Poster Competition Abstracts

BioXFEL

5th International Conference

A National Science Foundation Science and Technology Center

BioXFEL

Poster Blitz

Tuesday 4:45-5:15

Chairs: Rick Kirian (ASU) and James Fraser (UCSF)

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18	Jeremy Copperman	Extracting energy landscapes from non-equilibrium biological processes
19	Connie Darmanin	Serial crystallography experiments at synchrotron sources and XFEL: the struggle to determine structure of MyD88/MAL complex
26	Thomas Gruhl	Towards dynamics of rhodopsin photoactivation using time-resolved serial femtosecond crystallography
27	Jurek Zoltan	Dynamics of matter induced by intense X-rays
40	Romain Arnal	Prospects for ab initio phasing and XFEL imaging of 1D and 2D crystals
55	Manoj Saxena	Construction and characterization of a cytochrome C-Ti-Transferrin conjugate for inducing cancer cell death
58	Ghoncheh Mashayeki	Conformational landscape of a virus from single-particle X-ray scattering
59	Chenghua Shao	XFEL structure deposition and data architecture support in PDB
63	Timothy Stachowski	X-ray radiation induced Transforming growth factor β 1 (TGFβ1)
71	David Wojtas	Orientation and analysis of XFEL serial diffraction patterns from fibrous molecular assemblies
23	Indra Gonzalez	Towards the inhibition mechanism of lysozyme fibrillation by hydrogen sulfide

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Poster Session

Wednesday 7:00—9:00 in Jefferson Ballroom

Judging committee: James Fraser (UCSF), Richard Kirian (ASU), Anne Stone (Molecular Dimensions), Aina Cohen (SSRL), Russell Fung (UWM)

#	Name	Poster Title
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2	Hector D Arbelo-Lopez	Sulfheme isomeric structures dπ charge transfer and π conjugation leads to its deoxy and met like derivatives visible spectra
3	Salah Awel	Optical focusing of isolated particles for diffractive imaging experiments
4	Josue Benjamin-Rivera	Fluorescent studies to track Ti(IV) serum transferrin transport into human cells
5	Peter Berntsen	Serial crystallography development in partnership with the Australian Synchrotron
6	Hazel Borges Arias	Kinetic study of the HBI-SH2 complex at physiological conditions
7	Sarah Bowman	Complex crystallization, simple answers, identifying protein microcrystals at the High-Throughput Crystallization Screening Center
8	George Calvey	Towards routine time-resolved mix-and-inject serial crystallography
9	Julio Candanedo	Outrunning damage with fast electron beams
10	José A. Carmona-Negrón	Synthesis, characterization, and application of ferrocene-hormone complexes: an approach to design novel metal-based therapeutic drugs for breast cancer treatment
11	David Case	What can we learn from MD similuations of biomolecular crystals?
12	Alexander Castro Martínez	DNA-binding properties of Vibrio fischeri transcription factors OxyR1 and OxyR2
13	Ana Rosa Cebollero Lopez	Developing a high content analysis screening system for host cell phenotypes in Toxoplasma gondii infection
14	Joe Chen	When least is best – projection operators in phase retrieval
15	Matt Coleman	Using XFELs to characterize nanolipoprotein particles that form HDL and LDL complexes
17	Alice Contreras	Coronavirus envelope (E) protein viroporin structure-function studies
18	Jeremy Copperman	Extracting energy landscapes from non-equilibrium biological processes
19	Connie Darmanin	Serial crystallography experiments at synchrotron sources and XFEL: the struggle to determine structure of MyD88/MAL complex
20	Hasan DeMirci	Structure-based antibiotic development driven by ambient-temperature serial crystallography of small and large ribosomal subunits at high resolution
21	Adalberto Diaz	Spectroscopic studies and crystallization screening of the third FF domain of Homo sapiens Prp40 Homolog A
22	Leonie Flueckiger	Rapid mixing and injection for studying molecular dynamics in time-resolved liquid X-ray scattering experiments
23	Indra Gonzalez Ojeda	Towards the inhibition mechanism of lysozyme fibrillation by hydrogen sulfide
24	Rita Graceffa	Mixing experiments at European XFEL
25	Tom Grant	Ab initio electron density determination directly from solution scattering data
26	Thomas Gruhl	Towards dynamics of rhodopsin photoactivation using time-resolved serial femtosecond crystallography
27	Zoltan Jurek	Dynamics of matter induced by intense X-rays
28	Alan Kadek	Hitting proteins with a sledgehammer – combining native mass spectrometry with an XFEL

29	Christopher Kupitz	Phytochromes: The red-light photoreceptor
30	Dan Bi Lee	Supersaturation-controlled microcrystallization and visualization analysis for serial femtosecond crystallography
31	Chufeng Li	Developments of a matching-based auto-indexing algorithm SPIND and its applicability to Serial Protein Crystallography
32	Jose Liboy	Notch regulation of cell migration and invasion in triple negative breast cancer
33	Haiguang Liu	Structure and dynamics of chloride ion pumping rhodopsin
34	Stewart Malave	Investigating the role of an atypical variant surface glycoprotein (aVSG) in developmental progression to infectivity in Trypanosoma brucei
35	Darya Marchany-Rivera	Effect of pH on Fe-O2 bond in the oxygen reactive hemoglobins of L. pectinata by X-ray Crystallography
36	Hugh Marman	Radiation damage in protein microcrystallography
37	Jose Manuel Martin Garcia	Structure determination of inhibitor complexes with full length Human Taspase1: Towards rational drug design against a new cancer target
38	Derek Mendez	Probing the ultra-fast and fast structural transitions resulting from light-absorption in the bovine visual rhodopsin: A time-resolved solution scattering study
39	Derek Mendez & Natasha Stander	Determination of structural ensembles with data-guided molecular dynamics simulations
40	Romain Arnal	Prospects for ab initio phasing and XFEL imaging of 1D and 2D crystals
41	Mitchell Miller	Progress in the time-resolved studies of the blue/green cyanobacterial phytochrome, PixJ
42	Fabiola Moreno	Sulfmyoglobin and hydrogen sulfide metmyoglobin formation in metmyoglobin crystals
43	Nirupa Nagaratnam	Serial femtosecond crystallography of in vivo grown crystals
44	Reza Nazari	3D printed gas dynamic virtual nozzles to achieve fast liquid jets
45	Prakash Nepal	Difference structures from time-resolved SAXS/WAXS in the presence of shot noise
46	Jose Olmos & Suraj Pandey	Capturing enzymatic intermediates "on-the-fly" via mix-and-inject serial crystallography
48	Sofia Perez	Elucidating the mechanism of action of titanium (IV) complexes for a new anticancer drug design strategy
49	Ishwor Poudyal	Simulation of diffraction patterns of single protein molecule with realistic beam parameters
50	Sebastian Quintana	Reducing sample amount for serial femtosecond crystallography using water-in-oil droplets
51	Alina Roman-Hubers	Study of the stability of sulfhemoglobin and sulfmyoglobin
52	Keishla Sanchez	Photolytic studies of the carbon monoxide complex with deoxy myoglobin
53	Marcos Sanchez-Navarro	Avoidance over-conditioning impairs extinction of fear, induces persistent avoidance, and increases use of safety cues: implications for OCD
54	Lysmarie Santos-Velazquez	Sulfhemoglobin and its role as an endogenous hydrogen sulfide biomarker
55	Manoj Saxena & Xaymara Rodriguez	**Construction and characterization of a cytochrome C-Ti-Transferrin conjugate for inducing cancer cell death**
56	Gebhard F.X. Schertler	Exploring G protein coupled Receptors and membrane protein dynamics with X-ray free-electron lasers and solution NMR
57	Robin Schubert	Analyzing protein liquid dense clusters - intermediates in the nucleation process - as potential samples for future XFEL experiments

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58	Ghoncheh Mashayeki	Conformational landscape of a virus from single-particle X-ray scattering
59	Chenghua Shao	**XFEL structure deposition and data architecture support in PDB**
60	Megan Shelby	First results from LCLS X-ray diffraction studies of fiber-like crystalline ApoA1 nanodiscs
61	Andrew Shevchuk	Modeling of incoherent diffractive imaging from a virus using photoelectrons
62	Joshua Soto-Ocana	
63	Tim Stachowski	X-ray radiation induced Transforming growth factor β 1 (TGFβ1)
64	Natasha Stander	A new tool for efficient pattern selection from large SFX datasets
65	Ganesh Subramanian	Structural analysis of photoactive biomolecules using X-ray absorption spectroscopy: experiments and simulations
66	Shuo Sui	Graphene-integrated microfluidics for advanced crystallography
67	Miklos Tegze	Orienting diffraction patterns in single particle imaging experiments
68	Michael Thompson	Generalizing time-resolved crystallography and SAXS/WAXS with infrared laser-induced temperature-jump
69	Jennifer Vargas Santiago	Hydrogen sulfide (H2S) trapment by hemoglobin I from Lucina pectinata encapsulated in Sol-gels
70	Max Wiedorn	MHz serial crystallography at the European XFEL
71	David Wojtas	Orientation and analysis of XFEL serial diffraction patterns from fibrous molecular assemblies
72	Alexander Wolff	Optimizing sample delivery for time-resolved temperature-jump serial femtosecond crystallography
73	Iris Young	XFEL diffraction studies of the oxygen evolving complex of photosystem II

Alani Aldarondo-Torres, UPRM

H_2S induced structural changes of insulin protein in early lag phase: key to detaining amyloid formation

Aldarondo-Torres, Álani[1]; Colón-Ríos, Daniel[2]; Rosario-Alomar, Manuel F.[3]; López-Garriga, Juan[2]

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Amyloid fibrils are formed by soluble proteins that assemble into insoluble fibers associated to many types of diseases such as Diabetes Type 2, and Alzheimer's Disease. Amyloid formation can be divided in the lag phase where native protein unfolds and forms protofibrils, and the elongation phase which produces mature fibrils. By adding hydrogen sulfide (H_2S) to hen egg white lysozyme (HEWL), amyloid fibrils are inhibited (Rosario, 2014). To further explain the means by which H_2S inhibits amyloid fibrils, Insulin from Bovine Pancreas (IBP), was tested upon experimentation with H_2S concentrations of 1:0, 1:1, and 1:5 molar ratios (IBP: H_2S). ThT Fluorescence analysis revealed a decrease in peak intensity as the concentration of H_2S increased in each sample proving H_2S to be an inhibitor of insulin amyloids. Atomic Force Microscopy (AFM) of the control sample showed an array of fibrillary structures. As the concentration of H_2S increased samples went from having a combination of fibrillary structures and small conglomerated spherules to the sole appearance of small spherules. Far UV-Circular Dichroism spectra of the native protein and the protein interacting with H_2S were very similar. However, non-resonance Raman spectra revealed the presence of newly formed trisulfide bridges in the interaction between insulin and the H_2S species as opposed to the disulfide bridges found in native and fibrillated insulin protein. Obtained results suggest H_2S-protein interactions favor a structural change in the process of protein denaturing. The H_2S species intervenes with amyloid formation early on during the lag phase inhibiting the formation of protofibrils and potential mature fibrils.

Acknowledgement: Research reported in this publication was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

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Hector Arbelo-Lopez, UPRM

Sulfheme isomeric structures d_π charge transfer and π conjugation leads to its deoxy and met like derivatives visible spectra

Hector D. Arbelo Lopez[1], Angel D. Rodriguez-Mackenzie[1], Elddie M. Roman-Morales[1], Troy Wymore[2], and Juan Lopez-Garriga[1]

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Since the discovery, in the year 1863, of a new hemoglobin green derivative called “sulfhemoglobin” with a characteristic 618 nm absorption band, it has been published extensive literature suggesting the presence of diverse species and environments responsible for this red electronic transition. Thus, to define the landscape of the sulfheme visible region, time-dependent density functional theory (TDDFT) was used to calculate the modified sulfheme HOMO and LUMO molecular orbitals and their transitions. The resulting stable sulfheme isomers, episulfide three member ring (S_A) and episulfide five member ring (S_C) have unique a_{1u} and a_{2u} HOMO and LUMO orbitals energy and stability patterns. Overall, the interplay between the electron withdrawing and electron donating properties of the peripheral groups modulates the separation energy between the a_{1u} and a_{2u} orbitals, contributing to define and explain why other sulfheme proteins show a diversity of visible absorption transitions in the 620 nm region. It is suggested that the 620 nm and 715 nm visible q bands are associated with a charge transfer electronic transition between the sulfur orbitals and the heme iron d_π orbitals The sulfheme bands are product of charge transfer (CT) between the inserted sulfur atom into the heme group and the heme iron. We found that the overlap of the p orbitals of the sulfur with the heme π system allows the CT between the sulfur and the heme iron d_π orbitals. The S_A sulfheme isomer is the predominant specie between 50ms to 5 minutes timescale. At longer times the S_C sulfheme isomer becomes the predominant specie present.

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Salah Awel, DESY

Optical focusing of isolated particles for diffractive imaging experiments

Salah Awel[1,4], Daniel Horke[1,4], Rick Kirian[3], Xiaoyan Sun[1], Andrei Rode[2], Jochen Küpper[1,4,5], and Henry Chapman[1,4,5]

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Single-particle imaging is emerging as a new techniques at x-ray free-electron lasers (XFELs) that consists of directing a stream of randomly oriented bioparticles across the focus of the XFEL beam aiming to construct high-resolution 3D structure from diffraction patterns of multiple 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. In order to mitigate this problem, we have developed a technique for guiding aerosolized nanoparticles to the x-ray focus using spatially shaped optical laser beam [1]. Our current experiments aim at transversely confining streams of aerosolized particles as they exit an aerosol injector with a counter-propagating “hollow-core” quasi-Bessel beam. The experiment exploits radiation pressure and thermal (photophoretic) forces arising from the interaction of the particles with surrounding gas molecules to confine the particles within the low-intensity core of the laser beam [2, 3].

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[2] Eckerskorn et al., *Phys. Rev. Applied* **4**, 064001 (2015).

[3] Awel, et al. *Opt. Express* **24**, 6507-6521 (2016)

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Josue Benjamin-Rivera, University of Puerto Rico

Fluorescent studies to track Ti(IV) serum transferrin transport into human cells

Benjamin-Rivera, J.A,1, Tinoco, A.D.,1,Delgado,Y,2, Pandrala, M, 3, Vazquez,A,1,Vazquez, A 1

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Titanium is a metal commonly used in implants in alloy form. Ti in the implants is not as stable as previously thought and can interact with biomolecules in the body. This interaction results in Ti dissolving and releasng into the blood. This release increases the Ti (IV) concentration nearly 50 times greater (0.25 μM) than people with no such implants. Citrate is a small molecule, present in high μM amounts in blood and synovial fluid, this small molecule plays an important role in the solubilization and chelation of Ti (IV). The Ti(IV) citrate complex that forms in blood interacts with the protein human serum transferrin. This interaction regulates Ti(IV) speciation and protects the metal from hydrolysis. By understanding the aqueous speciation of the Ti(IV) serum transferrin (sTf) complex we will identify how the metal is transported and what is its biological fate. To gain insight into sTf coordination of Ti (IV) coordination we are interested in synthesizing a fluorescent derivative of citrate by modifying it with 5-aminofluorescein. This probe will help us elucidate the endocytotic transport of Ti(IV). The synthesis and purification of the 5-aminofluorescein derivative of citrate is described.

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Peter Berntsen, La Trobe University

Serial crystallography development in partnership with the Australian Synchrotron

Peter Berntsen[1], Marjan Hadian Jazi[1], Mick Kusel[2], Connie Darmanin[1],

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

1. Australian Research Council Centre of Excellence in Advanced Molecular Imaging, La Trobe Institute for Molecular Science, La Trobe University, Melbourne 3086, Australia. 2. ANSTO – Australian Synchrotron, 800 Blackburn Road, Clayton, Victoria 3168, Australia.

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

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Hazel Borges-Arias, UPRM
Alfredo Reyes-Oliveras, UPRM

Kinetic study of the HbI-SH_2 complex at physiological conditions

Borges-Arias, H.[1]; Reyes-Oliveras, A.[1]; López-Garriga, J. C.[1]

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

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Sarah Bowman, Hauptman-Woodward Institute

Complex crystallization, simple answers, identifying protein microcrystals at the High-throughput Crystallization Screening Center

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George Calvey, Cornell

Towards routine time-resolved mix-and-inject serial crystallography

George D. Calvey, Andrea M. Katz, and Lois Pollack

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Time-resolved mix-and-inject serial crystallography at x-ray free electron lasers provides an unprecedented opportunity to observe structural changes in biomolecules as they function. This technique has the potential to elucidate numerous biological processes and advance structure based drug design. However, up to this point, the experiment has required a team of trained mixing injector experts and has been more challenging than a standard serial femtosecond crystallography experiment. Here, we present a robust, modular mixing injector and microfluidic manifold that addresses these challenges. The manifold screws together, and is compatible with beamline equipment at both LCLS (CXI) and the European XFEL (SPB/SFX). The entire mixing injector and manifold can be assembled in an hour. This device advances mix-and-inject serial crystallography by making the technology and experiment accessible and routine.

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

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Julio Candanedo, ASU

Outrunning damage with fast electron beams

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The aim of this project is to investigate the possibility of our-running radiation damage using femtosecond MeV electron diffraction [1,2]. We use molecular dynamics simulations, similar to those used to study sample interactions with an XFEL [3]. In accordance with known cross sections, atoms are increasingly ionized as time passes, and ejected electrons tracked. Important differences between between X-ray and electron interactions include the larger elastic and inelastic scattering cross-sections for electron scattering, and greatly reduced amount of energy dumped in the sample by secondary electrons (of about 3 eV kinetic energy, with short range) per elastic scattering event, compared to the kilovolt energies of photoelectrons with much longer range. In addition, the gain (of about 10^5) of an XFEL is not available for the photoelectron electron sources which are used in both cases. Finally, electrons are scattered from the slow-moving atomic nucleus, unlike X-rays. We use GROMACS and the Lewis Dot notation to keep track of electron motion during the ionization cascade, in order to determine resolution loss in the electron diffraction patterns as a function of electron beam pulse duration. Supported by BioXFEL STC award.

1. Spence J.C.H. Struct. Dyn. 4, 044027 (2017).
2. Maxson J. et al et al Phys Rev Letts. 118, 154802 (2017)
3. Caleman, C. et al J. Mod. Optics 58, 1486 (2011)

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Jose Carmona-Negron, UPRM

Synthesis, characterization, and application of ferrocene-hormone complexes: an approach to design novel metal-based therapeutic drugs for breast cancer treatement

Carmona-Negrón, J.A.[1], Bauer W.J.[2], Rheingold A.L.[3], Snell E.S.2, Santana A.[1], and Meléndez E.[1]

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[2] Hauptman-Woodward Medical Research Institute, Buffalo, NY
[3] University of California-San Diego, Department of Chemistry, La Jolla, CA

Unlike common drugs that consist of entirely organic groups, incorporation of metals 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 limited the cisplatin drug application. Recently, ferrocene has been introduced for biological applications due its antineoplastic properties on Erlich ascite tumor, but also, due to their desired physical and chemical properties such as aqueous stability and high synthetic homology to benzene chemistry. 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 derivatives. These ferrocene complexes will be used to target estrogen dependent breast cancer (ER+), which account more than the 50% percent among the different breast cancers. The synthesized ferrocene-estrogen complex derivatives showed similar micromolar antiproliferative activity on hormone dependent MCF-7 breast cancer cell lines comparable to conventional therapeutic drugs activity such as tamoxifen and cisplatin. Computational studies of the interaction of the ferrocene complex with estrogen receptor protein demonstrated docking interactions of these ferrocene complexes in the protein's ligand binding pocket. In this work, we will also present the characterization by X -ray diffraction technique of the ferrocene complexes. Important fundamental aspect as selective precipitation of hormone-ferrocene conjugates isomers and their structural implication will be also discussed. In addition, our ongoing efforts to elucidate and validate the effectiveness of the hormone moiety part of the ferrocene complex to be recognized by macromolecules that is serving as a vector to target hormone-dependent breast cancer as a specific physiological target. 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.

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David Case, Rutgers

What can we learn from MD simiulations of biomolecular crystals?

Case, David A.

Dept. of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ 08854 USA

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

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

[1] D.S. Cerutti, P.L. Freddolino, R.E. Duke and David A. Case. Simulations of a protein crystal with a high resolution X-ray structure: Evaluation of force fields and water models. *J. Phys. Chem. B* **114**, 12811-12824 (2010).

[2] P.A. Janowski, D.S. Cerutti, J. Holton and D.A. Case. Peptide crystal simulations reveal hidden dynamics. *J. Am. Chem. Soc*. **135**, 7938-7948 (2013).

[3] C. Liu, P.A. Janowski and D.A. Case. All-atom crystal simulations of DNA and RNA duplexes. *Biochim. Biophys. Acta* **1850**, 1059-1071 (2015).

[4] P.A. Janowski, C. Liu and D.A. Case. Molecular dynamics of triclinic lysozyme in a crystal lattice. *Prot. Sci.* **25**, 87-102 (2016).

[5] A.H. Van Benschoten, L. Liu, A. Gonzalez, A.S. Brewster, N.K. Sauter, J.S. Fraser and M.E. Wall. Measuring and modeling diffuse scattering in protein X-ray crystallography. *Proc. Natl. Acad. Sci. USA* **113**, 4069-4074 (2016).

[6] S.P. Meisburger and N. Ando. Correlated Motions from Crystallography beyond Diffraction. *Acc. Chem. Res.* **50**, 580-583 (2017).

[7] K.A. Niessen, M. Xu, A. Paciaroni, A. Orecchini, E.H. Snell and A.G. Markelz. Moving in the right direction: Protein vibrational steering function. *Biophys. J.* **112**, 933-942 (2017).

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Alexander Castro-Martinez, University of Puerto Rico

DNA-binding properties of *Vibrio fischeri* transcription factors OxyR1 and OxyR2

Alexander Castro-Martínez, Paola A. Colón-Cruz, Sebastian F. Estarellas-Cobian, José Rodriguez-Martínez, Zomary Flores-Cruz

Univ. of Puerto Rico Rio Piedras, San Juan, PR

Transcription factors (TFs) are sequence-specific DNA-binding proteins that bear the key to cellular state. By controlling the expression of multiple genes, TFs also control how organisms respond to environmental stresses. OxyR is a LysR-type transcription factor that strongly binds to H_2O_2 and activates multiple genes that protect bacteria against oxidative stress. The genome of *Vibrio fischeri* encodes two different OxyRs (OxyR1, OxyR2). However, the collection of target genes of OxyR1 and OxyR2 in *V. fischeri* remains to be determined. Our research efforts are geared towards determining the intrinsic DNA-binding preferences of OxyR1 and OxyR2. Currently, we are working on over-expressing and purifying full-length OxyR1 and OxyR2. Purified proteins will be used to determine OxyR1 and OxyR2 DNA binding specificity using high-throughput SELEX (Systematic Evolution of Ligands by Exponential Enrichment). The determined DNA-binding specificities will be used to bioinformatically predict the genomic targets of OxyR1 and OxyR2. Our findings will contribute to our understanding of OxyR1 and OxyR2 roles in oxidative stress.

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Ana Cebollero-Lopez, University of Puerto Rico

Developing a high content analysis screening system for hsot cell phenotypes in *Toxoplasma gondii* infection

Cebollero-López, A., 1, LaDow, E., 2 and Reese, M., 2

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Toxoplasmosis, a disease that affects about a third of the world population, is caused by an obligate intracellular parasite called *Toxoplasma gondii*. In culture, *Toxoplasma* invasion is nonrandom; a subpopulation of cells become superinfected while other cells are uninfected. While the mechanisms for this are unclear, one potential contributor is cell cycle state. Previous research has shown that *Toxoplasma* invasion drastically increases when cells are in the S-phase of the cell cycle. Regardless of cell cycle state upon invasion, *Toxoplasma* infection causes otherwise quiescent cells to start cycling and shift to the S-phase of the cell cycle. How *Toxoplasma* detects and shifts host cell cycle state is unknown. Our goal is to develop a high content analysis assay for screens to look for both *Toxoplasma* and host cell factors that may contribute to these cell cycle phenotypes. We developed a computational pipeline for the automated analysis of *Toxoplasma* invasion. To study host cell cycle we successfully cloned a humanized form of a fluorescent ratiometric cell cycle marker, FUCCI, (HuFUCCI), and found it to be responsive to *Toxoplasma* invasion. With these methods we can now screen libraries of either host or *Toxoplasma* genetic manipulations to look for genes involved in essential infection processes.

ACL was funded, in part, by an REU supplement from the NSF. This work was funded, in part, by grants to MLR from the NSF and by the UTSW President's Research Council.

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BioXFEL

Joe Chen, ASU

When least is best - projection operators in phase retrieval

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Projection operators are operations that make the minimum possible change to an input such that the resultant output satisfies some predefined constraint. These operations are employed in iterative algorithms for solving constraint-satisfaction problems [1-2], of which phase retrieval, the procedure of recovering a structure from knowing only its Fourier magnitude, is a prominent example. A list of projection operators relevant to phase retrieval in the context of serial femtosecond crystallography will be discussed [3-4], along with their geometrical interpretations.

Acknowledgment

RAK acknowledges support from NSF STC Award (1231306).

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Matthew Coleman, LLNL

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

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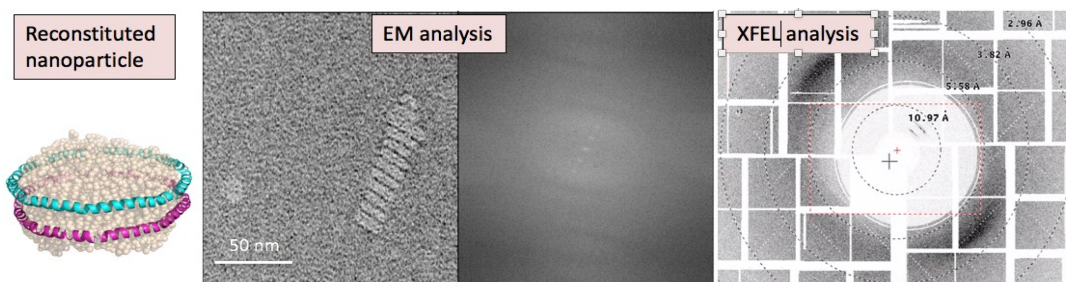
The overall goal of this project is to develop methods for high-throughput and high-resolution biological imaging that will enable temporal and spatial studies for formation of High Density Lipid (HDL) and Low Density Lipid (LDL) particles. HDL and LDL particles are involved in lipid and cholesterol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



This work was performed, in part, under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. This work was also supported by NIH grants R01GM117342 (NIGMS) and R21AI120925 (NIAID) and NSF STC ‘BioXFEL’ 1231306. Use of the LCLS, SLAC National Accelerator Laboratory, is supported by the U.S. Department of Energy, Office of Science, under contract no. DE-AC02-76SF00515.

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BioXFEL

Alice Contreras, ASU

Coronavirus envelope (E) protein viroporin structure-function studies

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Coronaviruses (CoVs) are large single stranded positive sense RNA viruses. The viruses infect a variety of hosts which includes, humans, bats, camels, and other types of domesticated and wild animals. CoVs are known to cause mild to moderate upper respiratory infections, however recently emerged Middle Eastern respiratory syndrome (MERS) CoV and severe acute respiratory syndrome (SARS) CoV cause severe lower respiratory infection and exhibit high mortality. The CoV virion contains four main structural proteins which includes the spike (S), membrane (M), nucleocapsid (N) and the envelope (E) protein. The E protein is a small membrane protein that plays a role in virion assembly. Additionally, the E protein is a viroporin that forms ion channels that play a not fully understood role in viral pathogenesis. SARS-CoV E protein is a virulence factor and recent data supports contributions of inflammasomes to lung injury during infection. Viroporins are attractive targets for antiviral therapeutic development. Understanding the structure and function of viroporins is critically important to identify and develop therapeutic molecules that interfere with the proteins. The lab has incorporated strategic mutations that may affect ion channel activity into mouse hepatitis virus (MHV) to disrupt said ion channel activity. To better understand the function of E, it is important to determine its structure. We have expressed the protein in *E. coli* and developed a purification strategy. Crystals have been produced in lipid cubic phase. Microcrystals were analyzed at the Linac Coherent Light Source (LCLS) and larger crystals were analyzed at the Advanced Proton Light Source (APS). However, the crystals do not diffract well. We are currently exploring additional expression systems and will attempt crystallizing proteins with strategic mutations that may yield improved crystals.

Current project focus:

- Express and produce the MHV E protein (wild type and mutants) in *E. Coli*
- Extraction and purification of E protein from *E. coli* using FPLC and size exclusion chromatography (SEC)
- Ion channel measurements of viroporin, both wildtype (WT) and mutations, to check for functionality

Future Project focus:

- Further crystallization optimization in lipid cubic phase (LCP)
- Further XFEL analysis of microcrystals

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Jeremy Copperman, UWM

Extracting energy landscapes from non-equilibrium biological processes

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In a non-equilibrium process, the distribution of work performed along different dynamical paths governs the system thermodynamics, with the reaction pathway connecting two states of interest dependent upon the protocol used to travel between them. Using a theoretical framework based upon geometric machine learning and stochastic thermodynamics, we are developing an algorithmic method capable of extracting the underlying free energy landscapes of molecular machines from non-equilibrium single-particle imaging experiments. Such a free energy landscape allows one to determine the underlying functional pathway in a protocol independent fashion, much as a Carnot cycle characterizes all heat engines. Without this underlying thermodynamic description, it is not possible to compare results from non-equilibrium measurements performed under different environmental or dynamical protocols. We demonstrate this concept using molecular dynamic simulations of non-equilibrium processes, and discuss the application to realistic time-resolved single-particle cryogenic electron microscopy (cryo-EM) and X-ray Free Electron Laser (XFEL) imaging. We hope our initial theoretical efforts will inform a new class of single-particle XFEL experiments, with the goal of determining the governing energetics and functional pathways of out-of-equilibrium biological processes.

Grant Acknowledgements: The research was supported by the US Department of Energy, Office of Science, Basic Energy Sciences under award DE-SC0002164, and by the US National Science Foundation under awards STC 1231306 and 1551489.

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Connie Darmanin, La Trobe University

Serial crystallography experiments at synchrotron sources and XFEL: the struggle to determine structure of MyD88/MAL complex

Darmanin, C.[1], Holmes, S.[1], Ve, T.[2], Meents, A.[3], Kobe, B.[4], Flueckiger, L.[1], Chapman, H.[3], Nugent, K.[1], Abbey, B.[1]

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Toll-like receptors (TLRs) detect pathogens and endogenous danger, initiating innate immune responses that lead to the production of pro-inflammatory cytokines. At the same time, TLR-mediated inflammation is associated with a number of pathological states, including infectious, autoimmune, inflammatory, cardiovascular and cancer-related disorders. This dual role of the pathways in protecting against infection and contributing to pathological conditions has attracted widespread interest from pharmaceutical and biotechnology industries.

Myeloid differentiation primary response gene 88 (MyD88) Toll/interleukin-1 receptor (TIR) domain, which is involved in Toll-like receptor mediated inflammation, has been associated with a number of pathological states including infectious, autoimmune, inflammatory, cardiovascular and cancer-related disorders. Cytoplasmic signaling by TLRs starts by their TIR (Toll/interleukin-1 receptor) domain (hereafter simply 'TIR' (Ve *et al.*, 2015) interacting with TIR-containing adaptor proteins such as MyD88 and MAL (MyD88 adaptor-like) (Ve *et al.*, 2012). Recruitment of these adaptors to the TLRs via TIR:TIR interactions orchestrates downstream signaling pathways, leading to induction of the pro-inflammatory genes. Although TLR pathways have been well characterized, molecular information on the signaling proteins is limited, impeding the development of therapeutic strategies, and the understanding of the effects of polymorphic variants on human disease. We have co-crystallized MyD88 and MAL and produced needle-like crystals with the dimensions of 10 to 0.5 µm (in the longest length) x 0.2 x 0.05 µm. Through a number of serial crystallography experiments at synchrotrons and different samples setups we had discovered the limitations and advantages of highly focused sources and the importance of sample delivery for determining structures from such small crystals. Here we present serial crystallography experimental data on the MyD88-MAL crystals data and ways we have indexed the data.

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Hasan DeMirci, Stanford PULSE Institute

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

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

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Adalberto Diaz, University of Puerto Rico
Gabriela Casanova, University of Puerto Rico

Spectroscopic studies and crystallization screening of the third FF domain of *Homo sapiens* Prp40 Homolog A

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Homo sapiens Prp40 Homolog A (*Hs*Prp40A) is a nuclear protein that is involved in pre-mRNA splicing. This modular protein has two WW domain followed by six FF domain. Previous studies shows that the WW domain-mediated interactions of *Hs*Prp40A are implicated in genetic disorders such as Huntington's disease and Rett syndrome. However, little is known about the biological implications related to *Hs*Prp40A's FF domains. Recently, we found that a *Hs*Prp40A peptide interacts with a calcium-binding protein named centrin. The interaction between the *Hs*Prp40A peptide and centrin was validated using isothermal titration calorimetry and two-dimensional infrared correlation spectroscopy. Interestingly, this centrin-binding site is located within the third FF (FF_3) domain of *Hs*Prp40A. Here, we present a comparative molecular biophysical study involving circular dichroism and two-dimensional infrared correlation spectroscopy to explore the molecular behavior of *Hs*Prp40A's FF_3 domain. Our next step is to obtain a high resolution structure of *Hs*Prp40A's FF_3 domain by X-Ray crystallography. Therefore, a crystal screening was performed using hanging drop vapor diffusion in order to find optimal conditions for protein crystal growth. These results may provide new insights about the stability of *Hs*Prp40A's FF_3 domain and its role in FF domain-mediated interactions.

This research material is supported by the National Science Foundation BioXFEL STC Award 1231306. We also thank the Alfred P. Sloan Foundation for its support in this research project.

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Leonie Flueckiger, La Trobe University

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

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

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

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Indra Gonzalez-Ojeda, University of Puerto Rico

Towards the inhibition mechanism of lysozyme fibrillation by hydrogen sulfide

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Amyloid fibrils are stable aggregates of misfolded proteins associated with many neurodegenerative disorders. It has been shown that hydrogen sulfide (H_2S), inhibits the fibrillation of lysozyme through the formation of trisulfide (S-S-S) bonds. However, the overall mechanism remains elusive. Here, the concentration dependence of H_2S effect was investigated using Atomic force microscopy (AFM), non-resonance Raman spectroscopy, Deep-UV Raman spectroscopy and circular dichroism (CD). It was found that small spherical aggregates with trisulfide bonds and a unique secondary structure were formed by adding concentrations of 25 mM and 50 mM of H_2S. This could indicate that H_2S might serve as a protecting agent for the protein. However, further characterization of these aggregates and their trisulfide bonds is needed to fully unravel the function H_2S has on protein fibrillization.

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Rita Graceffa, EuXFEL

Mixing experiments at European XFEL

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Mixing experiments provide important insights into the structure-function relationship of proteins *in operando*, by taking sequential snapshots at different time points after mixing [1]. Up to date XFEL mixing experiments on crystal suspensions, allow access to time scale of seconds [2]. However, the macromolecular conformational rearrangements that underlie signaling, transport, catalysis and assembly happen on the micro-milliseconds time scales.

Here we present a microfluidic device that allows probing conformational change in the milliseconds time scale, filling the existing experimental gap. The defined and reproducible geometry of the microfluidics channels provides accurate correlation between the spatial displacement of the crystals respect to the mixing point and the reaction time probed by the x-rays. The probed reaction time depends, in fact, on the fluid-dynamics of the system, including the mixing geometry and the distance travelled by the crystals after the mixing region. The device is made of glass, material compatible with a broad variety of samples and high pressure conditions. Its optical proprieties consent characterization of fluid-dynamics proprieties of the liquid before jet extrusion by visible light microscopy and spectroscopy. The device is integrated on the set-up of Single Particles, Clusters, and Biomolecules & Serial Femtosecond Crystallography (SPB/SFX) instrument of the European XFEL, by inline connectors that minimize the clogging events.

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Thomas Grant, University at Buffalo

Ab initio electron density determination directly from solution scattering data

Thomas D. Grant[1,2]

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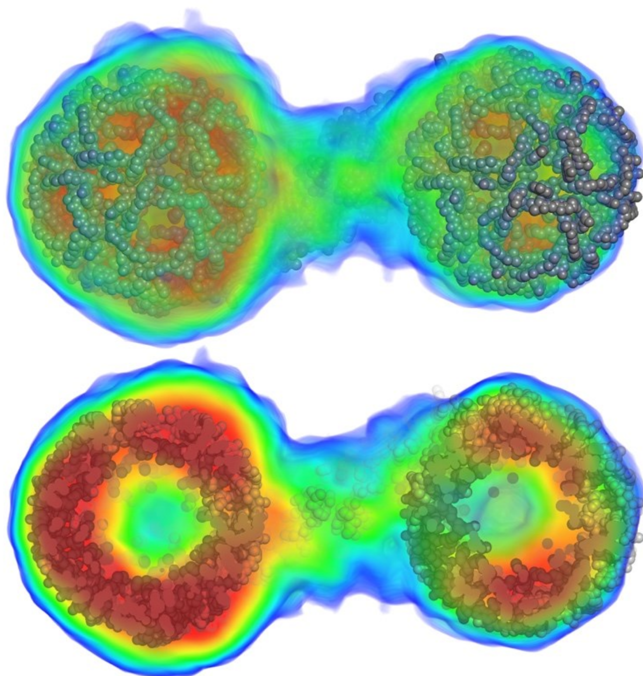
Small angle scattering is an experimental technique used to analyze the molecular structures of a wide variety of biological and non-biological samples in solution. In contrast to X-ray crystallography and cryo-electron microscopy, where 3D electron density maps are calculated, available methods for generating 3D structural information from 1D solution scattering data rely exclusively on modeling. Many modeling algorithms rely on an implicit assumption that electron density is uniform inside the particle envelope. This assumption breaks down at resolutions better than approximately 10 – 15 Å where fluctuations in electron density contribute significantly to scattering and for particles with large scale conformational dynamics or containing mixed density species. Here I present a method for calculating electron density maps directly from solution scattering data. Using solvent flattening as the only additional restraint, this method avoids many of the assumptions limiting the resolution and accuracy of conventional modeling algorithms. The algorithm has been applied to publicly available experimental scattering data from twelve different biological macromolecules. In each case the electron density maps closely match known atomic models, including complex shapes with multiple density components. These results demonstrate that accurate and complex electron density maps can be reconstructed from small angle scattering data and with significantly fewer restraints than imposed by existing modeling methods.



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Thomas Gruhl, Paul Scherrer Institute

Towards dynamics of rhodopsin photoactivation using time-resolved serial femtosecond crystallography

Thomas Gruhl, Tobias Weinert, Pikyee Ma, Petr Skopintsev, Jonas Mühle, Niranjan Varma, Ching-Ju Tsai, Jörg Standfuss, Valérie Panneels, Gebhard Schertler

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Rhodopsin is a member of the large family of G protein-coupled receptors, a group of membrane proteins having a key role in many cellular signalling pathways. In rhodopsin the absorption of a photon results in the isomerisation of the covalently-bound chromophore ligand (11-*cis*-retinal), inducing conformational changes in the receptor. These changes lead to the activation of the G protein transducin, which constitutes the initial event of dim-light vision. While the structures of dark[1] and fully active states[2] of rhodopsin have been characterised, the photoactivation mechanism at an atomic level remains controversial.

Recently, we have shown that serial femtosecond X-ray crystallography with X-ray free electron lasers (XFEL) is a powerful method to study dynamics of structural changes in the bacterial homolog bacteriorhodopsin[3]. Billions of microcrystals at room temperature are extruded one by one into the very intense X-ray pulse of a free electron laser and measured under the "diffraction before destruction" mode. For time-resolved studies, a pump laser photoactivates the protein in the microcrystals at different delays before diffraction. We have purified rhodopsin and obtained high density microcrystals suspensions required for serial crystallography. Rhodopsin microcrystals have been already tested at the two XFELs in LCLS in Stanford and SACLA in Japan. The final results will give insights on structural changes in the range of femtoseconds to milliseconds involved in the photoactivation process. These knowledge will be beneficial for other G protein-coupled receptors, one of the major targets in drug discovery.

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JUREK ZOLTAN, CFEL-DESY

DYNAMICS OF MATTER INDUCED BY INTENSE X-RAYS

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Matter becomes highly excited on femtosecond timescales when exposed to highintensity pulses from an X-ray free electron laser (XFEL). The radiation induced dynamics is complex due to the interplay of various processes occurring at the same time. Theory plays a key role in understanding the impact of these processes on the sample evolution, which is necessary for a correct interpretation of experimental results and proposing new measurements.

In this talk, we present the latest results from our theoretical simulations of XFEL irradiated systems such as small molecules, clusters and bulk matter [14]. In particular, we focus on the aspects relevant to the applications of XFELs in biosciences: electronic damage, charge rearrangement and atomic displacements. We also present our dedicated approach for calculating Bragg scattering patterns based on the simulated time evolution of irradiated nanocrystals [5].

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Alan Kadek, HPI / EuXFEL

Hitting proteins with a sledgehammer—combining native mass spectrometry with an XFEL

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Native mass spectrometry (MS) enables the ionization and transfer of intact non-covalent protein complexes into the gas phase. As such, it is a perfect tool to study proteins and their assemblies in a mass and conformation specific manner. This enables MS to probe structural transitions which proteins and their complexes undergo, e.g. during a viral lifecycle. Such transient states are of high importance for structural biology, but most often cannot be purified and are inaccessible for crystallography.

Despite its remarkable sensitivity and selectivity, the structural resolution in native MS alone is limited. The amount of structural information could be vastly increased by its combination with powerful hard X-ray free electron lasers (XFELs) such as the already established LCLS in Stanford or the European XFEL, the world's most intense light source so far, which has just become operational in Hamburg. These instruments promise an opportunity to obtain high resolution structures of single particles. Reciprocally, native MS could solve some of the issues with delivering sample into the beam and could also add another dimension of possibilities by manipulating and selecting charged molecules in the gas phase prior to their imaging.

This contribution will highlight the benefits of native MS for single particle imaging of transient protein intermediates at XFELs. It will also describe our plans and ongoing work to bring native MS to European XFEL single particle beamline as well as present our initial feasibility studies on achievable ion fluxes.

Acknowledgement:
This work has been funded by the German Federal Ministry of Education and Research (BMBF Verbundprojekt 05K2016). The Heinrich-Pette-Institut, Leibniz Institute for Experimental Virology is supported by the Free and Hanseatic City of Hamburg and the German Federal Ministry of Health.

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Christopher Kupitz, UWM

Phytochromes: the red-light photoreceptor

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Phytochromes are red-light photoreceptors that control light responses in bacteria, plants and fungi. First discovered in plants, similar proteins were later found in bacteria and given the name bacteriophytochromes (BphPs). Upon light absorption phytochromes interconvert between two states, Pr and Pfr. This activates an effector domain that very often has histidine kinase activity. We work with an unusual BphP from the myxobacterium *Stigmatella aurantiaca* (SaBphP1). What is fascinating about this protein is that it lacks the commonly conserved histidine residue near the chromophore that is thought to be vital for the Pr to Pfr transition in classical BphPs. Instead, it is replaced by a threonine residue. However, still stable Pr to Pfr transitions can be observed.

In this poster we present multiple methods of crystallization, as well as the structures of various constructs of the SaBphP1. The structures were initially determined at cryo temperatures at the Advanced Photons Source (Argonne, IL). Serial femtosecond crystallographic experiments were performed, as part of a large collaboration, at ambient temperature at both the Spring-8 Angstrom Compact free electron LAser(Harima, Japan) and the Linac Coherent Light Source (Menlo Park, CA) (Woitowich, 2017, under review). The X-ray data in combination with absorption spectra on solution provide a basis for the detailed mechanism of the Pr to Pfr transition in phytochromes.

This work was supported by NSF-STC “BioXFEL” (STC-1231306). E.A.S. is supported by NSF RUI grant BIO-MCB 1413360 and NIH grant T34 GM105549-01 NU-STARS. The XFEL experiments were conducted with the approval of the Japan Synchrotron Radiation Research Institute (JASRI) (proposal number 2016B8005).

*** For the full authors list of this work please see the following reference.

Nicole C. Woitowich, Andrei S. Halavaty, Patricia Waltz, Christopher Kupitz, Joseph Varela, Gregory Tracy, Kevin D. Gallagher, Elin Claesson, Takanori Nakane, Suraj Pandey, Garrett Nelson, Rie Tanaka, Eriko Nango, Eiichi Mizohata, Shigeki Owada, Kensure Tono, Yasumasa Joti, Angela C. Nugent, Hardik Patel, Ayesha Mapara, James Hopkins, Phu Duong, Dorina Bizhga, Svetlana E. Kovaleva, Rachael St. Peter, Cynthia N. Hernandez, Wesley B. Ozarowski, Shatabdi Roy-Chowdhuri, Jay-How, Petra Edlund, Heikki Takala, Janne Ihalainen, Jennifer Scales, Tyler Norwood, Ishwor Poudyal, Petra Fromme, John Spence, Keith Moffat, Sebastian Westenhoff, Marius Schmidt, & Emina A. Stojković, (2017), Structural basis for light control of cell development revealed by crystal structures of a Myxobacterial phytochrome, (IUCrJ, submitted, under review)

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Dan Bi Lee, Korea University

Supersaturation-controlled microcrystallization and visualization analysis for serial femtosecond crystallography

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Time-resolved serial femtosecond crystallography with X-ray free electron laser (XFEL) holds the potential to view fast reactions occurring at near-physiological temperature. However, production and characterization of homogeneous micron-sized protein crystals at high density remain a bottleneck, due to the lack of the necessary equipments in ordinary laboratories. We describe here supersaturation-controlled microcrystallization and visualization and analysis tools that can be easily used in any laboratory. The microcrystallization conditions of the influenza virus hemagglutinin were initially obtained with low reproducibility, which was improved by employing a rapid evaporation of hanging drops. Supersaturation-controlled microcrystallization was then developed in a vapor diffusion mode, where supersaturation was induced by evaporation in hanging drops for durations ranging from 30 sec to 3 min, depending on the protein. It was applied successfully to the microcrystal formation of lysozyme, ferritin and hemagglutinin with high density. Moreover, visualization and analysis tools were developed to characterize the microcrystals observed by light microscopy. The size and density distributions of microcrystals analyzed by the tools were found to be consistent with the results of manual analysis, further validated by high-resolution microscopic analyses. Our supersaturation-controlled microcrystallization and visualization and analysis tools will provide universal access to successful XFEL studies.

This work was supported by grants from the National Research Foundation of Korea (NRF) funded by the Korean government (MSIP) (2016R1E1A1A01942558) and the BK21 Plus program through the NRF of the ME.

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Chufeng Li, Arizona State University

Development of a matching-based auto-indexing algorithm SPIND and its applicability to serial protein crystallography

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We present a matching-based auto-indexing algorithm SPIND for diffraction patterns using the relative positions of only 5 Bragg peaks per pattern. Prior knowledge of the lattice parameters is needed as input for this auto-indexing algorithm. Alternatively, lattice type and constants can be determined from the virtual powder diffraction pattern. SPIND was invented for indexing sparse, small unit cell serial crystallography data, and successfully tested on a simulated data set from "magic triangle" I3C crystals to evaluate the robustness and practicability of the algorithm. All 400 patterns were indexed with an accuracy of within 0.1 degrees in Euler angles at the computation time cost of milliseconds per pattern in a Matlab R2014b environment (2.7GHz Intel). Recent developments and a few new features, including random-gradient-search-based lattice refinement and centering rejection, were included in the SPIND suite. To evaluate the effectiveness, efficiency and general applicability of SPIND to serial protein crystallography data, it is essential to have testing statics on multiple data sets collected from real world experiments. Four GPCR datasets deposited in the Coherent X-ray Imaging Data Bank (CXIDB) were re-analyzed using the CrystFEL 0.6.2 (1), and these results served as benchmarks in our testing of SPIND. We compared SPIND indexing of serial femtosecond crystallography (SFX) data from a human δ-opioid receptor (DOR, CXIDB #40) (2) with auto-indexing programs, MOSFLM, DirAx, with regard to indexing rate, data statistics, and computation time based on the same parameters and scripts as used in the previous work(1). The indexing rate using SPIND (53.5% after lattice refinement) was significantly higher than other auto-indexers individually. SFX figures of merit including R_{split}, CC*, along with signal to noise ratio were improved in the whole resolution range using SPIND. However, there are ~8% patterns indexed by MOSFLM that were not indexed by SPIND after lattice refinement. This indicates the potential of incorporating more capabilities in SPIND algorithm, such as multiple hits indexing. SPIND, written in Python, with updated features that are designed for protein SFX data is now publicly available via https://github.com/LiuLab-CSRC/indexing.

This work is supported by the STC Program of the National Science Foundation through BioXFEL under Agreement No. 1231306, ABI Innovation: New Algorithms for Biological X-ray Free Electron Laser Data under NSF grant No. 1565180, and the National Natural Science Foundation of China (awarded to H. Liu) #11575021, U1530401, and U1430237.

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Jose Liboy, University of Puerto Rico

Notch regulation of cell migration and invastion in triple negative breast cancer

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Triple negative breast cancer (TNBC) is a heterogeneous disease comprising several subtypes. The common characteristic of this class of diseases is that they do not express the estrogen receptor (ER-), progesterone receptor (PgR-), and HER2. For the most part, no effective treatment exists for TNBC. Our lab and others have shown that oncogenic Notch signaling is present in considerable numbers of TNBC and contributes to the pathology of the disease. The purpose of this project is to study the effect of Notch signaling on regulation of migration and invasion in TNBC cells. I used a scratch assay to assess the migration of TNBC cells under various conditions. To establish a positive control, I first analyzed the migration of Notch-wildtype MDA-MB-231 cells treated with JQ1 and enterolactone as possible anti-migratory drugs. JQ1 inhibits Notch signaling by blocking the family of bromodomain and extraterminal (BET) proteins, and enterolactone prevents extracellular matrix (ECM) remodeling in TNBC. Then, I used a scratch assay to study the migration of Notch-mutated MB-157 cells and its closely related metastatic line MDA-MB-157 in a gamma secretase inhibitor (GSI) treated condition. GSI is an inhibitor of Notch signaling that blocks the cleavage of the receptor protein by inhibiting gamma secretase. While JQ1 did not inhibit the migration of MDA-MB-231 cells, enterolactone showed inhibitory activity in this cell line. Notch regulation of cell migration could not be studied in MB-157, because this cell line is not invasive. GSI did not inhibit the migration of MDA-MB-157 cells.

This project was funded by the MARC Program, the Institute for Biomedical Informatics, the Biomedical Graduate Studies Program, and the Office of Research and Diversity Training – Perelman School of Medicine at the University of Pennsylvania. Also, I want to acknowledge the BioXFEL program for granting me the BioXFEL Travel Scholarship to attend this conference.

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Haiguang Liu, Beijing Computational Science Research Center

Structure and dynamics of chloride ion pumping rhodopsin

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

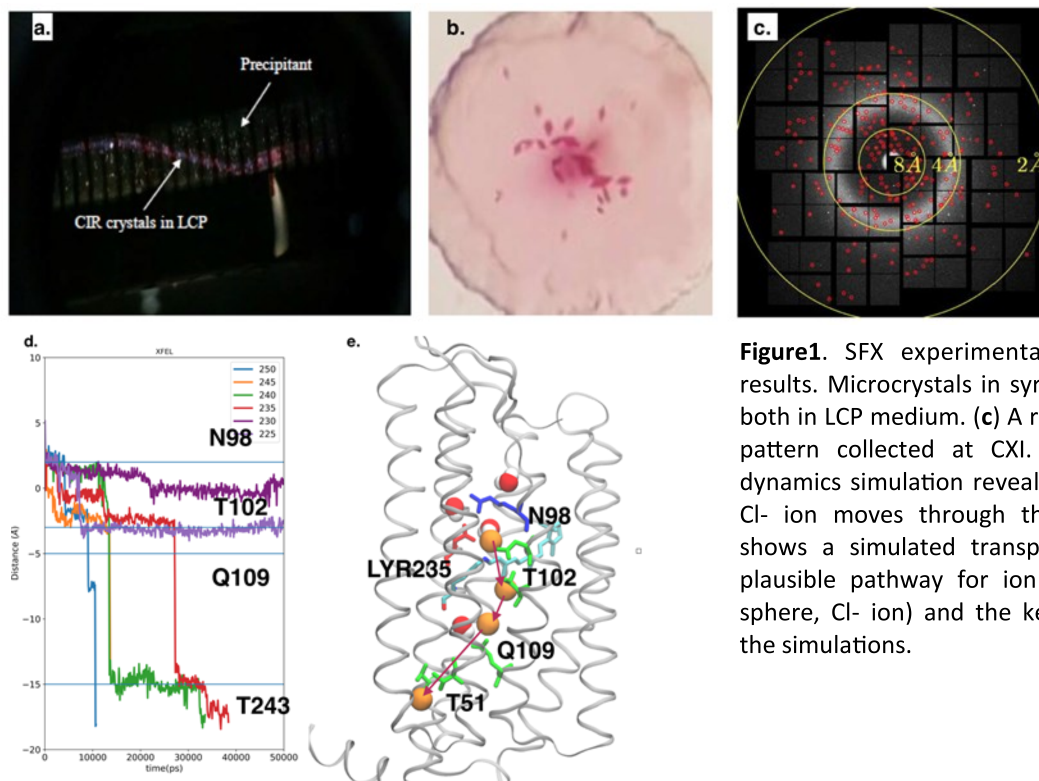

Figure1. SFX experimental data and simulation results. Microcrystals in syringe (**a**) and in plate (**b**) both in LCP medium. (**c**) A representative diffraction pattern collected at CXI. (**d**) Steered molecular dynamics simulation reveals intermediate states as Cl- ion moves through the channel,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

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

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Stewart Malave, University of Puerto Rico

Investigating the role of an atypical variant surface glycoprotein (aVSG) in developmental progression to infectivity in *Trypanosoma brucei*

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University of Puerto Rico at Mayaguez, Cabo Rojo, PR[1] and Yale School of Medicine, New Haven, CT[2]

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

Research reported here was supported by NIH grants AI028798 and AI110325 to C.T.

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BioXFEL

Darya Marchany-Rivera, University of Puerto Rico

Effect of pH on Fe-O_2 bond in the oxygen reactive hemoglobins of L. pectinata by X-ray crystallography

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The bivalve Lucina pectinata found in the coastal areas of Puerto Rico possess three haemoglobins from which two are oxygen reactive. Studies have shed some insight of how these two hemeproteins carry their function but raising some questions with it. Studies revealed that these proteins form dimers whether is a homodimer or a heterodimer. HbII has been extensively describe in the homodimer form and partially as a heterodimer with HbIII. The HbIII homodimer have proven to be a challenge to crystalographically describe. These hemeproteins characterize for a high association constant and a slow dissociation rate. We hypothesize that pH may trigger the oxygen release in these hemeproteins. Crystals were grown using the counter-diffusion technique and the pH variant of sodium formate or ammonium sulfate Triana© crystallization kits. Data sets were collected at the Stanford Synchrotron Radiation Source to a resolution range of approximately 2- 3Å.

Acknowledgement:

We acknowledge the SSRL staff for all their support and help, specially Dr. Aina Cohen, Dr. Clyde Smith, Dr. Ana Gonzalez and Dr. Sylvia Russi. This research was financially supported in part by the BioXFEL Educational Center at HWI and SSRL. We like to thank Dr. William Bauer for all his help. Use of the Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, is supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. DE-AC02-76SF00515. The SSRL Structural Molecular Biology Program is supported by the DOE Office of Biological and Environmental Research, and by the National Institute of Health, National Institute of General Medical Sciences (including P41GM103393). The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of NIGMS or NIH.

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Hugh Marman, La Trobe University

Radiation damage in protein microcrystallography

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Radiation damage in macromolecular crystallography (MX) experiments at third-generation synchrotrons presents a fundamental barrier to protein structure determination [1]. Accurate predictions of the dose (energy/mass) that a crystal will absorb during crystallographic data collection is a key component of planning an MX experiment. Current software packages have been shown to provide reasonably accurate dose predictions when using moderate to large (≥10μm) crystals and beam sizes (full-width-at-half-maximum (FWHM)) comparable to or larger than this. Until fairly recently this situation has been fairly typical of MX experiments.

Improvements in source and detector technology over the last decade, however, have permitted measurements to be made using micro-crystals (~1μm) and/or sub-micron focus beams. Under these conditions, the spatial distribution of energy arising from photoelectrons, which are the main cause of radiation damage at the energies typically used for MX, may lead to lower dose rates than predicted by currently-available tools [2] [3] [4] [5]. Here we describe work simulating the spatial distribution of energy deposited in a protein crystal based on Monte Carlo models of photoelectron trajectories and incorporating dynamic radiation damage effects to predict the rate of spot-fading under particular experimental parameters. Our results are compared to predictions obtained using the radiation damage simulator RADDOSE-3D [6] and experimental protein microcrystal diffraction data collected using third-generation microfocus synchrotron sources.

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Jose Martin Garcia, Arizona State University

Structure determination of inhibitor complexes with full length human taspase1: Towards rational drug design against a new cancer target

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Taspase1 (threonine aspartase 1) is an endopeptidase that is overexpressed in primary human cancers. It has been identified as a potentially potent anticancer drug target because loss of Taspase1 activity disrupts proliferation of human cancer cells in vitro and in mouse tumor xenograft models of glioblastoma. It functions as a non-oncogene addiction protease that coordinates cancer cell proliferation and apoptosis. Based on its promise as a novel anti-cancer therapeutic target, Taspase1 was accepted into the NCI Experimental Therapeutics (NExT) Chemical Biology Consortium (CBC) drug discovery and development pipeline. Several promising Taspase1 inhibitors have already been identified in high throughput screens. A high-resolution structure of the enzymatically active full-length Taspase1, and Taspase1 in complex with substrate and inhibitors would greatly enable optimization (through rational design) of pharmacologically potent Taspase1 inhibitors. Taspase1 is a highly conserved 50 kDa proenzyme. The biologically active form of Taspase1 is a heterotetramer that displays an overall αββα structure. Crystallographic structures of a truncated version of Taspase1 have been determined as apo enzyme and in complex with covalent inhibitors; however, this truncated form of the enzyme is catalytically inactive. The full-length protein contains an additional sequence that is anticipated to form a helical structure near the catalytic site. Until recently, no structure of active, full-length Taspase1 nor complexes of the catalytically active enzyme with inhibitors existed. Recently, first crystals of the full-length Taspase1 have been grown and data collected at the Advanced Photon Source (Chicago, IL). These crystals diffracted to ~ 3.5 Å but show a strong diffraction anisotropy. Taspase1 micro-crystals have been recently obtained and will be screened at LCLS during PCS beamtime P114 (run 16, December 15, 2017) for serial femtosecond crystallography (SFX) where we aim to determine the first high-resolution structure of the full length apo-protein and complexes with inhibitors of the enzyme. These structures should greatly benefit the structure-guided medicinal chemistry of Taspase1 inhibitors as anti-cancer therapeutics.

Acknowledgement:

This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Chemical Biology Consortium Contract No. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government

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Derek Mendez, Arizona State University

Probing the ultra-fast and fast structural transitions resulting from light-absorption in the bovine visual rhodopsin: A time-resolved solution scattering study

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Photo-activation of the bovine visual rhodopsin is explored using ultra-fast (fs-ps), fast (ps-ns) and intermediate (ns-μs) time-resolved small and wide angle solution scattering (TR-SWAXS). Using XFEL sources, we can probe the structural transitions that occur upon isomerization of the retinal which give rise to proposed meta-stable states involved in the visual process. Experimentally, rhodopsin is solubilized in CHAPS-detergent micelles, and injected into the path of the XFEL using 50 μm gas-focusing virtual nozzles. By examining the intensity difference between dark and optically-pumped solution snapshots we gain insights into the structural kinetics involved in photo-activation. Specifically, we observe a protein-quake in the fast time-regime, and our preliminary analysis has been dedicated to decoupling the rhodopsin contributions to this signal. To this end we employ molecular dynamics simulations of the isomerization (up to 50 ps), as well as singular value decomposition of our data. Conventional SWAXS analysis involves radially integrating the scattering patterns, losing useful signal that might arise in the azimuthal component of the snapshot solution scattering. We explore azimuthal asymmetries due to different fractions of the sample undergoing photo-activation, and consider how these signals couple to the underlying structural dynamics. Further, we discuss the novel technique correlated X-ray scattering (CXS), or fluctuation X-ray scattering (FXS), where angular correlations of scattered photons are accumulated across many snapshots, and we investigate the ability of CXS to extract high-resolution structural constraints from our TR-SWAXS data.

This work is supported by NSF awards *ABI Innovation: New Algorithms for Biological X-ray Free Electron Laser Data* (award number 1565180), and NSF-STC-1231306.

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BioXFEL

Derek Mendez, Arizona State University
Natasha Stander, Arizona State University

Determination of structural ensembles with data-guided molecular dynamics simulations

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X-ray crystallography remains the dominant method for solving atomic structures. However, for relatively large systems, the availability of only medium-to-low-resolution diffraction data often limits the determination of all-atom models. A new molecular dynamics flexible fitting (MDFF)-based approach, xMDFF, for determining ensembles of atomic structures from such low-resolution crystallographic data is reported. xMDFF employs a real-space refinement scheme that flexibly fits atomic models into an iteratively updated electron-density map. It addresses significant large-scale deformations of the search model (up to RMSD of 7 Å) to fit the low-resolution density, as demonstrated on the refinement of crystallographic data from membrane channels (Acta Cryst. D 70, 2344-2355; Nat. Struct. Mol. Biol. 21, 244), transporters (Structure 24, 2102-2114), and organic macrocycles (J. Amer. Chem. Soc. 137, 8810). Most recently, through the systematic refinement of a series of XFEL datasets from 5.0 to 3.5 Å resolution, xMDFF has delivered a structural ensemble representing the dark state of Photosystem II (PSII). Our ensemble-based analysis of the refined models provides an effective method for resolving data uncertainty with structural clustering - a practice generally applicable for interpreting low-resolution XFEL data. Showcased for PSII, this analysis is particularly beneficial for the refinement of flexible loops that often manifest poorly resolved regions. Finally, xMDFF provides a unique platform to integrate high-level quantum computations into crystallographic refinement, for example for the resolution of the Oxygen Evolving Complex within PSII.

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BioXFEL

Romain Arnal, University of Canterbury

Prospects for ab initio phasing and XFEL imaging of 1D and 2D crystals

Rick Millane and Romain Arnal

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Some biomolecular systems, such as fibrilar and membrane proteins, prefer to form 1D or 2D, rather than 3D, crystals. 1D crystals have traditionally been studied by x-ray diffraction by using specimens that contain many aligned crystals. However, recent work has shown the potential for measuring diffraction from single 1D crystals using XFEL sources [1,2]. Also, recent results have shown that XFEL sources can allow measurement of x-ray diffraction from single 2D crystal grains [3]. One advantage of using 1D or 2D crystals is that there is less sampling of the diffraction, improving the prospects for *ab initio* phasing.

In the case of a 1D crystal, if the molecular envelope deviates from a cylinder (of any cross-section) then the solution to the *ab initio* phase problem is theoretically unique [4]. If the ratio of the volume of the circumscribing cylinder to the volume of the molecular envelope exceeds about 1.2 to 1.5 then a unique solution is likely in practice.

For the case of a 2D crystal, the problem is not as well constrained as for the 1D crystal case, but sufficient molecular envelope information in this case can also render the solution unique. We have shown that if the "solvent content" (based on the volume between the envelope and a nominal unit cell) exceeds the normalised resolution (the resolution of the diffraction data divided by the maximum thickness of the 2D crystal), then there is a unique solution in theory [5]. This shows that if the ratio of the solvent content to the normalised resolution exceeds about 1.2 to 1.5 then a unique solution is likely in practice. We have conducted simulations of phase retrieval using the electron density of Aquaporin 1 [6], sampled 5A, and calculated 2D crystal diffraction amplitudes at 10A resolution as data. An envelope of 17A resolution is used as a constraint. Phase retrieval used an appropriate implementation of the difference map algorithm [7]. Reconstruction with noise-free data is possible in this case as shown below, and the results allow the prospects for *ab initio* phasing with actual XFEL data to be evaluated.

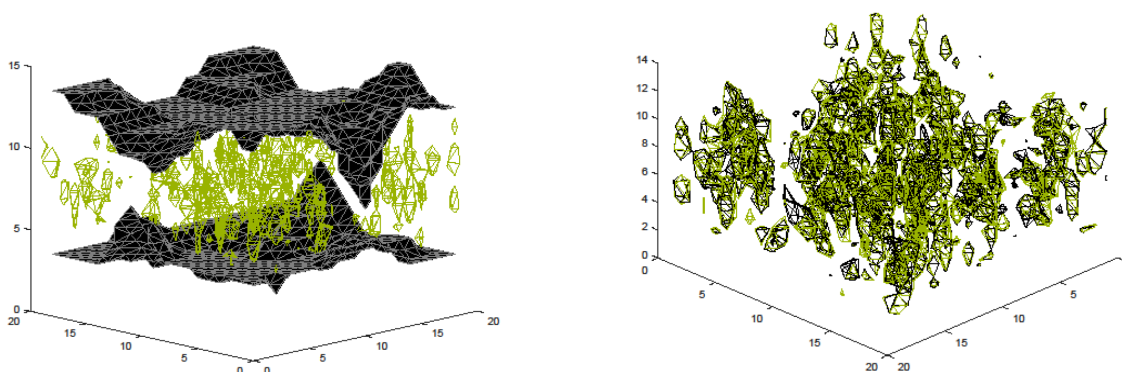

Figure 1: Left: AQP1 electron density (green) and 17A envelope (black). Right: True (black) and reconstructed (green) densities.

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BioXFEL

Mitchell Miller, Rice University

Progress in the time-resolved studies of the blue/green cyanobacterial phytochrome, PixJ

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Signaling proteins react to a stimulus in their receptor domain and change the conformation in a distant region. Phytochromes are bacterial “light switches” that can switch between two stable physical signaling states in response to light stimuli of particular wavelengths depending on the electrochemical nature of their chromophore. These proteins are interesting for their synthetic biology applications in addition to their physical and evolutionary biological properties. They are also of particular interest to the structural dynamics community because phytochromes are light triggered proteins, making them ideal to study in time resolved pump-probe experiments.

Here we report our progress studying PixJ, an interesting blue/green phytochrome from cyanobacteria. PixJ blue-shifts its spectra relative to other phytochrome family members by altering its chromophore via a second covalent bond from a cysteine sidechain. This bond is broken during the photoconversion from blue-absorbing to green-absorbing. We have studied this transition via pump-probe absorbance spectroscopy and have identified reaction intermediates.

We have also solved the crystal structure of PixJ in the blue-absorbing state to 1.2 Å. At this resolution, the chromophore is clearly visible and the chromophore binding pocket is well defined. As has been seem with other bilin chromophores similar to PixJ’s, the sample is very radiation sensitive at the site where the chromophore’s A ring binds to the protein. Previous XFEL structures have demonstrated that similar radiation damage can be “outrun” using the fs x-ray pulses characteristic of these sources.

Future studies utilizing both solutions and crystals of PixJ will help us to understand this interesting photoconversion reaction with both high spatial and temporal resolution.

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

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Fabiola Moreno, University of Puerto Rico

Sulfmyoglobin and hydrogen sulfide metmyoglobin formation in metmyoglobin crystals

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

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

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Nirupa Nagaratnam, Arizona State University

Serial femtosecond crystallography of *in vivo* grown crystals

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Even though protein crystals have been observed *in vivo* in various types of cells, their use in X-ray crystallography has been limited due to very small size. Advances in serial femtosecond crystallography (SFX) has overcome this limitation by using the extremely intense X-ray pulses from X-ray free-electron laser (XFEL) sources to image crystals within the cells. The main goal of our study is to screen a range of protein targets that are highly significant in disease states or major biological pathways for feasibility of *in vivo* crystallization and structure determination using SFX. The in vivo crystal structure determination pipeline consists of cloning of coding sequences for protein targets, recombinant baculovirus generation and amplification, baculovirus mediated protein expression in insect cells, protein yield analysis and optimization, crystal characterization, sample preparation for SFX experiments, SFX data collection / evaluation and structure determination. Of the many promising targets that exhibit *in vivo* crystallization, µNS (avian reovirus non-structural (NS) protein) crystals were further chosen for SFX experiments. Avian reoviruses are pathogenic viruses involved in several syndromes that may cause important economic losses in the poultry industry. The µNS protein encoded by avian reoviruses (ARVs) is an important scaffolding protein of viral factories which are globular inclusions found in the host cytoplasm. These structures aid in localization of viral components required for efficient replication of the virus. Thus, the molecular structure of µNS is vital towards understanding the mechanism by which ARVs replicate. SF9 insect cells harboring 5 – 15 µm sized µNS protein crystals were directly mixed with 12% agarose and subject to XFEL diffraction at LCLS (Linac Coherent Light Source). µNS crystals diffracted to 5 – 7 Å and SFX data analysis is currently in progress. While many other protein targets which showed successful *in vivo* crystallization need to be evaluated by SFX technique, currently, experiments are focused on improving the diffraction quality of *in vivo* grown crystals by exploring different types of insect cell expression systems, crystal extraction methods, SFX sample delivery media, etc. This will ultimately lead to the discovery of the 3-dimensinal structure of these important target proteins.

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Acknowledgements

This work is supported by the Flinn Foundation (No. 1991), the Center for Applied Structural Discovery at Biodesign Institute at Arizona State University, and the STC program at the National Science Foundation through BioXFEL (No. 1231306).

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Reza Nazari, Arizona State University

3D printed gas dynamic virtual nozzles to achieve fast liquid jets

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Gas Dynamic Virtual Nozzles (GDVN) produce microscopic flow-focused liquid jets and are widely used for sample delivery in serial femtosecond crystallography (SFX) and time-resolved solution scattering. Recently, 2-photon polymerization (2PP) made it possible to produce 3D-printed GDVN's with submicron printing resolution. Comparing with hand- fabricated nozzles, reproducibility and less developing effort and similarity of the performance of different 3D printed nozzles are among the advantages of using 3D printing techniques to develop GDVN's. Submicron printing resolution also makes it possible to easily improve GDVN performance by optimizing the design of nozzles. In this study, 3D printed nozzles were developed to achieve low liquid and gas flow rates, and high liquid jet velocities. A double-pulsed nanosecond laser imaging system was used to perform Particle Tracking Velocimetry (PTV) in order to determine jet velocities and assess jet stability/reproducibility. The testing results of pure water jets focused with He sheath gas showed that some designs can easily achieve stable liquid jets with velocities of more than 80 m/s, with pure water flowing at 3 microliters/min, and helium sheath gas flowing at less than 5 mg/min respectively. Our findings highlight the potential of making reproducible GDVN's with minimum fabrication effort, that can meet requirements of present and future SFX research.

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Prakash Nepal, University of Wisconsin-Milwaukee

Difference structures from time-resolved SAXS/WAXS in the presence of shot noise

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The problem of inverting time-resolved Small Angle X-ray Scattering (SAXS) / Wide Angle X-ray Scattering (WAXS) difference data is an important problem as it has been thought that there is not enough information to recover a solution. We have already reported a new idea by extending the concept of "difference Fourier method" which aimed to overcome the above limitation and demonstrated small time-resolved variations in the structure of Photoactive Yellow Protein by identifying the displaced residues.

We validated the approach with simulations in the absence of noise. The deduced difference electron density may be superimposed on a model of the dark structure in a particular orientation despite the fact that SAXS/WAXS comes from an ensemble of randomly oriented molecules. In order to study the effect of noise on the difference density, shot noise following Poisson distribution is introduced to experimentally realizable intensity. We show here that the effects of noise can be sufficiently suppressed by averaging over a sufficiently large ensemble which could possibly be obtained in experiments.

This material is based upon work supported by the National Science Foundation grant number MCB-1158138 and the STC Program of the National Science Foundation through BioXFEL under Agreement No. 1231306

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Jose Olmos, Rice University
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Capturing enzymatic intermediates "on-the-fly" via mix-and-inject serial crystallography

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

This work is available on bioRxiv, DOI: http://dx.doi.org/10.1101/202432

This research was carried out at the LCLS, a National User Facility operated by Stanford University on behalf of the U.S. Department of Energy, Office of Basic Energy Sciences. This work is supported by the NSF Graduate Research Fellowship Program under Grant No. R3E821 (to JLO) and the NSF STC BioXFEL Center Award No. 1231306.

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Sofia Perez, University of Puerto Rico

Elucidating the mechanism of action of titanium (IV) complexes for a new anticancer drug design strategy

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Titanium(IV) compounds show terrific promise as possible anticancer agents due to higher spectrum of activity, lesser toxicity and lack of cross resistance to platinum-based compounds, one of the major anticancer drugs in the market. This work focuses on studying the mechanism of action of two highly potent anticancer titanium(IV) complexes synthesized with a family of iron(III) chelators, whose binding sites mimic those of the protein serum transferrin. These complexes are known as TiHBED and Ti $(Deferasirox)_2^{2-}$. The mentioned strategy enables them to function by a two-prong method, allowing titanium(IV) to reach its intracellular targets triggering its cytotoxicity and lowering the iron(III) intracellular concentration. This suggests a mechanism of action partly based on the inhibition of iron dependent processes. Therefore, we worked with Jurkat cells to determine through electron paramagnetic resonance if the iron dependent enzyme ribonucleotide reductase I(RNR I) was being inhibited and to conduct a cell cycle assay using flow cytometry. We also measured the redox activity of the iron(III) complexes through cyclic voltammetry. The results showed that both complexes are inhibiting RNR I and causing an accumulation of cells in G1 phase. Also, both iron(III) complexes were redox inactive, but only TiHBED treated cells showed an increase in high spin iron(III) complexes. We conclude that a main characteristic of their mechanism of action is stopping DNA synthesis and that possibly the iron(III) complex being formed from $Ti(Deferasirox)_2^{2-}$ is not the bidentate form. These results bring us closer to elucidating their mechanism of action, which provides valuable insight that can help in the development of new viable anticancer drugs based on this strategy.

Acknowledgements: Research reported in this publication was supported by an Institutional Development Award (IDea) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475.

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Ishwor Poudyal, University of Wisconsin-Milwaukee

Simulation of diffraction patterns of single protein molecule with realistic beam parameters

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XFEL sources can be used to determine 3D structure of biomolecules and their complexes. Imaging of single particles at near atomic resolution is an effective way of obtaining structural information of large macromolecules that cannot crystallize, like membrane proteins. Crystal free 3D structure determination involves injection of many copies of biomolecules to serially diffract from intense X-ray pulses. Here we present simulations of diffraction patterns obtained from a single molecule of a full-length bacterial phytochrome. We calculate the coherent sum of scattering contributions from each atom, which yields the intensity in each Shannon pixel. For calculation of diffraction images, the important parameters are photon energy and fluence which were varied in our simulations. The intensity in each Shannon pixel was converted into a photon count by using Poisson noise generator.

By comparing the “noisy patterns” with noise free patterns we derive an R_{noise} similar to R_{merge} in crystallography. With this we estimated the quality of the “noisy” patterns as a function of fluence, wavelength and resolution, to assess how many patterns will be required to collect a full data set of sufficient quality at low (10 Å) and near atomic (2 Å) resolution.

*We acknowledge the support by NSF-STC “BIOXFEL” (STC-1231306).

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Sebastian Quintana, Arizona State University

Reducing sample amount for serial femtosecond crystallography using water-in-oil droplets

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Serial femtosecond crystallography (SFX) using an X-ray free electron laser (XFEL) has shown success for determining protein structures. One bottleneck of studying proteins using SFX with an XFEL is how resource intensive it is to make the large protein crystal suspension volumes required to obtain a full protein structure dataset. Furthermore, due to the XFEL pulsed mode, much of the protein crystal sample is wasted. For example, the XFEL at the Linac Coherent Light Source (LCLS) pulses at a maximum frequency of 120 Hz; and currently at the European XFEL (EuXFEL), kHz-bursts proceed in a 10 Hz train structure. Since there are no X-rays to irradiate crystals between pulses or bursts, any crystal sample injected during the X-ray duty cycle off-time is wasted. Using a 3D-printed microfluidic device, we have developed a water-in-oil droplet generation system that can inject aqueous droplets of protein crystal suspension at the frequency of the XFEL, while the oil carrier phase is delivered between the X-ray pulses. Thus, the amount of wasted protein crystal sample is reduced.

To generate aqueous droplets, we employ 3D-printing allowing rapid device design and testing. We designed a T-junction where two immiscible liquids, an aqueous crystal suspension and a fluorinated oil, are pressure-driven through the inlet capillaries and intersect at the T-junction. Based on the liquid flow rates and the interfacial forces, the aqueous phase can be sheared off into droplets by the oil phase. Furthermore, by adjusting the flow rate ratios, the droplet frequency and volume can be varied. Once generated, the droplets proceed to the outlet of a gas dynamic virtual nozzle (GDVN) and are then injected into the path of the X-ray beam.

To match the droplet generation to the XFEL duty cycle on-time, we generate droplets at 9 to 140 Hz. For the EuXFEL, our devices use 5 µL/min oil and 0.5 µL/min aqueous flow rates to generate 10 Hz droplets (about 0.7 nL/droplet). For SLAC, 12.5 µL/min oil and 7 µL/min aqueous flow rates yield 120 Hz droplets (about 1 nL/droplet). Compared to the typical sample consumption rates of 10 µL/min with GDVNs, our droplet method results in sample reductions of 30% and 95% at SLAC and EuXFEL, respectively. Furthermore, we tested this droplet generator most recently during the first user beam times at the EuXFEL with 3-deoxy-D-*manno*-octulosonate 8-phosphate synthase (KDO8PS) crystals. Droplet generation was monitored with an inline optical droplet detector, and diffraction data was collected. Preliminary data analysis has revealed characteristic diffraction of both the oil and aqueous phases, suggesting droplets were successfully injected into the XFEL. Data analysis for the obtained KDO8PS diffraction patterns is still ongoing. Future designs aim to incorporate an electrically induced droplet-on-demand system for improved synchronization at an XFEL.

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Alina Roman-Hubers, University of Puerto Rico

Study of the stability of sulfhemoglobin and sulfmyoglobin

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Easily detected by its characteristic repelling odor hydrogen sulfide (H_2S) has been historically known for its toxicity. Recent studies, now recognize H_2S as a distinguished biological molecule involved in many physiological processes. H_2S is synthesized in human and mammalian tissue and has the ability to modulate biological and physiological processes. In the presence of heme proteins, such as hemoglobin (Hb) and myoglobin (Mb); H_2S has the capacity of binding and heme modification resulting in the heme-derivatives sulfhemoglobin and sulfmyoglobin. H_2S role in human physiology and pathology is impeded by the lack of known specific biomarkers; which is fundamental for identifying physiological and pathophysiological processes regulated by H_2S. The presence of sulfhemoglobin (sulfHb) and sulfmyoglobin (sulfMb), could be an ideal biomarker of physiological H_2S. The formation of these sulfheme derivatives and their life cycle by the physiological turnover of red blood cells is considered a pathway of H_2S degradation in human tissue. This work evaluates and compares the physcial structure of the heme proteins and their stability as sulfheme complexes over a period of time by UV-Vis spectrophotometry. The heme proteins in the presence of hydrogen peroxide (H_2O_2) and H_2S wer monitored for 243 hours at a one to one ratio concentration to measure sulfHb and sulfMb stability by analyzing the visible fluctuations at spectrum spectrophotometry. The spectrum were analyzed to compare the quartenary stucture and affinity characteristics of Hb versus Mb single structure, and their ability and stability in binding with H_2S.

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Keishla Sanchez, University of Puerto Rico

Photolytic studies of the carbon monoxide complex with deoxy myoglobin

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Carbon monoxide binding by myoglobin has been studied as an intermediary model for other carboxy-myoglobin complexes. Met-myoglobin was treated with DTT to create deoxy-myoglobin which forms the carboxy complex. A myoglobin concentration of 5.0μM was used to obtain an absorbance of 1.5. In-vitro production of carbon monoxide was carried out using formic acid, mesyl chloride and trimethylamine (Morgan reaction). The instant CO reaction was generated in an anaerobic chamber with two compartments. The reaction between the CO reagents and the deoxy-myoglobin produced a rapid and great yield of carboxy-myoglobin. Results of UV-Vis spectrum showed bands at 423nm and 540nm, which indicate the presence of the carboxy complex. The next step of the project includes using a pump-probe flash photolysis to break the bond between myoglobin and carbon monoxide in order to study the kinetics of the reaction between myoglobin, hydrogen sulfide, and oxygen.

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Marcos Sanchez-Navarro, University of Puerto Rico

Avoidance over-conditioning impairs extinction of fear, induces persistent avoidance, and increases use of safety cues: implications for OCD

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Obsessive-compulsive disorder (OCD) is characterized by compulsive urges that can resemble avoidance of perceived danger. It is treated with exposure-with-response-prevention (ERP) therapy, in which patients are prevented from carrying out compulsions in response to triggers. Due to their repetitive nature, OCD compulsions are thought to resemble habit formation (Gillan et al., 2015), but little is known about the effects of repetitive avoidance on subsequent extinction. Using a platform-mediated avoidance task (Bravo-Rivera et al., 2014), we recently reported that a minority of rats persist in their avoidance following several days of extinction with a barrier that prevents access to the platform (extinction with response prevention, Ext-RP) (Rodríguez-Romaguera et al., 2016). A possible factor contributing to persistent avoidance after extinction is the development of habits over extended periods of training. Therefore, we trained two groups of rats with either 8 days (8d) or 20 days (20d) of avoidance conditioning, followed by 4 days of Ext-RP and a subsequent test with the barrier removed. Both groups showed similar avoidance conditioning. During Ext-RP, however, the 20d group showed impaired extinction of freezing (RM-ANOVA; $F(1, 28) = 57.37$, $p<0.001$), ending with elevated freezing levels. The following day, 20d rats showed increased avoidance at test compared to the 8d group (t-test; $t_{76} = 4.03$, $p<0.001$), but did not display differences in freezing. Thus, the ability to re-access the platform eliminated the excessive fear in this group. To assess whether 20d group interpreted the barrier as a safety signal, an additional test session was run with the barrier placed opposite to the platform. Under these conditions, avoidance was reduced in the 20d group (t-test; $t_{29} = 4.05$, $p<0.001$), suggesting the barrier signaled safety to these rats. We are currently using cFos to compare activity in prefrontal-striatal-amygdala circuits in the 8d and 20d groups. Our results suggest that repeated expression of avoidance-like compulsions may reduce the effectiveness of extinction-based therapies, and increase subjects' reliance on apparent safety cues.

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Lysmarie Santos-Velazquez, University of Puerto Rico

Fulfhemoglobin and its role as an endogenous hydrogen sulfide biomarker

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Hydrogen Sulfide (H_2S) had been conventionally considered as a toxic molecule until it was suggested for physiological function. H_2S is produced endogenously at low concentration by enzymatic pathways in humans, via cystathionine β-synthase (CBS), cystathionine ɣ-lyase (CSE), and 3-mercaptopyruvate sulfur transferase (MPST). H_2S is highly lipophilic, it can penetrate a cell by simple diffusion and interacts with some hemoproteins such as hemoglobin (Hb) in the presence of oxygen or hydrogen peroxide. This interaction generates a sulfhemoglobin complex as a result of a covalent heme modification in one of the pyrrole rings. The suggested H_2S concentrations that accomplishes physiological effects are at wide range between 10 and 300 uM. Clinical studies have emphasized the potential of modulating H_2S synthesis for therapeutic use, but the results of the investigations are limited due to the lack of reliable H_2S measurements in the body fluids and tissues, and a lack of known specific biomarkers. Sulfhemoglobin has not been evaluated by the scientific community as a biomarker of endogenous H_2S, even though it has been suggested. This research is directed to evaluate whether sulfhemoglobin can function as a biomarker of endogenous H_2S. The study was carried out evaluating the formation reaction of sulfhemoglobin at physiological conditions using different ratios. The sulfhemoglobin complex sample was prepared anaerobically by mixing the protein (Hb), with H_2O_2 and H_2S in a 6Q UV-Vis cuvette. The UV-Vis spectroscopy was used to monitor the sulfhemoglobin complex. The sample characteristic bands are: 420 nm, 626 nm and 720nm. The results showed a positive correlation between the sulfhemoglobin absorbance at 626nm and 720nm as a function of H_2S concentration. These in vitro results suggest that the species of sulfhemoglobin could be a potential biomarker of H_2S at physiological concentrations.

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Construction and characterization of a Cytochrome C-Ti-Transferrin conjugate for inducing cancer cell death

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Cancer is a disease that affects millions of people in the world. One of the greatest disadvantages of many oncological drugs is their "off target" effects. One way to minimize this unwanted effect is through targeted delivery of the drug to cancer cells. To stimulate cancer cell death using an apoptosis inducing protein, cytochrome c (Cyt c), we have designed a conjugate of Cyt c using a transporter of iron called transferrin (Tf). In human blood iron binds to Tf and this iron bound Tf is later taken up into the cells via transferrin receptor (Tfr). Cancer cells over expresses many receptors like Tfr which help them fulfill their increased demand of iron due to their rapid growth.

The Tf-Cyt c conjugate will allow the specific uptake of Cyt c into cancer cells expressing Tfr. To construct the conjugate, Cyt c and the titanium bound Tf were linked by SPDP cross-linker via a disulfide bond. The advantage of using the titanium bound Tf in comparison to iron-bound Tf is that it would transport the protein drug without feeding cancer cells with the iron. The resulting conjugate was purified on a Superdex 200 gel filtration column. The mass of the purified conjugate was characterized by SDS PAGE and MALDI. Two bands were observed in the SDS PAGE corresponding to mass of Cyt C (12,384 kDa) and Tf (80,000 kDa). Our MS results suggest that the of purified conjugate had two species of 92331.4688 Da (Tf + Cyt C) and 105236.3047 Da (Tf + 2 Cyt C). Chemical cross linkers could often change the structure of the proteins, resulting in loss of activity, hence we used the CD to probe any change in the secondary and tertiary structure of Tf and Cyt c. Our CD spectra shows that the structure of Cyt c and Tf remains largely unperturbed. The effectiveness of this conjugate in killing lung cancer cells would be tested in A549 cancer cells that overexpresses Tfr. In addition, normal lung cells (MRC5) would be used as a control to check the toxicity of this conjugate on normal cells.

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Gebhard Schertler, ETH Zurich-PSI

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

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

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Nature 530 (7589), 237-241 (2016)

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BioXFEL

Robin Schubert, University of Hamburg

Analyzing protein liquid dense clusters - intermediates in the nucleation process - as potential samples for future XFEL experiments

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At modern micro-beam synchrotron (SR) and Free-Electron-Laser (FEL) beamlines micro- sized crystals are preferred and mostly mandatory for diffraction data collection. Therefore, new, advanced and reliable methods to prepare and score 3D micro- and nano-crystal suspensions, most suitable for X-ray diffraction experiments need to be established in time. A better understanding of the nucleation process is of fundamental importance to grow crystals of desired size. Lately it was proposed that during crystallization biomolecules pass through a nucleation intermediate. However, till now the nucleation process is discussed in theory and experiment differently and no clear and unambiguous information is available. In order to obtain more insights about the process and to obtain supporting evidence for the tow-step nucleation mechanism and theory we investigated the nucleation process and early crystallization events for various proteins, applying complementary biophysical methods [1,2].

In particular we applied in situ dynamic light scattering (DLS), small-angle X-ray scattering (SAXS) and transmission electron microscopy (TEM) techniques. The obtained data strongly support the existence of a two-step mechanism of nucleation. The process is governed by the formation of liquid dense clusters as first step, followed by the transition to higher order assemblies. These liquid dense clusters can be used to combine single particle imaging experiments and serial femtosecond crystallography experiments at XFEL radiation sources in future.

After crystal nuclei have formed they continue to grow in size. The desired size for SFX experiments is preferably in the upper nanometer or lower micrometer regime. This guides to a strong demand to develop and establish new methods to analyze, score and optimize protein nano- and micro crystal suspensions for serial crystallography. To support and facilitate this demand a new microscope based setup was designed and constructed, based on detecting second harmonic generation (SHG) signals of the particles in sample suspensions. This method has the advantage that it allows to distinguish reliably between amorphous and crystalline particles. The setup and instrument enhances the already available signal sensitivity to such extend that detection of relative small crystals and crystals with higher symmetry, known to produce rather weak signals, is now possible. Further, the instrument is equipped with additional channels, which are capable to detect the third harmonic generation signal (THG) as well, and three-photon excited UV-fluorescence all in parallel, to provide complementary information about the crystalline sample suspension. Details and experimental data will be presented, as well as the potential of liquid dense clusters to serve as a bridge between single particle imaging and serial femtosecond crystallography experiments.

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[2] Schubert *et al.,* Crystal Growth and Design, Issue 17 (3), 954–958, (2017)

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Ghoncheh Mashayeki, University of Wisconsin-Milwaukee

Conformational landscape of a virus from single-particle X-ray scattering

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Using a manifold-based analysis of experimental diffraction snapshots from an X-ray free electron laser, we determine the three-dimensional structure and conformational landscape of the PR772 virus to a detector-limited resolution of 9 nm. Our results indicate that a single conformational coordinate controls reorganization of the genome, growth of a tubular structure from a portal vertex and release of the genome. These results demonstrate that single-particle X-ray scattering has the potential to shed light on key biological processes.

Grant Acknowledgements: The research was supported by the US Department of Energy, Office of Science, Basic Energy Sciences under award DE-SC0002164, and by the US National Science Foundation under awards STC 1231306 and 1551489.

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Chenghua Shao, RCSB Protein Data Bank-Rutgers

XFEL structure deposition and data architecture support in PDB

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

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

This work is supported by NSF, NIH, and DOE (DBI-1338415).

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Megan Shelby, Lawrence Livermore National Laboratory

First results from LCLS X-ray diffraction studies of fiber-like crystalline ApoA1 nanodiscs

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XFELs unique capabilities are utilized to characterize interactions between lipids and apolipoproteins with an eventual goal to study membrane protein dynamics. We present first results from X-ray diffraction studies performed at the LCLS on microcrystals of nanolipoprotein (NLP) particles, or “nanodiscs”. Cell-free expression of apolipoprotein A1 (ApoA1) in the presence of lipids leads to the formation of discs consisting of a 5 nm thick lipid bi-layer surrounded by a 10-20 nm diameter “belt” of apolipoprotein. These represent both a stable intermediate state in LDL formation and a membrane-model system into which membrane proteins can be inserted and thus solubilized.

Conditions for crystallization were screened first by a high-throughput survey of lead conditions at the Hauptman Woodward Institute followed by validation and optimization at LLNL. A condition producing a moderately high number density of rod-like crystals ~50 - 100 μm in length was repeated to harvest crystals for measurements at the LCLS. Crystals of ApoA1 NLPs were loaded in a humidified chamber onto patterned Si chips containing ~40,000 8 μm diameter pores. SFX experiments took place at the MFX instrument at LCLS using a 3 μm FWHM X-ray beam and the CSPAD detector. Implementation of the Roadrunner fast scanning fixed target system developed by CFEL allowed for 60 Hz repetition rate data collection, generating ~1200 diffraction images at a ~3% hit rate from a single chip. Bragg peaks at low resolution are apparent within the fiber-like diffraction of the ApoA1 hits. The average layer-line distances along the meridian correspond to the known height of the A1 disc as characterized by electron microscopy, dynamic light scattering, SAXS and SANS, about 60 Å, while the distances between Bragg peaks within layer lines correspond to the disc width, about 100 Å. This is consistent with a model in which NLPs “stack” in solution via contacts between lipid bilayers, as has been previously documented by TEM. Diffuse arcs centred around the axis perpendicular to the meridian are observed at 1/d of ~4.5 Å that are likely due to the partial ordering of lipids within the discs and will be the subject of further analysis. This study represents critical initial steps in both obtaining a structural picture of ApoA1 NLPs and developing this system as a scaffold for future structural characterization of inserted membrane proteins and was enabled by recent advances in fixed stages.

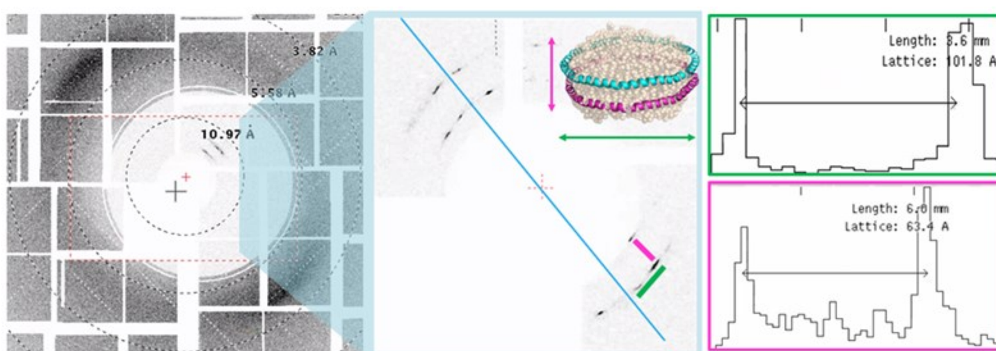

Figure: Fiber-like diffraction features are recorded to 11 Å and reflect known disc geometry.

This work was performed, in part, under the auspices of the U.S. DOE by LLNL under Contract DE-AC52-07NA27344. This work was supported by NIH grants R01GM117342 (NIGMS) and R21AI120925 (NIAID). Use of the LCLS, SLAC National Accelerator Lab, is supported by the U.S. DOE, Office of Science, under contract no. DE-AC02-76SF00515.

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Andrew Shevchuk, Arizona State University

Modeling of incoherent diffractive imaging from a virus using photoelectrons

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With one particle per shot, the limited amount of elastic scattering, low hit rate, particle inhomogeneities, and errors arising during particle orientation determination and phasing during data merging currently limit resolution in the single-particle (SP) mode. In this project, we consider the use of the much stronger photoelectron emission signal for image reconstruction.

This emission, and also inner-shell X-ray fluorescence, is normally treated as incoherent and isotropic, with no interference possible between wavefields emitted by different atoms. Classen et al. (2017), however, have proposed a method ("incoherent diffractive imaging" or IDI) in which they point out that for an incident pulse which is briefer than the lifetime of an excitation, interference will occur between the X-ray fluorescence emitted by different atoms. By analogy with the Hanbury-Brown and Twiss intensity interferometer, the cross-correlation function between intensities emitted within the coherence time then contains phase information. They propose detecting inner-shell X-ray fluorescence over a wide range of angles (ideally using a spherical detector), from which an angular cross-correlation function can be formed and inverted (despite a random phase shift between different internal sources) to give the spatial distribution of emitting atoms. For a 2D object the theory resembles the van-Cittert Zernike theorem. With sub-fs pulses becoming a reality at XFELs, the method, which would in principle provide a three-dimensional reconstruction of the selected atomic species from a single shot, may soon be practical. Challenges include the achievement of adequate signal-to-noise, the limitation of there being only one emitted X-ray per atom per shot, the resolution-limiting long wavelengths of light element inner-shell emission and the problem of "after-glow" due to secondary processes continuing the emission beyond the excitation lifetime.

The afterglow problem may be addresses by detecting the photoelectrons rather than the inner-shell fluorescence, since ejection of photoelectrons is a direct process, with lifetime given by the reciprocal of the incident beam energy spread, the reciprocal of the bandwidth for the band from which the electron was ejected, or the additional energy losses due to inelastic scattering, as the photoelectron traverses the sample. Experimentally, this approach would require an angle-resolved energy-filtering photoelectron detector, in which time-of-flight analysis can enhance the interference effect by spectral filtering. Here we show simulations for these non-isotropic emission patterns and their inversion for two cases. Figure 1 shows the pattern from just two atoms and its inversion, and Figure 2 shows emission from the vertices of a single icosahedral virus.

Classen, A., Ayyer, K., Chapman, H. N., Röhlsberger, R., von Zanthier, J., “Incoherent Diffraction Imaging via Intensity Correlations of hard X-rays”, Phys. Rev. Lett. 119, 053401 (2017)

This work is supported by BioXFEL award 1231306.

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Tim Stachowski, HWI-RPCI-UB

X-ray radiation induced transforming growth fact β1 (TGFβ1)

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Understanding the basis of cellular response to radiation is a major goal in the search for effective cancer treatments. Research over the past several years has generated mounting evidence that extracellular signaling proteins orchestrate complicated behaviors between cells that collectively direct the future of tissues, representing a promising new class of targets for biomodulation. Transforming growth factor beta 1 (TGFβ1) is of particular interest because in response to radiation damage it initiates downstream signaling pathways that control a number of cancer related processes such as proliferation, migration, and invasion. Normally, the 25 kDa dimer of TGFB1 is secreted with the 55 kDa dimer, Latency associated peptide (LAP), that renders TGFβ1 inactive, and together are known as Latent-TGFβ1 (LTGFβ1). Dissociation from this arrangement allows the now "activated" TGFβ1 to bind cognate receptors that initiate signaling pathways and ultimately alter gene expression. X-ray radiation induced TGFβ1 dissociation from LAP was first observed in the immuno-histochemical staining of irradiated mammary gland cells and was later shown to also be activated through reactive oxygen species (ROS) (Barcellos-Hoff *et al.*, 1996). Additionally, mutagenesis work showed that two cysteines in LAP are necessary for proper LTGFB-1 complex formation (Brunner *et al.*, 1989). As our lab and others have shown that disulfide bonds are the protein elements most sensitive to radiation damage, we aim to show through small-angle x-ray scattering (SAXS), x-ray crystallography, and complementary structural techniques that direct disulfide bond cleavage in LAP by x-ray radiation leads to TGFβ1 activation (Sutton *et al*., 2011). This project aims to not only determine and characterize a novel TGFβ1 activation mechanism that is relevant for radiation biology, but also to develop a platform for studying large scale conformational changes in proteins.

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Natasha Stander, Arizona State University

A new tool for efficient pattern selection from large SFX datasets

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XFEL datasets collected using a fixed target chip[1] have higher hit rates than datasets collected from a liquid jet. This makes much more efficient use of protein sample and XFEL beamtime and results in much larger datasets, including over 500,000 indexed diffraction patterns from microcrystals of photosystem II in the dark adapted state, collected at MFX, LCLS in November 2016. The output files from autoindexing with CrystFEL ("stream files") for this dataset are ~350 GB in total, making it computationally expensive to run analysis on the entire dataset at once. We present new pattern selection software for efficiently and intuitively selecting data subsets for merging and phasing. Any parameters in the stream files can be used to select data subsets, then write a new "stream file" for further analysis. Working with subsets of large datasets is not only faster, but also allows comparing experimental conditions, exploring correlations in the data (e.g. diffracting resolution version orientation on the chip), or selecting various criteria in order to optimize the quality of merged SFX datasets.

The software is demonstrated on the November 2016 photosystem II dataset comparing unit cell tolerance (i.e. distribution of unit cells determined by autoindexers that are accepted as solutions) and per-pattern diffraction resolution limit (calculated by CrystFEL). A comparison of per-pattern resolution versus unit cell size showed better merged-dataset diffraction resolution limits (based on $CC_{1/2}$ values) for patterns with unit cell *a* axis close to 133 Å. Three subsets with around 20,000 patterns each were created, with the same resolution cut off according to $CC_{1/2}$. The first had a very narrow unit cell distribution with the *a* axis between 132.8 and 133.2 Å. The second had only patterns with diffraction resolution limit better than 4.1 Å. The third was a combination with unit cell *a* axis between 132.5 Å and 133.6 Å and diffraction resolution limit better than 4.8 Å. Initial analysis of these subsets shows that the per-pattern diffraction resolution plays a bigger role than unit cell anisomorphism on the final R_{Free} for photosystem II microcrystals.

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Ganesh Subramanian, Arizona State University

Structural analysis of photoactive biomolecules using X-ray absorption spectroscopy: experiments and simulations

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Macrocycle complexes (such as porphyrins and corrins) are known to be photosensitive. This sensitivity is not just visible through their activity in the natural biological paradigm but also in novel synthetic applications spanning across disciplines. [1, 2] Ability to understand the structure-function relationship of such molecules, however, requires an ability to measure and interpret both the electronic and geometric structure simultaneously, when in action.

Here we present results from measurements of the X-ray absorption spectra [3] on Cobalt based porphyrins and corrins. The experiments were carried out at the Advanced Photon Source (11-ID-D) where both ground-state and time-resolved measurements (using an optical laser pump) were performed. DFT calculations were performed using Gaussian09 and ORCA programs in combination with the FEFF8.4 code to interpret the XAS fine-structure. These computational studies were performed using allocations from the Arizona State University Advanced Computing Center (A2C2).

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Acknowledgements:

This work was supported by the STC Program of the National Science Foundation through BioXFEL under Agreement No. 1231306.

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Shuo Sui, University of Massachusetts Amherst

Graphene-integrated microfluidics for advanced crystallography

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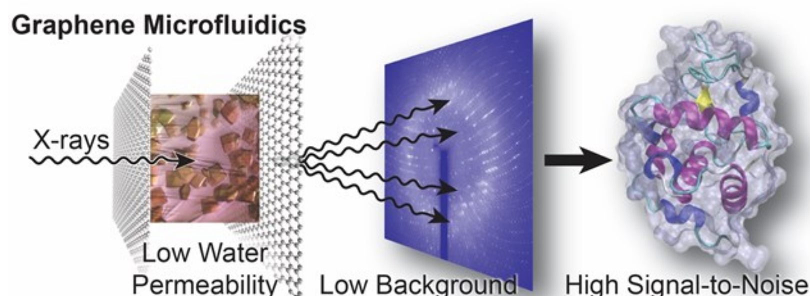
X-ray crystallography has long been the main workhorse technique for the structure determiantion of proteins and other biomacromolecules. The increasing briliance of X-ray sources has enabled the use of ever smaller and more weakly diffracting crystals and has accelerated the quality and rate of structure determination. However, this push towards high-throughput crystallography is limited by traditional manual harvesting and manipulation of crystals. Microfluidic strategies have been used to enable the growth and subsequent *in situ* crystallographic analysis of large numbers of crystals, avoid the challenge of harvesting micro-crystals, and have the potential to facilitate the structural characterization of protein targets that have been resistant to single-crystal strategies. We have developed a robust strategy for the incorporation of single-layer graphene as an ultra-thin window material and vapor-diffusion barrier into microfluidic devices. This architecture allows for a total material thickness of less than 1 µm, facilitating on-chip X-ray diffraction analysis while creating a sample environment that is stable against significant water loss over several weeks. These devices enable the collection of high quality, room-temperature diffraction data with excellent signal-to-noise. Furthermore, these devices have significant potential to enable continuous diffraction/diffuse scattering experiments, as well as the analysis of oxygen-sensitive targets because of the low background and barrier properties of the graphene layers. We are also exploring the possibility of utilizing the conductivity of graphene as an X-ray compatible integrated electrode for the application of an electric field for voltage-jump triggering of protein structural dynamics. Although this work is focused on the use of graphene for protein crystallography, we anticipate that this technology should find utility in a wide range of both X-ray and other lab-on-a-chip applications.



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Miklos Tegze, Wigner Research Centre for Physics

Orienting diffraction patterns in single particle imaging experiments

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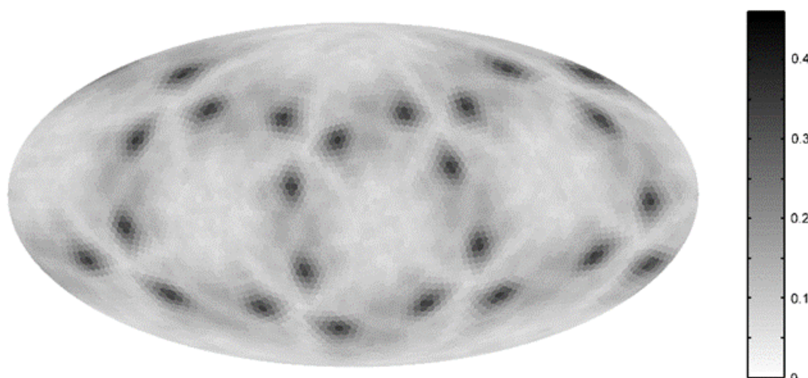
In a single particle imaging experiment, identical particles are injected into the XFEL beam with random orientations. Diffraction patterns can be recorded in a 2D detector before the particle is destroyed by radiation damage. Individual diffraction patterns of small particles or molecules are noisy and contain insufficient information to solve the structure of the particle. Therefore, to assemble a consistent 3D set of diffraction data, thousands of diffraction patterns must be recorded. The crucial step is to find the relative orientations of the individual patterns. Orientation methods fall into two categories: common line methods [1,2] using the information in the intersection of the patterns, and iterative methods [3-5] using all the patterns at the same time to find a consistent 3D data set.

We have developed a simple and very efficient iterative method based on correlation maximization (CM) for orienting the diffraction patterns of single particle imaging experiments [5]. Here we show that the CM method can find the correct orientations of noisy diffraction patterns (each containing only few thousands of scattered photons) of biological molecules and assemble them to a 3D data set. The CM algorithm is also able to select identical objects from a mixture [6] and find the symmetry of symmetrical objects. We introduced correlation maps (**Figure**) and a figure of merit as useful tools to test the progress and convergence of the orientation algorithm [7]. They were developed in the context of the CM method [5], but can be used to validate the results of any other orientation algorithm as well. Using the CM method and phasing algorithms, we show on simulated diffraction images that the structure of large biological molecules can be recovered with atomic resolution.

This work was funded by grant K115504 of the National Research Development and Innovation Office – NKFIH.

Figure – Correlation map for ferritin after convergence, showing 24 peaks according to the symmetry of the molecule



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Michael Thompson, University of California-San Francisco

Generalizing time-resolved crystallography and SAXS/WAXS with infrared laser-induced temperature-jump

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Time-resolved X-ray scattering and diffraction are among the most information-rich experimental techniques in structural biology. To date, systems that have been most rigorously 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 utilize 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, most of which are not subject to direct photoactivation. Towards this goal of generalizing time resolved X-ray techniques for structural biology, we are pioneering the use of infrared laser-induced temperature-jump (T-jump) in these dynamic structural experiments. In contrast to photochemical excitation, which is highly specific to a particular chromophore, temperature perturbation is a universal method for stimulating conformational dynamics of biomolecules, whose intrinsic motions are thermally -driven. We will present the results of both T-jump crystallography and SAXS/WAXS experiments, performed at X-ray free electron laser and synchrotron light sources respectively. Our results demonstrate the successful application of T-jump methods to high-resolution studies of protein conformational dynamics, and confirm that because laser T-jump methods exploit the photochemistry of the solvent, and not the protein molecules, they are universally applicable as a tool for studying protein dynamics.

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Jennifer Vargas-Santiago, University of Puerto Rico

Hydrogen sulfide (H_2S) trapment by hemoglobin I from Lucina pectinata encapsulated in sol-gels

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

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Max Wiedorn, CFEL

MHz serial crystallography at the European XFEL

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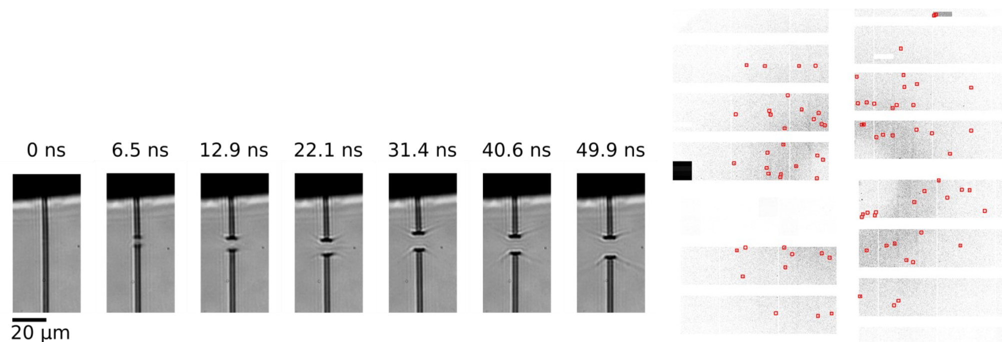
Serial crystallography has become a standard technique for the structure determination of proteins[1] and many structures have been deposited into the PDB using this technique. At x-ray free-electron laser (FEL) sources, data recorded from many thousand diffraction patterns is used to estimate the structure factors of the sample molecule. The advent of high repetition-rate x-ray FELs makes it possible to collect such a data set in a few seconds, and thereby, allowing rapid screening of enzymatic dynamics or the of ligand binding to proteins of medical relevance. While new detectors like the AGIPD or the Jungfrau allow collecting data at the rate of the FEL, sample delivery needs to be adapted to those rates.

Methods commonly used for sample delivery for serial diffraction experiments at x-ray FELs are fixed targets, viscous jets, liquid jets and aerosol streams[2]. While the former two cannot easily be accelerated to supply fresh crystalline sample at MHz rates, the latter suffers from efficiency deficits as the hit fraction of x-ray pulses is very low. A promising approach to deliver samples for MHz serial crystallography experiments is the use of fast liquid jets.

We have designed and developed a nozzle that can deliver liquid jets with velocities of up to 80 m/s and demonstrated their feasibility as a sample delivery method at the FLASH FEL facility in Hamburg. Further, we have conducted the first serial crystallography experiment using MHz x-ray pulse repetition rates at the SPX/SFX instrument at the European XFEL facility in Hamburg.



Left: A water jet exposed to x-ray pulses at the FLASH facility in Hamburg. Right: A diffraction pattern collected during the first user experiment (XFEL2012) at the European XFEL. The red boxes indicate peaks identified by Cheetah.

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David Wojtas, University of Canterbury

Orientation and analysis of XFEL serial diffraction patterns from fibrous molecular assemblies

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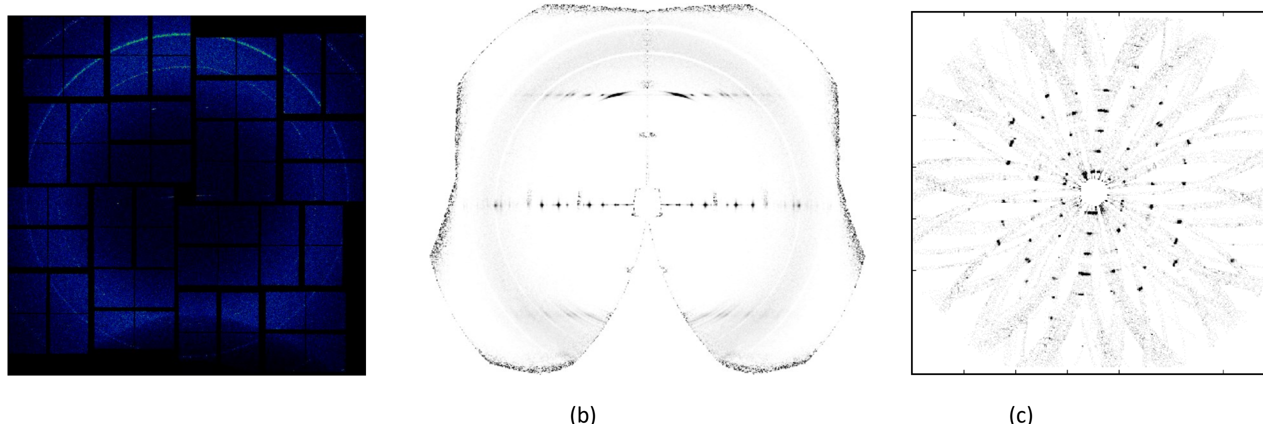
The application of XFELs by serial femtosecond nanocrystallography (SFX) has been highly successful in structural biology [1], with over 100 structures in the PDB determined by SFX [2]. Most of these studies use 3D crystals – an artifice that increases the diffracted signal strength. Fibrilar biomolecular assemblies are long, slender systems that are periodic along their axial direction but exhibit little or no crystallinity in the lateral plane. Such assemblies have traditionally been studied by "X-ray fiber diffraction analysis" where the specimen consists of a large number of aligned, but randomly rotated, molecules [3]. The resulting diffraction is cylindrically averaged, significantly reducing its information content. XFELs offer the possibility of measuring diffraction from single such assemblies, overcoming this limitation.

We have collected diffraction data at the LCLS, using a liquid jet, from an amyloid-forming oligopeptide of unknown structure. Diffraction patterns typically show only 2-4 sharp spots (Fig. 1a), and their analysis allows identification of patterns due to single fibrils. For each hit, the orientation of the fibril is estimated and the diffraction data merged into 2D reciprocal space (Fig. 1b). From the 2D merge, the unit-cell dimensions are determined. By considering orientations of the Ewald sphere that bring each spot into a diffracting position that belongs to the reciprocal lattice, the rotation of the fibril about its axis can be determined. The data can then be merged into 3D reciprocal space (Fig. 1c), and the full set of structure amplitudes determined. Structure determination using this data is in process. This approach offers a new route to the study of fibrilar systems in biology. The experiments described here were conducted as part of a large collaboration between investigators at UC, CFEL, LCLS and ILL



(b) (c)

Figure 1: (a) Single shot, (b) 2D merge, and (c) 3D merge on a single plane in reciprocal space.

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Optimizing sample delivery for time-resolved temperature-jump serial femtosecond crystallography

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While pump-probe crystallography experiments can reveal exquisite details about protein conformational dynamics (Suga et al., 2017), they require precise delivery of microcrystals to the laser–sample interaction point. Precise sample delivery is a balancing act, one requiring a thorough exploration of sample space. For a pump-probe experiment, the time delay between excitation and measurement, and the degree of excitation, need to be well-controlled to induce a consistent effect on the sample. Both of these depend upon uniform sample flow and the monodispersity of the crystalline slurry. With the recent advent of serial crystallography, these topics are just entering the published literature on protein crystallography. Using human cyclophilin A (CypA) as a model system, we explored the protein's phase space and discovered a set of parameters that allowed us to crystallize CypA in batch. Further adjustments to the preparation gave us much greater control over crystal size and monodispersity. Next, we explored different strategies for injecting this crystalline slurry for time-resolved temperature-jump XFEL experiments. We began by utilizing the microfluidic electrokinetic sample holder (MESH) injection system (Sierra et al., 2012), which minimized sample consumption and allowed us to keep the crystalline slurry in its native solution. We also tested an LCP-style injector (Weierstall et al., 2014), which offered slower and more uniform jet velocities. Using the LCP injector required us to increase the viscosity of our crystalline slurry, adding another dimension to sample optimization. Nonetheless, both injector systems allowed us to solve high-resolution, radiation-damage-free, CypA structures in the dark state. These data enabled us to examine whether vastly different injection strategies affect CypA's structure. Furthermore, these adventures in optimization led to a workable solution for sample delivery for time-resolved temperature-jump crystallography, a technique which aims to generalize the study of protein structural dynamics.

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XFEL diffraction studies of the oxygen evolving complex photosystem II

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

(IDY was supported by NIH / NIGMS grant GM102520 to NKS.)

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