focusing nozzle, (c) inner jet visualized with fluorescent dye (overlay of dark and bright images).

Many protein crystal buffers include either high salt or high PEG concentrations increasing challenges of using GDVN for sample injection. Both salt and PEG leave residues inside the nozzle, which change the jetting properties of the nozzle over time. In order to improve the nozzle performance, we have developed a double-flow focusing nozzle (DFFN) that employs an outer sheath liquid to encapsulate the sample stream. Ethanol was found to employ most positive effects as the sheath liquid. Low surface tension makes ethanol easy to jet while its low melting point prevents the formation of icicles, which have hindered the data collection during past experiments with standard GDVNs. Since the outer liquid controls the jet performance, it is possible to drastically reduce the sample flow rate while still maintaining a high hitrate. Improvements of 5-10 time reduced sample consumption (compared to a GDVN) could be achieved with the double-flow focusing nozzle.

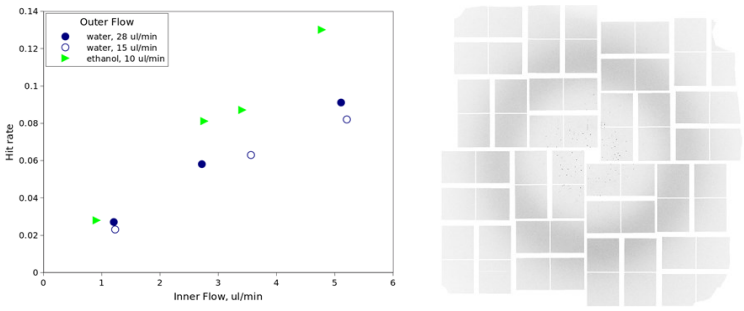
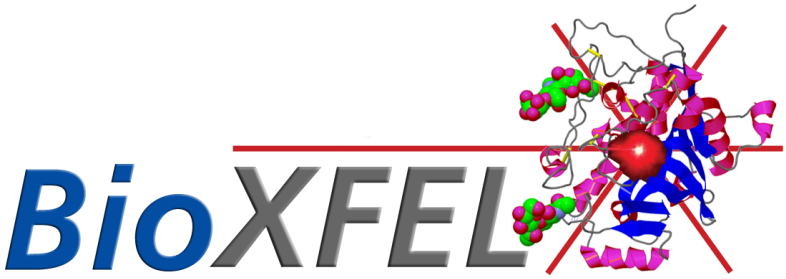


FIG 2: (a) Achieved hit rate per sample flow rate for Granulovirus at LCLS and (b) RNA Polymerase II diffraction pattern enabled by the use of a DFFN.

BioXFEL Education & diversity Programs



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BioXFEL Education and Diversity Opportunities

X-ray free electron lasers (XFELs) present unique capabilities that have the potential to revolutionize structural biology and its ability to probe the biological world. As the NASA Apollo era captured a generation's interest in outer space, these unique capabilities have the potential to capture the imagination of students, teachers, and potential scientists interested in inner space. **We aim to foster this effect through our education and diversity programs…**

Official BioXFEL Membership

There are two levels of BioXFEL membership available to students and postdocs, the Association of BioXFEL Young Scientist (ABYS), and BioXFEL Scholar. The ABYS group is open to any student, postdoc or young scientist who is interested in learning more about our Center and the research performed by our scientists. To get involved in ABYS, email Bill Bauer at wbauer@hwi.buffalo.edu. All ABYS members are welcome to attend our online activities described below. BioXFEL Scholars must meet a distinct set of criteria that includes being a member of a participating BioXFEL laboratory and working directly on a related project. BioXFEL Scholars are provided unique benefits such as our graduate student cross-training scholarship and conference and workshop travel reimbursement.

Data Processing Workshops

One of the greatest challenges faced by BioXFEL scientists is how to deal with the large and fundamentally new types of data sets generated by serial femtosecond crystallography studies. This annual workshop brings students and postdocs together with leading data analysts and software developers to discuss the latest methods for tackling data analysis problems. The workshop combines lecture presentations with practical exercises and tutorials with state-of-the-art software tools.

The next workshop— *Serial Crystallography Data Analysis with Cheetah and CrystFEL: Concepts and Tutorials*—will be held at the 2016 ACA Annual Meeting in Denver Colorado, Friday July 22nd.

BioXFEL at the 2016 SLAC/LCLS Users' Meeting

ABYS meets at the annual Users' Meeting to discuss current events in the broader XFEL field, progress with LCLS upgrades, and the content of BioXFEL's education curriculum. Our members enjoy the opportunity to network with each other and leading scientists working in structural discovery. Tours of the LCLS facility are available.

BioXFEL Online Education Programs

The BioXFEL STC manages several online courses through their website and via live webcasts. We are currently creating a collection of **online BioXFEL lectures,** an **online Crystallography Course,** and a library **of BioXFEL Journal Clubs** led by our BioXFEL Scholars. Please feel free to visit these resources and share this information with any interested colleagues. If you would like to participate in the live events, please contact Bill Bauer at **wbauer@hwi.buffalo.edu** to be added to our mailing lists.

Graduate Student Cross-training Scholarship

BioXFEL is committed to providing our students with opportunities to diversify their skill sets and integrate themselves into our highly multidisciplinary Center. In addition to the workshops, conferences and online courses, we will now be offering our students the opportunity to train in a new technique in another laboratory within the Center. This support will come in the form of a scholarship that can be used to pay for travel, housing and incidental costs up to ~$5,000. For more information, visit our web page: www.bioxfel.org/resources/CTS.

BioXFEL 3RD INTERNATIONAL CONFERENCE

Opportunities for University of Puerto Rico Students

BioXFEL is dedicated to sharing resources with the University of Puerto Rico to help engage them in all types of activities within the Science and Technology Center. These activities will be occurring throughout the year and are described below. More information can be found at www.bioxfel.org/education/upr.

BioXFEL STC Annual Conferences in January

The BioXFEL National Science Foundation Science and Technology Center is pleased to announce that it will be hosting its annual international conferences Puerto Rico. Every year the members of the Center convene to discuss their recent results, share ideas, and forge new collaborations with their colleagues. This is a great opportunity for UPR faculty and scholars to learn more about the STC's research, meet our world-class scientists and become involved in our educational outreach programs.

UPR Graduate Student Internships

BioXFEL is offering **4 month graduate student internships** to all eligible* and interested UPR graduate students. This program allows UPR graduate students to bring their graduate projects to a BioXFEL laboratory where they will receive hands-on training in XFEL related biochemical and biophysical techniques specifically designed to enhance their research. Students interested in this program should be able to provide unofficial transcripts from their home institution; a resume, CV, or Biosketch; and a letter of support from their current PI.I

*Students should be finished with their classes, must be currently enrolled in a PhD program, have a genuine interest in learning new XFEL-related techniques and should be interested in pursuing a career in a related science. If you are interested in participating in this program, please contact Dr. Bill Bauer at internships@bioxfel.org for more details.

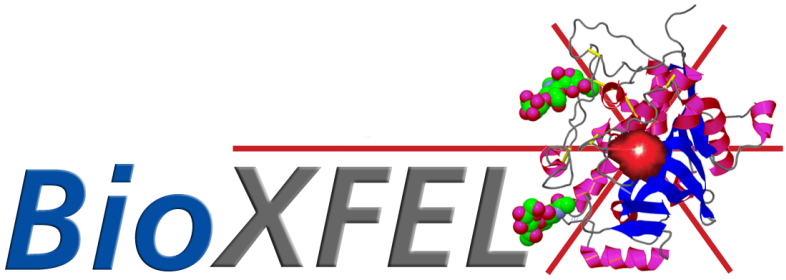
UPR Undergraduate Summer Internships

BioXFEL runs a **10 week summer internship for undergraduates** interested in pursuing careers in physics, biochemistry, biophysics, engineering, programming, mathematics or other related fields. This is a highly competitive program where students will be given hands-on training in XFEL-related laboratory techniques. At the end of the 10 weeks, students present their research results to the group. Internships run from the end of May through the first week of August. For more information on the program and how to register, please visit our website (www.bioxfel.org/education/ undergraduate-internships). Applicants must be US residents or US nationals. Women and minorities underrepresented in STEM fields are strongly encouraged to apply.

SSRL/BioXFEL Remote Data Collection Workshop

On the Tuesday following the conference (**January 19th, 2016**) SSRL and BioXFEL are holding a workshop on remote access synchrotron data collection workshop. The workshop will be held at the University of Puerto Rico's Rio Piedras campus and is designed to train users in Remote Access Data Collection. It will include a live remote access demonstration where you will connect to SSRL beamlines to present the features accessible to remote users. This workshop will enable UPR students and faculty to utilize SSRL's state-of-the art facilities for remote data collection.

2016 BioXFEL Conference Attendees



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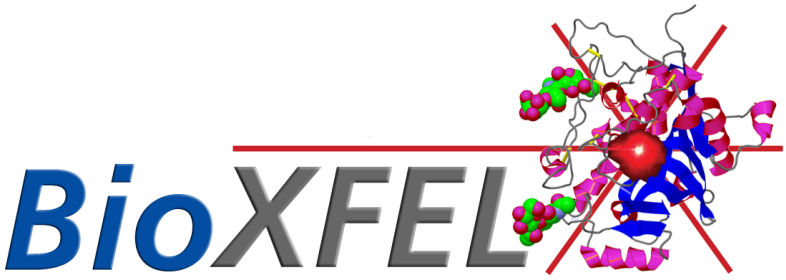

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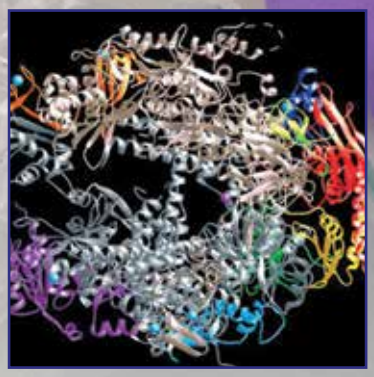

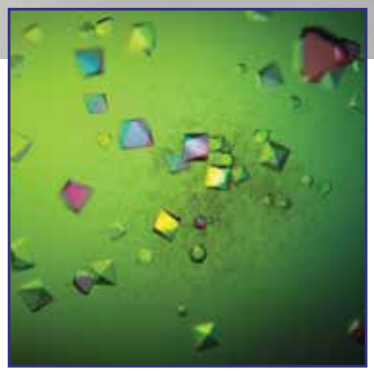

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MX340-HS
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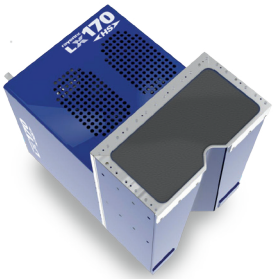

rayonix LX series HS

The only X-ray detectors designed with cutout for simultaneous SAXS/WAXS

LX170-HS
LX255-HS

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Smaller format, high speed

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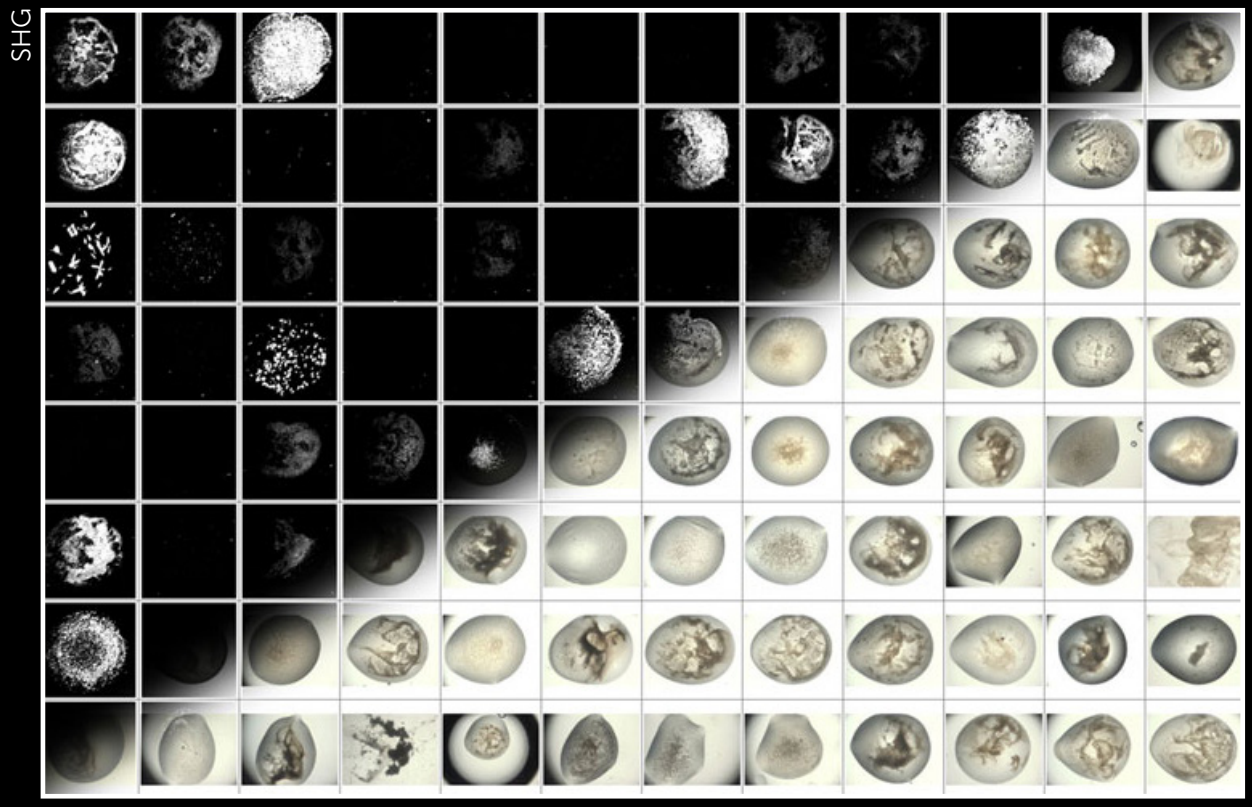
rayonix XFEL DETECTORS

FORMULATRIX
crystallization accelerated

remove the guesswork

SONICC

finds crystals other technologies cannot



detection of microcrystalline protein material for XFEL and electron diffraction applications, or for scaling up using conditions found to grow bigger crystals

SONICC
Second Order Nonlinear Imaging of Chiral Crystals

definitively identify chiral crystals, such as proteins, using state-of-the-art Second Harmonic Generation (SHG) technology

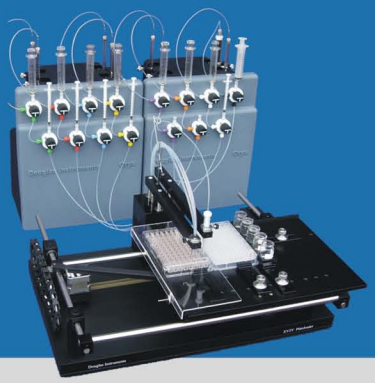
discern between protein and salt crystals utilizing built-in Ultraviolet Two-Photon Excited Fluorescence (UV-TPEF) technology

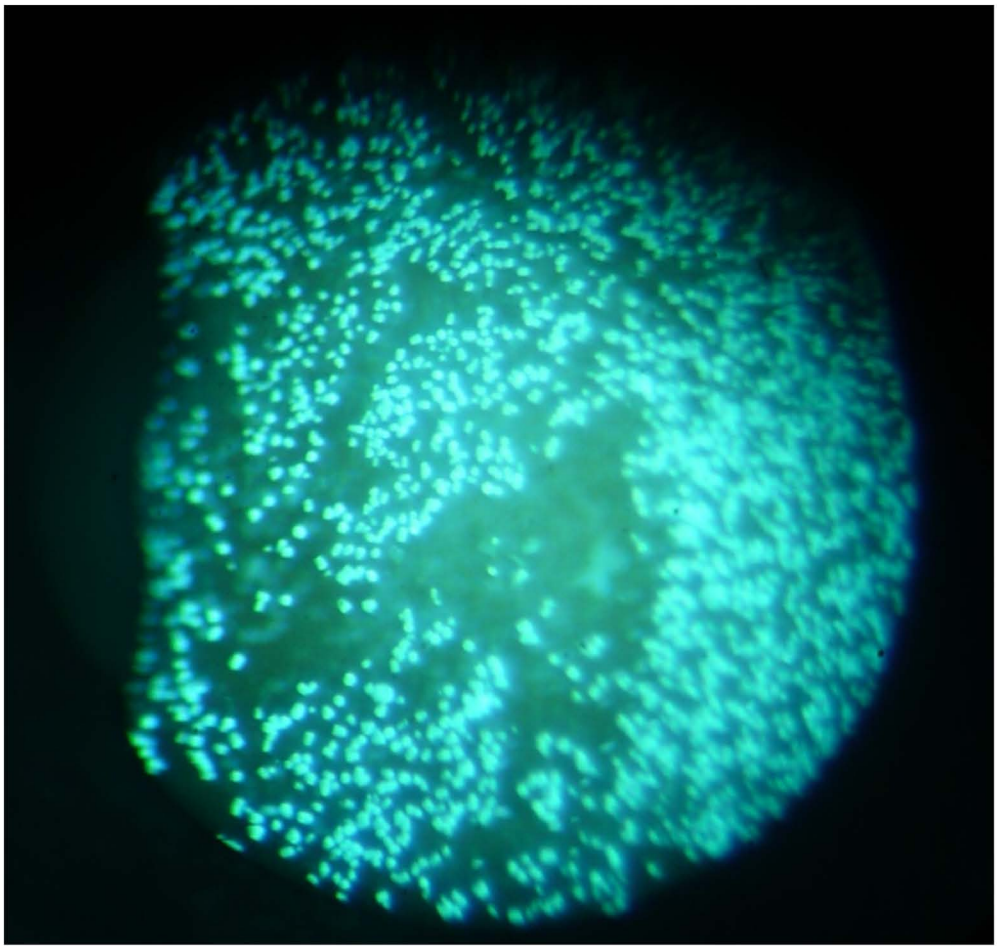
crystals appear white against a stark black background, helping you to quickly identify crystals even in murky environments

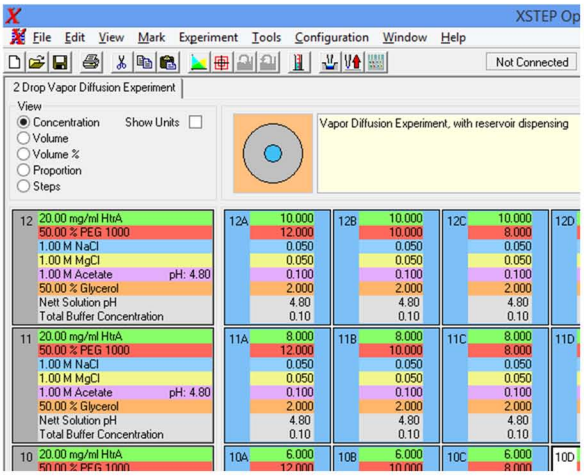
SONICC can detect extremely thin crystals, microcrystals <1 µm, and crystals obscured in birefringent LCP

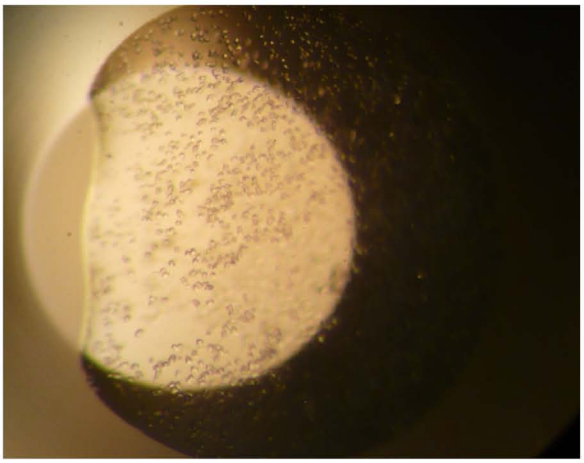
for more information about SONICC, visit us at
www.formulatrix.com or email info@formulatrix.com

How to get great results with an Oryx robot from Douglas Instruments . . ?









Batch crystallization: *from screening to final production*

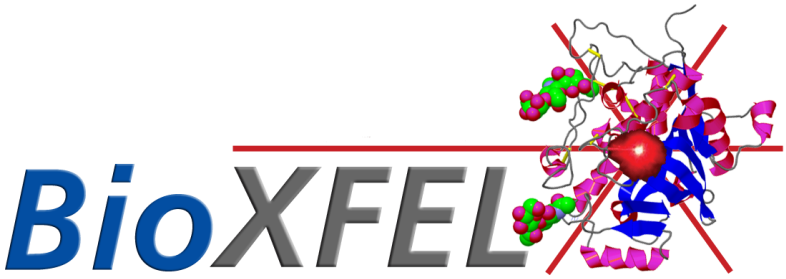
Oryx offers:

- Microbatch-under-oil screening and rMMS microseeding
- Only 10 µL protein and 1 µL seed stock required for a 96 well microbatch screen
- Microbatch optimization - 7 dimensional multivariate auto design
- Create gradients or custom designs with up to 7 ingredients

Douglas Instruments

www.douglas.co.uk

Local Attractions



3RD International Conference

A National Science Foundation Science and Technology Center

Old San Juan

Old San Juan is the oldest settlement within Puerto Rico and is the historic colonial section of San Juan, Puerto Rico. It was listed on the U.S. National Register of Historic Places in 1972 as "Zona Histórica de San Juan", and declared a National Historic Landmark District in March 2013. Here you can do any of the following:

- San Juan National Historic Site: The San Juan National Historic Site (World Heritage Site) is comprises Castillo San Felipe del Morro, Castillo San Cristobal, most of the city walls, the San Juan Gate and Fort San Juan de la Cruz just across the entrance to the bay. The Spanish spent over 250 years fortifying the prized location of Puerto Rico, since through it, Spain controlled all access in and out of the Caribbean. This dramatic fortress rises 140 feet above the sea on a rocky promontory, and is composed of six huge levels of ramps, barracks, dungeons, turrets, towers and tunnels.
- Salsa Dancing in any of the local bars.
- Fortaleza Street: Enjoy traditional Puerto Rican and Caribbean fusion cuisine along Restaurant Row.
- Paseo la Princesa: Walk from the main fountains all the way to the ocean along this road that hugs the city wall. It is best seen at sunset but during the day on weekends you can see street performers and visit local vendor stalls.
- La Casa Blanca: Built in 1521 as a home for Governor Juan Ponce de Leon, today this historic landmark contains two interesting museums of Indian and colonial artifacts.
- La Rogativa: La Rogativa is a famous bronze statue located at the Plazuela de la Rogativa in Caleta de las Monjas and very near La Puerta de San Juan. Rogativa is a Spanish word that derives from the verb “rogar” meaning to plea or supplicate. According to historians, British troupes led by Sir Abercrombie took control of the city through a naval blockage on April 30, 1797. The San Juan governor, desperate, ordered a “rogativa” (large marching procession-type plea to God). The women of the city, along with the bishop, paraded through the streets at night starting at the Cathedral singing hymns, carrying torches, bells, and praying for their city to be saved. The British mistook the sights and sounds of that night as evidence that reinforcements that outnumbered them had arrived to protect San Juan. This led Abercrombie’s fleet to abandon the city promptly. Today, there are four bronze statues commemorating this event. These statues are grouped together in Old San Juan in what is called the Plazuela de la Rogativa as they pay tribute to the efforts of the townspeople and their Bishop leader.
- Tour Old San Juan: This area boasts many different types of tours including night tours, Segway tours, food and wine tours, architectural tours, and more!