Poster Competition Abstracts

BioXFEL

6th International Conference

A National Science Foundation Science and Technology Center

BioXFEL

Poster Blitz

Wednesday 3:45-4:00

Chair: Thomas Grant (UB)

#	Name	Poster Title
20	Joshua Dickerson	Calculating temporally resolved X-ray dose on an XFEL source
22	Martin Fuchs	Ultra-fast raster-scanning synchrotron serial micro-crystallography
27	Thomas Gruhl	Rhodopsin dynamics using pump-probe serial femtosecond crystallography
41	Sankar Raju Narayanasamy	Computational fluid dynamic characterization of liquid sheets suitable for XFEL and synchrotron experiments
23	Deepshika Shamraj Gilbile	Development of a universal platform for fixed target SFX using XFELs

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Poster Session

Wednesday 7:00—9:00 in Regatta Pavilion

Judging committee: Thomas Grant (UB), Shatabdi Roy-Chowdhury (ASU), Sarah Perry (UMass Amherst), Anne Stone (Molecular Dimensions), David Bushnell (Stanford)

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2	Romain Arnal	Molecular-replacement ab initio phasing in protein crystallography
3	Josue Benjamin	Studies of Ti(IV) in living cells to understand a possible endocytosis route using fluorescents molecules
4	Hazel Borges	Kinetic study of the HBI-SH2 complex at physiological conditions
5	Sabine Botha	De novo protein structure determination by SIRAS phasing in LCP using serial millisecond crystallography
6	Stephen Burley	Supporting XFEL/SFX deposition with extended data content in the wwPDB OneDep System
7	Julio Candanedo	Dynamics of electron dissociation of Homodiatomic Crystals
8	Juan Carlos-Villalobos	Expression of the human a4b2 nAChR by recombinant baculovirus transduction and Ion-channel functional characterization
9	José A. Carmona-Negrón	Biological interaction of ferrocene-hormone conjugates with human serum albumin using fluorescence spectroscopy
10	Melissa Carrillo	Crystal structures of a phytochrome from the non-photosynthetic Myxobacterium S. aurantiaca
11	Cecelia Casadei	From two-dimensional crystal serial diffraction to a three-dimensional intensity set: paving the way to the time-resolved study of large-scale movements in membrane proteins
12	Alexander Castro	Protein-DNA interactome of oxidative-stress transcription factors in Aliivibrio fischeri
13	Joe Chen	Shape transform phasing
14	Robert Kin Yip Cheng	X-ray Free Electron Laser: Opportunities for drug discovery
15	**Aina Cohen**	**Sample Delivery**
16	Matt Coleman	Diverse biotechnology and imaging applications using reconstituted high density lipoprotein nanoparticles
17	Natalia Crespo-Rosado	Structural studies using nanolipoprotein particles (NLP)
18	Leishla Cruz-Collazo	Hydrogen sulfide limits amyloids development in hemeproteins
19	Ali Dashti	Conformational dynamics and energy landscapes; pursuit of function from single particle imaging
20	Esmarline De Leon Peralta	3D printing of elastomeric strain reliefs for the optimization of clinical tethered capsule endomicroscopy device
20	Joshua Dickerson	Calculating temporally resolved X-ray dose on an XFEL source
21	Austin Echelmeier	Time-resolved mix and inject serial crystallography facilitated by 3D-Printed microfluidics
22	**Martin Fuchs**	Ultra-fast raster-scanning synchrotron serial micro-crystallography
23	Deepshika Shamraj Gilbile	Development of a universal platform for fixed target SFX using XFELs
24	Patricia Gonzalez	Exploring the anticancer potential of titanium (IV) salicylate as an inhibitor of intracellular iron
25	Indra Gonzalez-Ojeda	Hydrogen sulfide (H2S) limits amyloid development in hen egg white lysozyme (HEWL) as a function of concentration
26	Thomas Grant	Solving the phase problem in solution scattering
27	Thomas Gruhl	Rhodopsin dynamics using pump-probe serial femtosecond crystallography

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28	Rick Hewitt	Simulations of kilohertz serial femtosecond crystallography with the ASU compact X-ray light source
29	**Izumi Ishigami**	**Snapshot of an oxygen intermediate in the catalytic reaction of cytochrome c Oxidase**
30	Antonio Kalil-Aulet	Modelling and crystallographic studies of the cytoplasmic domain of Wsc1p
31	Andrea Katz	Mix-and-inject serial crystallography at EuXFEL
32	Daihyun Kim	Revolution in sample reduction for SFX : droplet-based sample delivery and triggering
33	Ji-Hye Lee	Time-resolved pH jump studies using serial femtosecond X-ray crystallography
34	Dan Bi Lee	
35	**Yinfei Lu**	**A novel sample delivery system based on native mass spectrometry for X-ray Free-electron Lasers**
36	Rafael Maldonado	Preparation and biophysical characterization of nAChR for high-resolution studies
37	Darya Marchany-Rivera	UV-Vis to determine time-points for serial mix-and-inject X-ray diffraction
38	**Sebastian Günther**	**Roadrunner III & IV: High-speed fixed-target sample delivery**
39	Osamu Miyashita	Cryo-cooling effect on DHFR crystal studied by replica-exchange molecular dynamics simulations
40	**Diana Monteiro**	Protein activation methods: Photocaging and microfluidics at monochromatic synchrotron sources
41	**Sankar Raju Narayanasamy**	**Computational fluid dynamic characterization of liquid sheets suitable for XFEL and synchrotron experiments**
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45	Nadia Opara	Demonstration of femtosecond X-ray pump X-ray probe diffraction on protein crystals
46	Valerie Ortiz Gomez	Biophysical characterization of novel biomimetic peptide-polymer conjugate using the properties of antimicrobial peptide Maximin H5
47	Suraj Pandey	Structural basis for light control of cell development revealed by crystal structures of a myxobacterial Phytochrome*
48	Surya Pulavarti	X-ray free electron lasers meet NMR: Structural enzymology of β-lactamase
50	Angel Rodriguez	Oxy-myoglobin's interaction with hydrogen sulfide: A pathway from compound III to compound 0
51	Jessica Rodriguez-Rios	Uncovering DNA binding specificity of cardiac transcription factors complexes of GATA4, NKX2-5 and TBX5
52	Alexander Rose	Towards web molecular graphics for multi-scale models in space and time
53	Keishla Sanchez Ortix	Optimization and characterization of protein crystals to study molecular structure using crystallography methods
54	Lysmarie Santos Velazquez	Sulfhemoglobin and its role as an endogenous hydrogen sulfide biomaker

55	Manoj Saxena	Diverse applications of reconstituted high-density lipoprotein nanoparticles
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57	Alex Sedlacek	Rigaku Innovative Technologies, Inc. provides meter-class, multi-stripe coated mirrors to Stanford Linear Accelerator Facility for use on the Linear Coherent Light Source
58	**Megan Shelby**	**Fixed target delivery for SFX of weakly-diffracting objects**
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60	Jonah Shoemaker	Diffractive stereo imaging
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67	**Martin Trebbin**	**Microfluidic reaction control for time-resolved structure determination at XFELs and Synchrotrons**
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70	**Uwe Weierstall**	**Sample Delivery**
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72	Stella Lisova	GDVN / double-flow focused nozzles
73	Ishwor Poudyal	Proteins and enzymes: How many single-particle snapshots we need?
74	Iris Young	Water oxidation reaction in photosystem II studied by X-ray spectroscopy and crystallography
75	Liz Santiago	Novel biomimetic membrane for water filtration purposes using Lipidic Cubic Phases
76	Jessika Pazol	Using electrospray deposition technique for enzyme adsorption onto Di-block copolymer self-assembled nano films for water treatment applications
77	**Hao Hu**	**Microjet sample delivery for SFX**

*** Posters for Sample Delivery Mini Workshop in bold.**

Alani Aldorando-Torres, University of Canterbury

Hydrogen Sulfide (H2S) Inhibits Insulin Amyloid Fibril Formation

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

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Insulin from bovine pancreas (IBP) is one of about thirty human proteins that form amyloid fibrils. These amyloid fibrils obtain a b-sheet structure and assemble into insoluble fibrils, causing many amyloidosis diseases. Amyloidosis caused by insulin protein is an acquired systemic disease. Developing procedures to eradicate amyloid fibril precursors is key to detaining amyloid fibril formation. To explore amyloid inhibition, native insulin protein was exposed to different concentrations of H_2S, and protein unfolding was induced. Atomic Force Microscopy (AFM) imaging evidenced overlaying spherical structures, as the concentration of H_2S increased in each sample as opposed to the fibrillary structures in the control sample. 3D AFM contour plots of the overlaying spheres due to protein-H_2S interaction show smoother peaks of greater height contrary to the sharp smaller peaks of the insulin amyloid fibrils. Results from Far UV-Circular Dichroism spectra suggest that insulin protein in presence of H_2S conserves its native insulin structure. These results are independent of pH value and are observable at physiological pH. Deep Ultraviolet Resonance Raman Spectroscopy further explains protein-H_2S interaction suggesting the formation of trisulfide bridges in insulin-H_2S sphere structures. Results suggest H_2S inhibits insulin amyloid fibril formation by detaining the development of insulin fibril precursors.

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Romain Arnal, University of Canterbury

Molecular-replacement AB Initio Phasing in Protein Crysallography

Romain D. Arnal[1], Markus Metz[2,4], Andrew J. Morgan[2], Henry N. Chapman[2,3,4] and Rick P. Millane[1]

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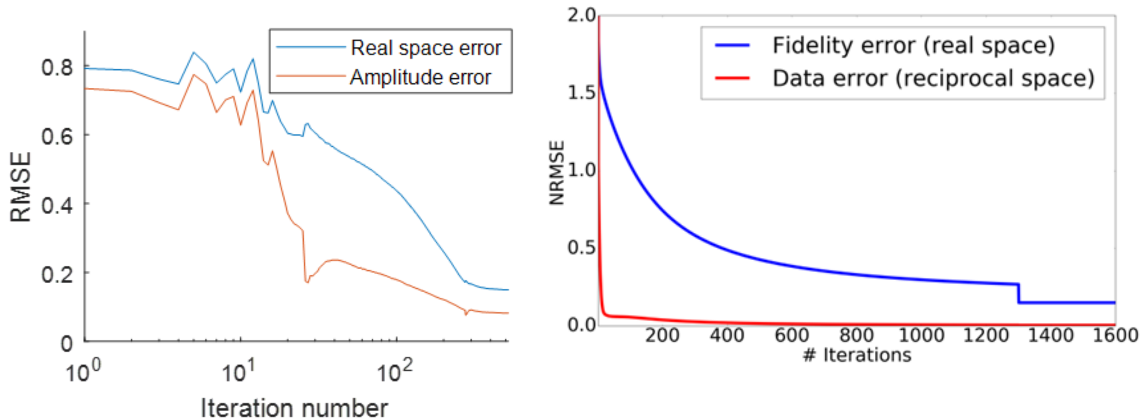
Despite advances in protein X-ray crystallography, the phase problem remains a limiting factor in structure determination. Molecular replacement (MR) is extremely powerful as it leverages structural information in the PDB, but it can be affected by model bias and, in cases where homologous models are not available, is ill-suited for finding new structural folds. Here we propose a phasing approach related to MR that does not require a model structure. The approach uses diffraction measurements of the same molecule crystallised in two or more different crystal forms. Such intensity data sets are generally independent, providing additional information that makes the solution to the phase problem more tractable.
To solve the phase problem, we have developed an iterative projection algorithm that matches the diffracted amplitudes for each crystal form to the measurements, as well as enforcing equivalence of the molecular electron density in each crystal form and any other real space constraints. For practical ab initio phasing, the molecular envelope and the relative molecular positions and orientations in the different crystals would need to be determined, but for initial experiments we assume that these are known.
We have investigated two different situations by simulation. In the first, we used two crystal forms of Lysozyme with different space groups. In the second, we used Photosystem II in three slightly different unit cells with the same space group (corresponding to the cell dimensions observed during an LCLS experiment due to variations in humidity in a fixed target delivery system). Using error-free simulated data, our results are as follows. For two Lysozyme crystal forms, phasing is successful given an initial 10A-resolution envelope (below left), whereas it is unsuccessful using data from a single crystal form. For three Photosystem II crystal forms, phasing is successful using a 6A-envelope (below right), whereas a 1.5A-envelope is necessary for phasing using only one of the crystal forms. Thus, although more work is needed to make this a practical phasing method, our results so far show the potential of this approach.

Supported by the NZ Marsden Fund.



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Josue Benjamin, UPR-Rio Piedras

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

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Titanium is a transition metal present in the human body. Some sources of Ti are toothpaste, white paint, food and implants (alloy form). People with titanium containing implants can reach 50 times higher (0.25 - 2 M) Ti than levels than normal in the blood. There is great concern that elevated Ti(IV) levels can be detrimental to the body. Human serum transferrin (HsTf), the iron cellular transport protein, plays an important role to keep the Ti4+ soluble in the blood with the help of a small molecule known as citrate. Considering the potential of Ti(IV) to affect Fe(III) uptake into cells, our goal is to study the endocytotic transportation of the Ti(IV) in the cells by HsTf using fluorescent probes. To perform the studies, we modified the citrate with a fluorescent molecule (5-aminofluorecine, green dye). The product was then purified and characterized using MS, 1H-NMR, and FT-IR. To perfome the experiment we need to labeling the protein with a fluorescent blue molecule (BFP or CFP) and the cell membrane with red fluorescent molecule (FM 4-64). To see the three fluorescent molecules at the same time. The modification of the protein and citrate can allow us to make an indirect probe of Ti4+ in presence of hTf and citrate. To confirm the stability of the computational modeling will be doing using Maestro Schrödinger software. Furthermore, the technique confocal microscopy will be used to understand the endocytosis process of the complex. The study will answer whether the hTf is responsible for the regulation of the metal ion

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Hazel Borges, UPR-Mayaguez

Kinetic study of the HBI-SH2 complex at physiological conditions

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Hydrogen Sulfide is a well-known poisonous gas whose toxic effects have been studied for many years. Recently, 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. As result studies of H_2S are focused as signaling molecule involved in various physiological processes. 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 using compounds that release or promote production of H_2S in biological samples for its therapeutic attribution known as H_2S donors. These studies had demonstrated that treatment with H_2S could ameliorate pathologies and thus the quality of life of patients, but they have shown some disadvantages. 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. This research study the viability of the protein HbI from Lucina Pectinata for the delivery of H_2S in biological systems to obtain a reliable H_2S donor. In preliminary kinetic experiments, results have shown low release of H_2S from the complex (k_{off}:$0.42x10^{-3}s^{-1}$) and the high affinity of the H_2S for the protein regardless of physiological parameters studied. If this H_2S-delivery protein continue demonstrating a successful and beneficial administration of H_2S in physiological conditions, the results could lead us to the generation of new therapies based on H_2S.

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Sabine Botha, ASU

De novo protein structure determination by SIRAS phasing LCP using Serial Millisecond Crystallography

[A] *S. Botha[1,2], D. Baitan[1,3], K. J. E. Jungnickel[4], D. Oberthür[5], C. Schmidt[1], S.Stern[5], M. O. Wiedorn[2,5,6], M. Perbandt[1,2], H. N. Chapman[2,5,6], C. Betzel[1,2]*
[B] *S. Botha [1,2], J. Martin-Garcia[2,3], H. Hu [2,3], U. Weierstall [2,3], M. Fuchs[4], W. Shi[4], B. Andi[4], J. Skinner[4], H. Bernstein[5], P. Fromme[2,3], N. Zatsepin [1,2]*

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Serial femtosecond crystallography (SFX) is an up and coming method for protein structure determination [1] and it has been shown that data can be phased *de novo* using this method. This method of data collection has further been adapted to synchrotron application, termed serial millisecond crystallography (SMX) [2, 3, 4]. SMX substantially reduces radiation damage incurred by the individual protein crystals compared to conventional, oscillation data collection approaches, facilitating structure solution from micrometer sized crystals at synchrotrons. However *de novo* phase retrieval remains difficult and is rarely applied to serially collected SFX and SMX data. Here we present *de novo* phasing results from Proteinase K SMX data applying [A] single isomorphous replacement with anomalous scattering (SIRAS) and [B] native Sulphur single-wavelength anomalous dispersion (S-SAD). Protein crystals were injected into the X-ray interaction region in a free-standing LCP jet for serial data collection.

[A] Using the model system Proteinase K and its mercury derivative, successful *de novo* phase retrieval is demonstrated, applying SIRAS to serial millisecond crystallography data collected at the P11 beamline at PETRA III [5].

[B] Successful Sulphur-SAD phasing of serial snapshot millisecond crystallography data from native Proteinase K crystals collected at the NSLS-II at the Brookhaven National Laboratory is presented.

Acknowledgements:

[A]This research was supported by the excellence cluster `The Hamburg Centre for Ultrafast Imaging' by the Deutsche Forschungsgemeinschaft (DFG). We would further like to acknowledge the P11 staff at DESY.

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

References: [1] Chapman H, et al. Nature 2011 Feb 3;470(7332):73-7, [2] Gati et al., 2014 IUCrJ 1:87, [3] Stellato et al., IUCrJ. 2014 Jul 1; 1(Pt 4): 204–212, [4] Botha et al., Acta Crystallogr D Biol Crystallogr. 2015 Feb;71(Pt 2):387-97, [5] Botha et al., IUCrJ. 2018 Sep; 5: 524-530

Stephen Burley, Rutgers University

Supporting XFEL/SFX Deposition with Extended Data Content in the wwPDB OneDep System

Chenghua Shao[1], Jasmine Y. Young[1], Ezra Peisach[1], Zukang Feng[1], John D. Westbrook[1], Yuhe Liang[1], Irina Persikova[1], and Stephen K. Burley[1,2]

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The Protein Data Bank (PDB) is the single global repository for experimentally determined three-dimensional structures of biological macromolecules and their complexes with ligands. The Worldwide Protein Data Bank (wwPDB) is the international collaboration that manages the PDB archive according to the FAIR Principles: Findability, Accessibility, Interoperability, and Reusability. PDB archive now holds and freely disseminates more than 145,000 experimentally-determined structures of biological macromolecules, with rapid growth in the number of structures coming from X-ray Free-Electron Lasers (XFEL) and Serial Femtosecond Crystallography (SFX).

To faithfully represent XFEL experiments, the Worldwide Protein Data Bank partnership (wwPDB, wwpdb.org) has worked with the wwPDB PDBx/mmCIF Working Group and experts from the XFEL community to develop new PDBx/mmCIF metadata extensions that support the XFEL/SFX metadata collection at the wwPDB OneDep system (https://deposit.wwpdb.org/) for deposition, validation, and biocuration.

This presentation will describe how depositors can now provide details of sample delivery and measurements within the OneDep system such as focusing optics, beam dimensions, pulse energy, frequency, and number of frames and crystals. The wwPDB is committed to working closely with XFEL/SFX users to ensure faithful preservation and representation of their data in the PDB core archive.

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

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Julio Candanedo, ASU

Dynamics of Electron Dissociation of Homodiatomic Crystals

J. Candanedo, J. Spence, and C. Caleman

The objective of this paper is to understand radiation damage by high-energy femtosecond electron pulses traversing a thin for crystal, in order to determine the experimental conditions required to out-run damage using fast electron beams. To achieve this,Monte Carlo (MC) and Molecular Dynamics (MD) simulations were used to model radiation induced dissociation and ionization. The Monte Carlo (MC) simulation calculates the potential energy/surface changes caused by the ionizing beam, while the Molecular Dynamics (MD) simulation, calculates subsequent time evolution of atomic/nuclear coordinates on this new potential energy landscape. In these simulations the bright (pulse quanta of 0.7, 7, 70, and 700 e/å2) ultrafast (durations of 1.2, 12, and 120 fs) high energy (136, 544, and 1360 keV) electron beam irradiates a 1000 unit cell homodiatomic (H2, Li2, N2, O2, F2, S2, Cl2, Br2, I2) crystal. This project is to serve as a stepping stone for triatomics, more complicated molecules, and eventually proteins.

Supported by NSF STC award number 1231306

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Juan Carlos-Villalobos, University of Puerto Rico, Rio Piedras

Expression of the Human a4b2 nAChR by Recombinant Baculovirus Transduction and Ion-Channel Functional Characterization

Juan C. Villalobos-Santos1,2, José A. Lasalde-Dominicci1,2

1. University of Puerto Rico, Río Piedras Campus, San Juan PR, 00931; 2. University of Puerto Rico, Molecular Sciences Research Center, San Juan PR, 00926.

Nicotinic acetylcholine receptors (nAChR) are widely known for their role in fast cellular responses, and their relevance in many physiological pathways. The human a4b2 nAChR is the most abundant nAChR in the human brain, and has been directly linked to nicotine addiction and other common neurodegenerative conditions. Recently, two 3-dimensional structures of the a4b2 nAChR were reported using X-ray crystallography and cryo-electron microscopy (cryo-EM). The functional characterization of the a4b2 nAChR was performed in HEK cells using the whole-cell patch clamp method. Along this line, these studies did not provide a functional characterization of the a4b2-nAChR-detergent complex (a4b2-nAChR-DCs). Two-electrode voltage clamp (TEVC) studies on reconstituted muscle-type nAChR-Detergent complexes (nAChR-DCs) in Xenopus oocytes, showed that the detergents used in the latter studies (n-dodecyl-b-D- maltoside or DDM), abolish ion-channel function, and due to the similarity of neuronal and muscle-type receptors, similar results may be observed in a4b2-nAChR-DCs. The reason behind this loss of function has been reported to be mainly related to detergent induced delipidation, causing unfavorable changes in the lipid composition of nAChR-DCs leading to denaturation. We aim to express the a4b2 nAChR by recombinant baculovirus transduction of HEK GnTI- cells, and solubilize membranes containing the receptor for further functional characterization. The receptor will be purified by strep tag affinity chromatography, and analyzed through fluorescent size exclusion chromatography (FSEC). The resulting a4b2-nAChR-DCs with detergent variations will be injected in Xenopus oocytes to measure ion-channel activity using the TEVC technique. Furthermore, we will characterize the lipid composition of the solubilized receptor to define the lipid basis for the ion-channel function of the a4b2-nAChR-DCs.

This project was funded by NIH Grant: 1R01GM098343 and 1P20GM103642, also by RISE: 5R25GM061151-17.

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José A. Carmona-Negrón, UPRM

Biological interaction of Ferrocene-hormone conjugates with human serum albumin using fluorescence spectroscopy

José A. Carmona-Negrón[1], José M. Liboy-Lugo[2], and Enrique Melendez[1]

[1] University of Puerto Rico, Department of Chemistry, Mayagüez, PR
[2] University of California-San Francisco, TETRAD Graduate Program, San Francisco, CA

Previously, ferrocene incorporation into the principal structural component of biologically active molecules resulted in enhanced cytotoxic activity against hormone-dependent MCF-7 and T-47D and hormone-independent MDA-MB-231 breast-cancer cell lines. Here we explore the biological interaction of three ferrocene estrogen conjugates at position 16 and 3 of the estrogen hormone with Human Serum Albumin by using fluorescence spectroscopy. The biomolecular quenching constant, as defined in the Stern–Volmer equation, was determined by performing a binding assay of a human serum albumin (HSA) and ferrocene-estrogen conjugates at physiological pH at three temperatures. The temperature dependency of the quenching constant allows estimation of thermodynamic parameters which in turn permits to assess the nature of the intermolecular interactions involved in the protein-ligand complex. The thermodynamics parameter that govern the protein-ligand interaction is presented. Docking studies were performed in order to obtain a suggested *in silico* interaction with the amino acid residues in the protein ligand cavity.

Melissa Carillo, Northeastern Illinois University

Crystal Structures of a Phytochrome from the Non-Photosynthetic Myxobacterium S. aurantiaca

Melissa Carrillo1, Juan Sanchez1, Moraima Noda1, Marius Schmidt2 and Emina A. Stojković1

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

Bacteriophytochromes (BphPs) are red light photoreceptors found in photosynthetic and non-photosynthetic bacteria. They are dimeric proteins that consist of a photo-sensory core module (PCM) and an enzymatic domain, which are often histidine kinases. The PCM consists of the Per/Arnt/Sim (PAS) domain, the guanosine monophosphate phosphodiesterase / adenylyl cyclase / FhlA (GAF) domain, and a phytochrome-specific (PHY) domain. The PHY domain is covalently linked to an effector domain with histidine kinase activity. The GAF domain contains a covalently bound chromophore called biliverdin (BV) which is a heme-derived, open chain tetrapyrrole. The BphPs reversibly interconvert between the red-light absorbing Pr state and the far-red light absorbing Pfr state. The non-photosynthetic myxobacterium Stigmatella aurantiaca contains two bacteriophytochromes (BphPs) denoted SaBphP1 and SaBphP2. Interestingly, SaBphP1 lacks a conserved histidine in the GAF domain, which stabilizes the Pr state by forming a hydrogen bond with the D-ring of BV. The same histidine also stabilizes the Pfr state by forming a hydrogen bond with the C-ring propionate side chain. SaBphP2, like classical phytochromes, contains the mentioned conserved histidine and shares the same domain composition as SaBphP1. Recently, we determined two crystal structures of SaBphP2 PCM: a) in the wild-type and b) in a mutant form that contains threonine instead of conserved histidine in the GAF domain. These two variants of SaBphP2 PCM crystallize in the same space group (P21) and diffract to 1.9 and 2.2 Ångstrom resolution, respectively. When compared, the structural differences between two proteins are observed at the dimer interface and in the chromophore-binding pocket, explaining the lack of photoactivity in the SaBphP2 mutant. Through our recent developments of highly-diffracting SaBphP2 microcrystals, we propose SaBphP2 to be a suitable model for investigations at free electron lasers using Time-Resolved Serial Femtosecond X-ray crystallography (TR-SFX). The proposed experiments will provide insight in the progress of the photoreaction and in the formation of intermediates during of the Pr to Pfr transition. This will then help to determine how enzymatic activity is controlled and regulated by the light stimulus in phytochromes in general.

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BioXFEL

Cecelia Casadei, PSI

From two-dimensional crystal serial diffraction to a three-dimensional intensity set: paving the way to the time-resolved study of large-scale movements in membrane proteins

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

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

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Alexander Castro, University of Puerto Rico, Rio Piedras

Protein-DNA interactome of oxidative-stress transcription factors in *Aliivibrio fischeri*

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The LysR type transcriptional regulators (LTTRs) comprise the largest prokaryotic family of transcription factors. Transcription factors (TFs) are sequence-specific DNA-binding proteins that dictate cell function by controlling selective usage of genomic information. The transcription factor OxyR functions as a defense mechanism against hydrogen peroxide-induced oxidative stress. It has been noted that *Aliivibrio fischeri*'s genome encodes for OxyR1 and OxyR2 (OxyRs). The OxyRs share functional and sequence similarities across different bacterial species, but respond differently depending on the amount of hydrogen peroxide concentration. Studying the OxyRs protein-DNA interactomes will allow us to identify key differences between them. Currently, we are working on over-expressing and purifying full-length and DNA binding domain of OxyR1 and OxyR2. To determine the protein-DNA interactomes of the OxyRs, High-Throughput Systematic Evolution of Ligands by Exponential Enrichment (HT-SELEX) will be used. SELEX provides information regarding the *in vitro* DNA-binding preferences of a target TF, but not all binding sequences identified in a genome might be related to the regulation of the TFs in cells. The determined DNA-binding specificities will be used to bioinformatically predict the genomic targets of OxyR1 and OxyR2. Evaluating the specificity profile of DNA binding proteins is a nontrivial challenge that hinders the ability to decipher gene regulatory networks.

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JOE CHEN, ASU

SHAPE TRANSFORM PHASING

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

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

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

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Robert Kin Yip Cheng, leadXpro AG

X-ray Free Electron Laser: Opportunies for Drug Discovery

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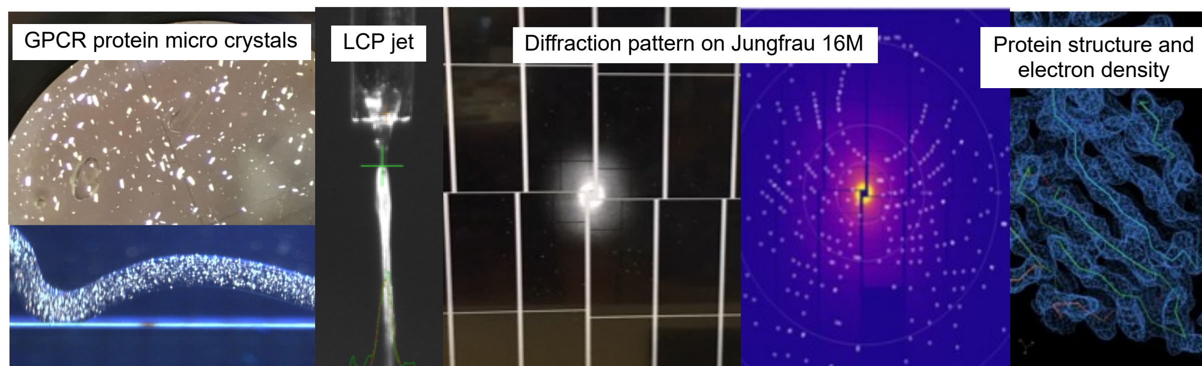
Past decades have shown the impact of structural information derived from complexes of drug candidates with their protein targets to facilitate the discovery of safe and effective medicines. However, membrane protein drug targets like ion-channels, transporters and GPCRs still represent a significant challenge. Recent developments in single particle cryo-electron microscopy have significantly improved the options to derive structural information for ion-channels and complexes of GPCRs with G protein. LeadXpro is a structure based lead discovery company focusing on challenging membrane protein drug targets, including G-protein coupled receptors (GPCRs), ion channels and transporters.

Advances in serial crystallography are a pre-requisite to use the unique properties of X-ray Free Electron Laser (XFEL) with unmet peak brilliance and beam focus, which allows successful structure determination from smaller and weakly diffracting crystals. Serial crystallography at synchrotron has already been shown to be instrumental for structure determination and here we present an example in which a GPCR structure was solved using such a method. To further capitalize on the XFEL advantage which allows the capturing of dynamic processes of drug molecule binding and associated conformational changes with great impact to the design of candidate drug compounds, innovations in crystal sample delivery for the X-ray experiment, data collection and processing methods are required and some recent developments will be shown.

In August 2018, the SwissFEL facility was used for the very first biostructure experiments. We performed successfully the structure determination of a GPCR at the ALVRA beamline using the LCP jet and the brand-new Jungfrau 16M detector.

Figure: First biostructure experiment at SwissFEL – a GPCR structure!



Acknowledgement: We thank all colleagues from PSI for contribution to the work and Heptares Therapeutics for protein supply.

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Matthew Coleman, LLNL

Diverse biotechnology and imaging applications using reconstituted high density lipoprotein nanoparticles

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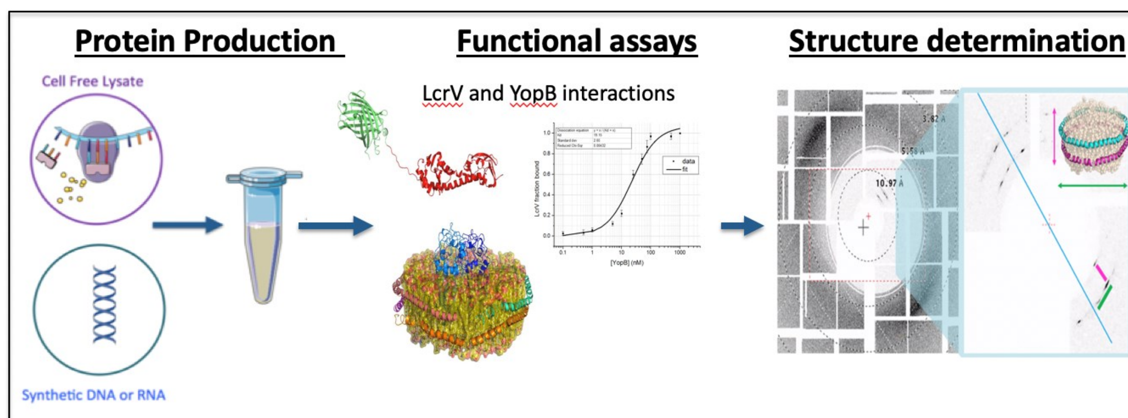
We have developed several methods for high-throughput and high-resolution biological imaging based on formation of High Density Lipid (HDL) particles. HDL particles are involved in lipid and cholesterol transport and form proteolipid nanoparticles. These nanolipoprotein particles (NLPs; aka nanodiscs), form 10-25 nm discs and can be reconstituted in vitro. 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 formation. Here, we discuss how our laboratory has used NLPs technologies to produce and support trans-membrane proteins for on-going as well as future XFEL-based studies. First, we have focused on using NLPs to support functional and structural analysis of membrane proteins. We have produced over three dozen membrane bound proteins and multi-protein complexes that were fully functional. This includes receptors such as rhodopsins, G-protein coupled receptors (GPCRs), kinases, cytokines, antibodies, proteases, outer membrane proteins, and multiple secretion system complexes. Second, the NLPs have shown utility for use as in vitro and in vivo delivery of functional proteins as well as therapeutics. Correctly folded membrane proteins were shown to fully recapitulate protein signaling in cells. In addition, NLPs have been shown to increase bioavailability and enhanced therapeutic efficacy of molecules complexed with NLPs. Finally, we have demonstrated that NLPs are amenable to multiple structural techniques to include fluorescence correlation spectroscopy (FCS), circular dichroism (CD), electron microscopy (EM), small-angle X-ray as well as neutron scattering (SAXS/SANS) and X-ray diffraction at LCLS. These structural techniques have provided a dynamic scale of imaging for understanding HDL particles and NLP-supported membrane proteins. Overall, NLPs represents a unique solution to address multiple bottlenecks in the production, functional and structural characterization of lipid-bound proteins that were previously difficult to obtain. On-going studies will help refine the function and structure of the aforementioned proteins of interest.



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Natalia Crespo-Rosado

Structural studies using nanolipoprotein particles (NLP)

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Nanolipoprotein particles (NLPs) are self-assembled, discoidal phospholipid bilayers that allow the study of membrane associated proteins in a near native environment without the use of detergents. NLPs are composed of two antiparallel membrane scaffold proteins that wrap around the lipids. NLPs can act as detergent substitutes and are particularly useful for structural and enzymatic studies of membrane proteins in near-native environments. Advances in NLP applications would benefit the study of membrane proteins using solution scattering, single particle imaging, and potentially cryoEM.

Structural information on NLPs is lacking, but is also necessary to guide the engineering of new, more functional constructs. We aim to realize the full potential for structural and enzymatic studies, a new approach was developed for potentially optimizing their applicability to structural studies. Here we present the initial results of a mutation of an NLP construct, MSP1D1, that allows for the formation of a linear network of covalently linked NLPs via disulfide bonds and describe our initial crystallization results.

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Leishla Cruz-Collazo, ASU

Hydrogen Sulfide Limits Amyloids Development in Hemeproteins

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Conformational diseases are characterized by structural conversion of proteins to alternative forms, which subsequently convert into protein fibrils. The accumulation of these protein fibrils as amyloid deposits in the brain is implicated in a number of neurodegenerative diseases, including Alzheimer's, Parkinson's, Huntington's, among others. Hemeproteins play an important role keeping the functions in the cell, including from gaseous exchange to redox reactions. Myoglobin (Mb) and Hemoglobin (Hb) are the most studied proteins of all, because of their biological significance of oxygen-binding. There has been less publications evidence for amyloid-like aggregation of hemoglobin and myoglobin under physiological conditions. Amyloid formation of myoglobin (Mb) and hemoglobin (Hb) upon addition of 2,2,2-trifluoroethanol (TFE) in 45% v/v were analyzed using ThT fluorescence and Circular Dichroism (CD). ThT data of Mb and Hb in presence of 45% v/v TFE reveals the rapid increment in fluorescence intensity after 180 minutes proposing the formation of amyloid fibrils under the studied conditions. Furthermore, CD spectra of Mb and Hb confirms β-sheet conformation indicating the formation of amyloid fibrils. Overall, the data suggest that under the conditions presented Mb and Hb can transform its structure to fibrils derivatives. We also studied the effect of hydrogen sulfide (H2S) in the formation of amyloid-like fibrils. The results showed that in the presence of H2S, the amyloid-like fibrils formation of Mb and Hb are inhibited. The ThT data confirmed no fluorescence increment and CD spectra showed α-helical conformation in the presence of H2S. This is the second research study that showed the inhibition effect of H2S over amyloid fibrils formation.1

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Ali Dashti, UWM

Conformational dynamics and energy landscapes; pursuit of function from single particle imaging

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We present a new approach to determining the conformational changes associated with biological function from single -particle cryo-EM snapshots of ryanodine receptor (RyR1), a Ca2+-channel involved in skeletal muscle excitation/ contraction coupling. The functional motions differ substantially from those inferred from discrete structures. The differences include the conformationally active structural domains, the nature, sequence, and extent of conformational motions involved in function, and the way allosteric signals are transduced within and between domains.

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Esmarline De Leon Peralta, UPR-Mayaguez

3D Printing of Elastomeric Strain Reliefs for the Optimization of Clinical Tethered Capsule Endomicroscopy Device

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Imaging technology has emerged as a powerful resource that enables physicians to provide a rapid and accurate diagnosis of different diseases. State of the art endoscopy has limitations for the detection of diseases of the gastrointestinal tract. Dr. Tearney's laboratory has developed a new tethered optomechanical pill that captures three-dimensional microscopic images of the gastrointestinal (GI) tract in unsedated patients. The capsule uses a 10-µm-resolution cross-sectional microscopic imaging technology called Optical Coherence Tomography (OCT) that enables the diagnosis and monitoring of premalignant conditions like Barret's esophagus, a precursor to esophageal cancer. This tethered capsule endomicroscopy (TCE) procedure is non-invasive, allows the adjustment of the capsule position in the GI tract, provides a more accurate diagnosis, can be performed in minutes, and does not require advanced or expensive equipment. These features of TCE make it a promising tool for screening for a wide array of upper GI tract conditions. One of the key requirements for TCE as a viable screening modality is low procedural cost, facilitated by reusing the device multiple times following disinfection. A key component of the TCE device that enables multiple use is a flexible, silicone part called the strain relief that prevents the capsule from breaking off the tether and protects the tether-capsule junction. One of the challenges that the lab has experienced is outsourcing these strain reliefs, which is expensive and time consuming due to the unique requirements of the silicone injection molding process. 3D printing technology provides an alternative way to fabricate flexible strain reliefs for the capsule. In this research we investigated the use of an innovative 3D printer solution to directly fabricate the capsule's strain relief. By providing an easily manufactured and customizable strain relief for TCE, the output of this project has the potential to benefit patients by accelerating the translation of this technology so that it can be used for screening for premalignant gastrointestinal conditions.

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Joshua Dickerson, University of Oxford

Calculating temporally resolved X-ray dose on an XFEL source

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Radiation damage has plagued X-ray diffraction studies since the early days of macromolecular crystallography (MX). The femtosecond pulses of XFELs coupled with their high peak brilliance can theoretically allow diffraction patterns to be collected before radiation damage levels are detectable[1]. However, radiation damage to proteins has been seen at XFEL sources[2] and whether radiation damage is outrun depends on a number of parameters, including the pulse length, beam fluence, incident photon energy, and crystal composition.

The quantity of absorbed dose (J/kg) is independent of parameters such as time and incident beam energy and has thus been used as a universal '*x*-axis' against which to plot various metrics to monitor the progress of radiation damage[3]. The program RADDOSE-3D can be used to calculate temporally and spatially absorbed dose during synchrotron MX experiments[4].
However, RADDOSE-3D did not accurately estimate dose on the short femtosecond timescales of XFEL pulses since diffraction images are collected before primary photoelectrons have lost all of their kinetic energy and come to rest. RADDOSE-3D estimated dose values will therefore not be representative of the level of damage in the crystal. We have now extended RADDOSE-3D to estimate absorbed dose on the femtosecond timescale for use at XFELs. As well as being a useful quantity for radiation damage studies at XFELs, it has the potential to aid predictions of pulse lengths at which we would expect to see radiation damage in the diffraction pattern for a given incident beam fluence, crystal size and composition.

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Austin Eichelmeier, ASU

Time-resolved mix and inject serial crystallography facilitated by 3D-printed microfluidics

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Ultrashort, brilliant X-ray free electron laser (XFEL) pulses have been utilized in conjunction with a pump-probe optical laser for time-resolved serial femtosecond crystallography (TR-SFX) to study intermediates of photoactivated proteins [1]. However, many reactions are not photoinitiated but instead are triggered by a chemical change such as a ligand binding to a protein or a pH change, and the intermediates for these chemically-triggered reactions are an exciting forefront in TR-SFX. Several mix and inject serial crystallography (MISC) studies have recently been accomplished at XFELs [2][3], but there is still room for optimization. Since mixing by diffusion, the typical method for chemical triggering in MISC, is much slower than light activation, several time constraints must be considered. Ideally mixing should occur quickly, at millisecond or sub-millisecond time scales, and the mixing should be uniform such that a single reaction intermediate is observed. Additionally, the ability to probe multiple time points along a reaction coordinate is desired in order to construct a molecular movie; therefore, a method that is easily adaptable to multiple time points while maintaining the previous requirements is ideal. We have developed a 3D-printed microfluidic mixer that uses hydrodynamic focusing to mix by diffusion for MISC at an XFEL. Mixing times range from millisecond to sub-millisecond, and the mixing times have been characterized by numerical models (COMSOL) and verified with fluorescence microscopy. The reaction time is determined based on the distance from mixing location to the X-ray interaction region and the velocity at which the crystals travel that distance. This is most easily tuned by the length and inner dimensions of the outlet that connects the mixer to the injection nozzle. We have developed two main types of mixing devices: a) a capillary-coupled mixer that is interfaced to a nozzle *via* a fused silica capillary, and b) a hybrid device that has a nozzle integrated into the 3D-printed design containing a hydrodynamic mixer. The capillary-coupled mixer can be constructed with an outlet length of 0.5 mm or greater, being able to reach reaction time points between milliseconds up to minutes. With the hybrid device, reaction time points of <10 ms can be achieved with an outlet length of <1.5 mm. We have utilized the capillary-coupled mixer with a long outlet capillary to study the catalytic oxidation of cytochrome *c* oxidase (CcO), a transmembrane protein that is responsible for reducing oxygen to water and for pumping protons in mitochondria. Electron densities of the reduced, oxidized, and P_R intermediate were observed for CcO at the 8 s time point using the 3D-printed capillary-coupled mixer. We have also begun developing a concentric mixer which reduces mixing times by about a factor of two and reduces time point variation to <10% due to reduced variation in crystal velocity. Future designs will reduce the number of fluidic inlets from three to two for a simplified setup.

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Martin Fuchs, Brookhaven National Laboratory

Ultra-Fast Raster-Scanning Synchrotron Serial Micro-Crystallography

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In recent years, serial micro-crystallography at storage rings has undergone a series of developments that greatly widen its appeal to the structure determination of challenging proteins. Increases in beamline brightness and new sample delivery methods allow crystallographers to obtain structures from crystals that previously would have been considered intractable. The new crystallography beamlines at the National Synchrotron Light Source-II [1,2] provide beams of unprecedented brightness, stability and versatility. The micro-focusing Frontier MX beamline, FMX, delivers a flux of 3.5×1012 ph/s at 1 Å into a 1×1.5 µm (V×H) focus. Its maximum flux density of up to 2×1012 ph/s/µm2 surpasses current MX beamlines by up to two orders of magnitude, capable of delivering dose rates >500 MGy/s.

The high dose rate presents a great advantage for raster-scanning serial micro-crystallography in cutting measurement times from hours to under a minute. To harness the full potential of this new dose rate regime, we built the FastForward Goniometer – a high-speed, high-precision goniometer based on a unique XYZ piezo positioner [3]. We obtained high quality diffraction-data sets up to the Eiger 16M's maximum frame rate of 750 Hz (using the 4M central region), using the full flux of the beamline. By collecting multiple rotation diffraction images per crystal, we required fewer crystals for a complete dataset compared to acquiring still images. The shutter-open time for a complete dataset was under 20 s [3]. A dedicated data processing pipeline for the partial rotation datasets employs cluster analysis to obtain the final merged dataset.

New micro-patterned sample holders facilitate buffer removal to optimize background scattering [4]. With the high speed, any crystal distribution on a membrane can be scanned, obviating the need to load crystals into a fixed-target matrix sample holder.

Complementing this development for LCP grown crystals, we are establishing serial crystallography data collection with a high viscosity extrusion injector in a partner-user collaboration with researchers from Arizona State University [5].

This flexible sample delivery beyond standard cryo-crystallography allows tailoring the experiment to the protein-crystallization environment, to the temperature range of interest, and to size and amount of available crystals – a prerequisite to add serial crystallography to the standard repertoire of the synchrotron MX community.

This research is supported by Brookhaven National Laboratory LDRD program 16-006 and uses resources of the NSLS-II, BNL (Contract No. DE-SC0012704). The FMX beamline is further supported by US NIH (NIGMS grant P41GM111244), and DOE Office of Biological and Environmental Research (KP1605010).

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Deepshika Shamraj Gilbile, University of California Davis

Development of a universal platform for fixed target SFX using XFELs

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The goal of this work is to develop a universal fixed target Serial Femtosecond Crystallography (SFX) platform for room temperature structural studies of both 2D and 3D protein crystals using X-ray Free Electron Lasers (XFELs). Our primary focus is on developing substrates that control and maintain sample hydration while contributing minimally to background scatter. This approach promises to make SFX more available to samples that are weakly diffracting and difficult to produce in large quantities. Our secondary focus is optimizing ease of substrate and sample preparation, cost effectiveness and, importantly, pliability to in-situ crystal growth and patterning.

To address the low-background requirement, our group developed techniques to produce and transfer clean, crackless, few layer graphene and ultra-thin polymer films that can span large (1” x 2”) micropatterned silicon chips with almost 100 % area coverage, providing mechanically robust support for delicate samples. We also developed techniques to embed samples between two such films with a thin layer of water or buffer to maintain crystal integrity. This poster will focus on evaluating the use of few layer graphene and polymer thin films for a variety of samples: (1) robust 3D microcrystals of rapid encystment protein (REP24), (2) weakly-diffracting 2D streptavidin crystals and (3) weakly-ordered nanolipoprotein particle (NLP) crystals, and present interesting findings that inform our future work towards making 100 % polymer-based fixed target XFEL substrates.

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. LLNL-ABS-XXXXX.

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BioXFEL

Patricia Gonzalez, University of Puerto Rico

Exploring the anticancer potential of titanium (IV) salicylate as an inhibitor of intracellular iron

Arthur D. Tinoco, Kavita Gaur, Manoj Saxena and Patricia González

Salicylic acid is a beta hydroxy acid that occurs as a natural compound in plants. It has direct activity as an anti-inflammatory agent and acts as a topical antibacterial agent due to its ability to promote exfoliation.[1] Salicylic acid and some other salicylates have been reported to form protonated iron(III) complexes. Knowing this it can be expected to form stable titanium (IV) complexes. Since acetylsalicylic acid, aspirin, does not display cytotoxicity at low concentrations, we expected salicylic acid to have the same behavior. We found that for both our cell lines, A549, human lung carcinoma cells, and MRC5, human lung cells, salicylic acid did not display any cytotoxicity. In this work we synthesize a titanium salicylate complex using salicylic acid and potassium titanium oxide oxalate dehydrate. We recrystallized and used the information from X-Ray crystallography, a titanium colorimetric assay and elemental analysis to characterize the product. Using these tools, we found that our complex may have a molecular of $Na_2TiOC_{14}H_8O_6$, which means two salicylate molecules are complexed to titanium. As a potential anti-cancer complex we will work to determine the cytotoxicity of this complex in the future in the cell lines A549 and MRC5.

(1) *Salicylic Acid;* SDS No. 338 [Online]; PubChem:
Bethesda, MD, December 18, 2018.
https://pubchem.ncbi.nlm.nih.gov/compound/7236#section=Top

Grant 5T34GM007821-39

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BioXFEL

Indra Gonzalez Ojeda, University of Puerto Ricco, Mayagüez

Hydrogen Sulfide (H2S) Limits Amyloid Development in Hen Egg White Lysozyme (HEWL) as a Function of Concentration

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Amyloid fibrils are conformations of misfolded proteins with a stable β-sheet structure. These structures aggregate in various parts of the human body and disrupt the healthy functioning of vital organs, causing diseases known as amyloidosis. Lysozyme is a bacteriolytic enzyme that is synthesized in macrophages, gastrointestinal cells, and hepatocytes and it is commonly found in human saliva and tears; when this protein misfolds, its amyloidosis can have devastating effects on the health, causing sicca syndrome (dry eyes and mouth), rheumatoid arthritis and even renal failure. Here, it is shown that by adding hydrogen sulfide (H2S) to hen egg white lysozyme (HEWL) the formation of amyloid fibers is inhibited. This inhibition results in small spherical aggregates of unordered protein that exhibit almost no cytotoxicity. This effect was further investigated by exposing HEWL to various concentrations of H2S and exploring the protein's conformational changes. This was done through atomic force microscopy (AFM), non-resonance Raman spectroscopy and circular dichroism (CD). The results show that when H2S was present, the protein did not undergo fibrillation and instead kept a secondary structure alike its native structure, with this effect increasing as a function of H2S concentration. Additionally, it is seen that HEWL does not form fibers and instead organizes itself in spherical aggregates and that these new aggregates have a higher abundance of trisulfide bonds (S-S-S). This fact provides valuable information about the mechanism through which H2S might inhibit the pathogenic fibrillation. Further studies are being conducted to unravel the exact mechanism through which hydrogen sulfide prevents the aggregation of lysozyme; However, the effects observed open the door to a possible preventive therapy for deadly amyloidosis disorders.

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Thomas Grant, University at Buffalo

Solving the phase problem in solution scattering

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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[1] for calculating electron density maps directly from solution scattering data. Using only few simple restraints such as solvent flattening, 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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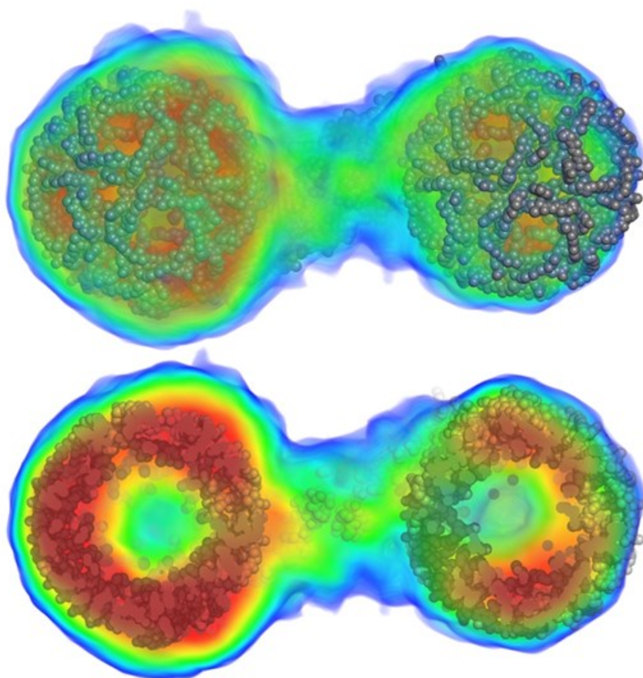

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Thomas Gruhl, PSI - ETH Zürich

Rhodopsin dynamics using pump-probe serial femtosecond crystallography

Thomas Gruhl, Niranjan Varma, Antonia Furrer, Sandra Mous, Petr Skopintsev, Ching-Ju Tsai, Pik Yee Ma, Takashi Tomizaki, Przemyslaw Nogly, Dan James, Tobias Weinert, Jörg Standfuss, Gebhard Schertler, Valérie Panneels

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Rhodopsin is a member of the large family of G protein-coupled receptors, an intensely studied group of membrane proteins with a key role in many cellular signalling pathways. In rhodopsin, the absorption of a photon results in the isomerization of a covalently-bound chromophore ligand (11-cis-retinal), inducing conformational changes in the receptor. These changes, constituting the initial event of dim-light vision, ultimately result in the activation of the cytoplasmic G protein transducin. The 'static' structures of dark[1 -2] and active[3-5] states of rhodopsin have been characterized by X-ray crystallography in cryogenic conditions. Obtaining high-resolution structures of photoactivated intermediates in a time-resolved manner and at room temperature would provide important insights on the detailed mechanism of rhodopsin activation, e.g. cis-to-trans retinal isomerization, rearrangement of amino acid side chains and water molecules, and changes in protonation states (e.g. at the E(D)RY motif). It has been recently shown that pump-probe serial femtosecond X-ray crystallography[6] with an X-ray free electron laser (XFEL) is a powerful method to study the dynamics of structural changes in proteins. For instance, the complete photoactivation mechanism of the proton pump bacteriorhodopsin[7-9] has been determined at an atomic level at the LCLS (Linac Coherent Light Source) and the SACLA (SPring-8 Angstrom Compact Free Electron Laser), by obtaining data from ultrafast changes in the femtosecond range until the millisecond scale. We have now prepared and characterized crystals of wild-type mammalian rhodopsin diffracting to a resolution better than 2 ⍰. The crystals are grown at high density in a lipidic cubic phase in order to use a viscous delivery system offering an optimised constant speed of sample delivery. First SFX tests at SACLA and TR-SFX tests at LCLS showed a satisfactory hit rate, which allowed to obtain diffraction patterns of a 1 microsecond rhodopsin intermediate at a similar resolution as the dark state.

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Rick Hewitt, ASU

Simulations of kilohertz serial femtosecond crystallography with the ASU compact X-ray lightsource

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The Compact X-ray Light Source[1] (CXLS) being constructed at Arizona State University, in collaboration with MIT, SLAC and DESY, is due to deliver first light in 2019. Its unique beam characteristics and widely tunable energy range will open up novel experimental opportunities spanning a wide range of X-ray science. The CXLS is anticipated to deliver 1E8 photons/pulse (at 5% bandwidth) in 300 fs pulses arriving at 1 kHz. To determine the optimal data collection conditions for serial crystallography at the CXLS, we are developing *mcsim,* a program for Monte Carlo simulations of serial snapshot crystallographic data powered by *bornagain,*[2] a GPU-enabled python package for simulating X-ray diffraction under the Born approximation. Monte Carlo sampling on the GPU enables *mcsim* to carry out fast simulations of serial snapshot crystallography diffraction patterns (using PDB files as input) involving broad distributions in X-ray beam divergence, bandwidth, crystal rotational mosaicity and finite mosaic domains, as well as various sample delivery media and flexible geometry.

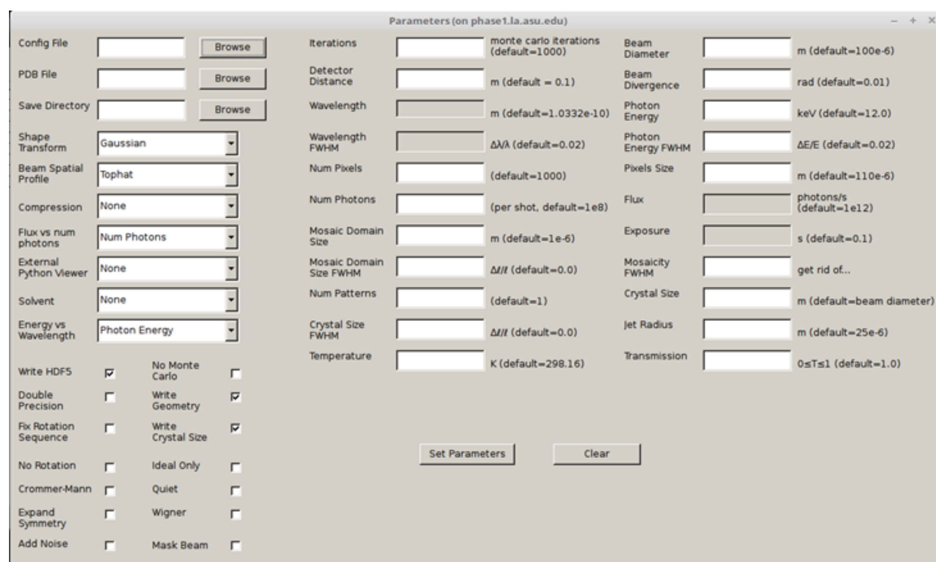

Figure 1: A screenshot of the GUI for *mcsim* under development. Many of the parameters *mcsim* can use to simulate experiments are listed here.

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Hao Hu, ASU

Liquid microjet sample delivery

A reliable sample delivery method is essential for serial femtosecond Crystallography (SFX) experiments. Delivering with a liquid microjet is one of the most popular methods, since it can continuously and stably renew the sample after each X-ray pulse. We have developed two types of devices for microjet delivery: Lipidic Cubic Phase (LCP) injectors for high-viscosity samples, and Gas Dynamic Virtual Nozzles (GDVN) for low-viscosity samples. Both have been successfully and broadly applied to many SFX experiments in X-ray Free Electrons Lasers (XFEL) and synchrotrons world-wide.

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BioXFEL

ISHIGAMI IZUMI, ALBERT EINSTEIN COLLEGE OF MEDICINE

SNAPSHOT OF AN OXYGEN INTERMEDIATE IN THE CATALYTIC REACTION OF CYTOCHROME C OXIDASE

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Cytochrome c oxidase (CcO), the terminal enzyme in the electron transfer chain, translocates protons across the inner mitochondrial membrane by harnessing the free energy generated by the reduction of oxygen to water. Time-resolved spectroscopic studies indicate that the dioxygen reduction reaction has several intermediates and follows a sequential mechanism. However, how the oxygen reaction is coupled to proton translocation remains unproven. By using time-resolved serial femtosecond crystallography, we trapped and identified a key oxygen intermediate of bovine CcO. It is assigned to the PR-intermediate, which is characterized by specific redox states of the metal centers and a distinct protein conformation. The heme a3 iron atom is in a ferryl (Fe4+=O2-) configuration and heme a and CuB are oxidized while CuA is reduced. A Helix-X segment is poised in an open conformational state; the heme a farnesyl sidechain is H-bonded to S382; and Loop-I-II adopts a distinct structure. These data offer new insights into the mechanism by which the oxygen chemistry is coupled to unidirectional proton translocation.

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Antonio Kalil-Aulet, University of Puerto Rico—Mayaguez Campus

Modelling and crystallographic studies of the cytoplasmic domain of Wsc1p

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Saccharomyces cerevisiae, the budding yeast, must remodel initial cell shape and cell wall integrity during vegetative growth and pheromone-induced morphogenesis. The cell wall remodeling is monitored and regulated by the cell wall integrity (CWI) signaling pathway. Wsc1p belongs to a family of highly O-glycosylated cell surface proteins that function as stress sensors of the cell wall in Saccharomyces cerevisiae. This cell surface protein has the main role of activating the CWI signaling pathway by stimulating the small G-protein Rho1p, which subsequently activates protein kinase C (Pkc1p) and a mitogen activated protein (MAP) kinase cascade that activates downstream transcription factors of stress-response genes. Wsc1p possesses a cytoplasmic domain where two conserved regions of the sequence have been assessed to be important for Rom2p interaction. One of the two conserved regions of the cytoplasmic domain is composed of a stretch of serine residues including Ser-319, Ser-320, Ser-322 and Ser-323 that can be modified through phosphorylation that inhibits or regulates Rom2p interaction (Fig. 1). In addition, there are Tyr-303 and another conserved region that includes Leu-369, Val-371, Gln-373 and Asp-375 which are involved in further protein-protein interaction when the cytoplasmic domain lacks phosphorylation of the aforementioned serine residues.

There is a lot of research about Wsc1p function in activating the CWI due to stress. Nevertheless, no mechanism for the protein-protein interaction between Wsc1p and Rom2p has been proposed at the molecular level. For this purpose, molecular structure of the cytoplasmic domain of Wsc1p was generated using the standard and fully-automated ORCHESTAR procedures provided by the Sybyl-X 2.1.1 program. The protein model was validated with Procheck-PDBsum and ProSA-web tools. To prove the aforementioned model experimentally, a synthetic peptide encompassing the amino acid positions from Lys-301 to Asp-378 was purchased from Biomatik (ON, Canada) and analyzed through Blue Native PAGE. The results have shown that the synthetic peptide requires further purification.

The purification of the corrected peptide size will be achieved by FPLC using an AKTA Explorer 100 chromatography system and HiPrep™ Sephacryl® S-200 HR column. The purified peptide will then be used for crystallographic analysis with the aim to validate our computer generated model.

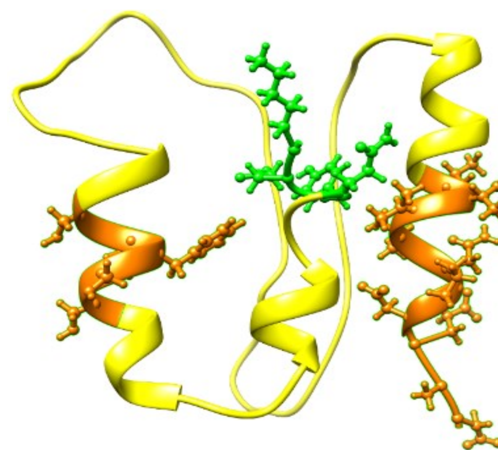

Fig. 1 Modeled tridimensional structure of the cytoplasmic domain of Wsc1p.
The ribbon model shows the N-terminal region in green color (the first three amino acids), the serine residues (at the left) and the C-terminal region (at the right) are in brown color. The protein structure was visu-

Grant acknowledgement: Travel Scholarship for the 2019 International BioXFEL Conference.

The BioXFEL program will be useful for providing the forum to expose our research and significance in a better understanding of how yeast cells response to stress at the molecular level.

Andrea Katz, Cornell University

Mix-and-Inject Serial Crystallography at EuXFEL

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The EuXFEL's high pulse repetition rate creates the potential to collect more diffraction patterns in the same amount of beamtime, making it appealing for serial femtosecond crystallography experiments. This gain will especially benefit mix-and-inject experiments, in which several datasets are collected for each sample at different time points after mixing. However, mixing injector technology requires modifications for use at each new source. Here, we detail a newly designed nozzle rod system that meets the space demands of the SPB/SFX sample environment while allowing for efficient injector loading and changes. A custom feedthrough, placed at the top of the nozzle rod, creates a simple and robust vacuum seal around liquid supply lines, allowing more space for the injector inside the sample chamber itself. Preliminary results from early mix-and-inject beamtimes at EuXFEL hint at a bright future for mix-and-inject experiments at this new, high repetition rate source.

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Daihyun Kim, ASU

Revolution in sample reduction for SFX: droplet-based sample delivery and triggering

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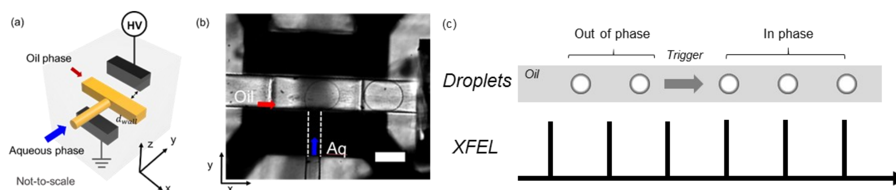
Serial femtosecond crystallography (SFX) utilizing X-ray free electron lasers (XFEL) is a revolutionary technique to determine structures of biomolecular complexes. This method benefits the study of atomic resolution structures of large membrane protein complexes and time-resolved reactions with crystallography. A major challenge of SFX studies with XFELs, however, is the large consumption (up to one gram) of protein crystal sample required to acquire a complete X-ray diffraction data set for structure determination. This is due to the pulsed nature of current XFELs where any pristine crystal sample transported in the path of the X-ray beam during the beam's off-time, is wasted. This sample wastage increases costs and effort by increasing the amount of sample, sample preparation time and XFEL experimentation time. Current XFELs like the Linac Coherent Light Source (LCLS) pulse at a maximum frequency of 120 Hz and the European XFEL (EuXFEL) has MHz-bursts of X-ray pulses that proceed in a 10 Hz train structure. Thus, it is important to deliver sample efficiently to increase X-ray-sample hit rates and reduce sample consumption.

To address this large sample consumption requirement due to the intrinsic pulsed nature of XFELs, we developed a water-in-oil droplet generation system that can inject aqueous droplets containing protein crystals, while the oil carrier phase is delivered in the path of the X-ray beam between the X-ray pulses. We have demonstrated that this droplet generator can produce crystal containing droplets at frequencies compatible with LCLS and EuXFEL instruments. We further developed a 3D-printed microfluidic device with integrated metal electrodes for water-in-oil droplet generation that can be dynamically triggered and manipulated for droplet generation. We demonstrated on-demand droplet generation using DC potentials as well as the ability to tune the frequency of a continuous stream of droplets through the application of AC potentials. More importantly, to assist with the synchronization of droplets and XFEL pulses, we show that the device can induce a phase shift in the base droplet generation frequency. This novel electric triggering approach for enhanced control of droplet generation has the potential to reduce sample waste by more than 95% for SFX experiments with XFELs and can operate under low and high-pressure liquid injection systems.



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Ji-Hye Lee, Korea University

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

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

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

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BioXFEL

Yinfei Lu, Heinrich Pette Institute-Leibniz Institute for Experimental Virology

A novel sample delivery system based on native mass spectrometry for X-ray Free-electron Lasers

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The start of user operation of currently the brightest X-ray Free-Electron Laser (XFEL) at the European XFEL paves the way for single-particle imaging (SPI) with unprecedented high resolution. So far SPI has been mainly achieved by the injection of a crystallized molecular beam, which interacts with the XFEL pulse, in order to acquire diffraction patterns of individual nano-crystals. One major bottleneck of this technique is that it cannot be applied to transient states, which are normally low populated and cannot be purified, and therefore are inaccessible to crystallography. In order to overcome this challenge, a sample delivery system, which is based on native mass spectrometry (MS) technique, is proposed for high-resolution structural imaging of transient conformational states of molecules. The key property of native MS is that it brings non-covalent protein ions into gas phase without any conformational changes. The gaseous intact protein ions are then transferred, selected, and analyzed based on their m/z ratios, where the resulting mass spectra reveal the conformation information on proteins and their assemblies. Consequently, it is possible to analyze transient states, such as proteins and their complexes during a viral life cycle, by native MS with remarkable selectivity and sensitivity. However, the study of transients by native MS alone is limited due to its poor structural resolution. As a result, SPI, which combines native MS with XFEL beam, can greatly increase our knowledge on the structural information for transient states of molecules. In addition, a protein orientating device is implemented to select protein molecules based on their mass and conformations, which will vastly decrease the volume of data sets required for the reconstruction of a single structure. Here a detailed layout of the novel sample delivery system, which is a hybrid of several cutting-edge native MS and protein orientation and separation techniques, will be illustrated and discussed, and some preliminary results from our system under development will be presented.

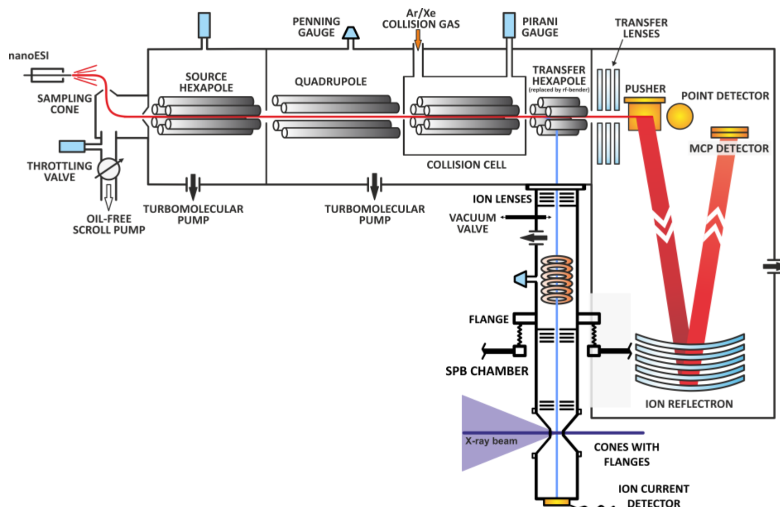

Figure 1 Schematic overview of the sample delivery system layout

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Rafael Maldonado, University of Puerto Rico, Rio Piedras

Preparation and biophysical characterization of nAChR for high-resolution studies

Rafael Maldonado Hernández 1,3, José A. González3, José Orlando Colón4, Claude Maysonet1, 3, Orestes Quesada2, 3 & José Lasalde1, 3

1. University of Puerto Rico Department of Biology; 2. Department of Physical Sciences; 3. Molecular Sciences Research Center; 4. Puerto Rico Medical Sciences Campus4

The nicotinic acetylcholine receptor (nAChR) has long been the holy grail of membrane protein research. These receptors are important as a biological target for the therapeutic development of a large number of diseases such as Alzheimer's Syndrome, Myasthenia Gravis Disease, Parkinson's Syndrome, Schizophrenia and nicotine addiction. Over the past 5 years we have developed a multi-attribute analytical platform that has allowed us to prepare milligram amounts of a functional, high-pure, and stable Torpedo (muscle-type) nAChR detergent complex (Tc-nAChR-LFC16) to achieve a high-resolution structure. We have developed a purification process, which yields crystallization-quality preparations of functional nAChRs for structural studies. Native nAChRs was extracted from the electric organ of Torpedo californica (Tc), using LFC-16 as a detergent, followed by multiples step of chromatography purification. We use MALDI TOF TOF and Nano-LC-MS/MS to identify various important peptides form Tc-nAChR, to demonstrate its purity. A total of 105 target peaks were detected, resulting in confident protein identification by Proteome Discoverer software version 2.1. Also, we characterized the glycan profile of the Tc-nAChR. The most abundant glycan's are mannose 8 and mannose 9. Furthermore, we used circular dichroism spectroscopy, dynamic light scattering, zeta potential, and a two-electrode voltage-clamp assay to examine the stability and functionality of the Tc-nAChR-LFC16. Finally, we performed fluorescence recovery after photobleaching (FRAP) assays displayed a lower mobile fraction with cholesteryl hemisuccinate (CHS) incorporation compared to the implementation of cyclodextrin, suggesting that the removal of the CHS increase the mobile fraction significantly, in comparison with CHS. Our next goal is used theses protein preparation to obtain high-resolution X-ray data of the Tc-nAChR.

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Darya Marchany-Rivera, UPR, Rio Piedras

UV-Vis to determine time-points for serial mix-and-inject X-ray diffraction

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UV-Visible Absorption micro-Spectrophotometry (UV-Vis AS) can help characterize samples for X-ray diffraction by examining ligand binding affinities or reaction rates. UV-Vis AS has proven useful in observing reactions within single crystals and minimizing radiation damage. UV-Vis AS can also help optimize experimental design for structural enzymology at XFELs and serial synchrotron beamlines by timing events of interest along the reaction. We intend to set up a mixing protocol suitable for the UV-Vis ABS system at SSRL to monitor reactions. Here we show preliminary results for the reaction of a model enzyme Isocyanide hydratase (ICH) with its substrate p-nitrophenyl isocyanide (p-NPIC). Crystals were grown in previously determined conditions and cryocooled with 40% glycerol for data collection. Spectra were collected with the UV-Vis AS at BL9-2 at room temperature. Analysis of the spectra revealed that a putative ICH intermediate formed 30 s after mixing.
This work was funded by the National Science Foundation STC award number 1231306, BioXFEL UPR Graduate Student Fellowship. The 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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Sebastian Günther, DESY

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

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

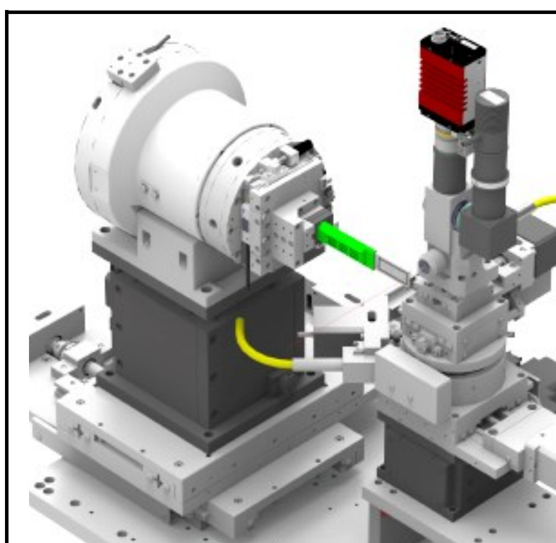

Figure 1: Roadrunner III goniometer for high-speed fixed target serial crystallography experiments.

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

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

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

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

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Osamu Miyashita, RIKEN CCS

Cryo-cooling effect on DHFR crystal studied by replica-exchange molecular dynamics simulations

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Cryo-cooling is routinely performed before X-ray diffraction image collection, to reduce the damage to crystal due to ionizing radiations. It has been suggested that, while backbone structures are usually very similar between room-temperature and cryo-temperature, cryo-cooling may hamper biologically relevant dynamics. In this study, crystal environment of Escherichia coli dihydrofolate reductase (DHFR) at the temperatures from 180 K to 300 K is studied with replica-exchange molecular dynamics (REMD) simulation and results are compared with crystal structure determined at cryo-temperature and room-temperature with time-averaged ensemble method [1, 2].

We analyzed the temperature dependence of physical quantities such as volumes and solvent accessible surface areas, and described how the arrangements of proteins within crystal changes at lower temperatures (Fig. 1). We then discuss the temperature dependent fluctuation of the protein, showing that backbone structure can also be affected by cryo-cooling. Such effects are quite heterogeneous, indicating the complexity of the underlying energy landscape (Fig. 2). While these observations are in accord with experiment, it is found that the protein structure at low temperature can be more heterogeneous than the ensemble of structures reported by using time-averaged ensemble method, putting an alarm to overinterpretation of one average structure and encouraging further development of ensemble refinement method.

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Funding: FOCUS for Establishing Supercomputing Center of Excellence & JSPS Kakenhi

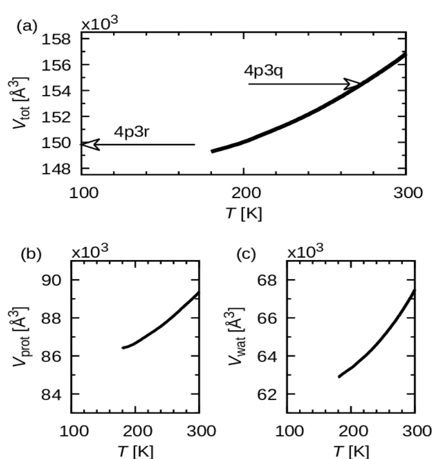

Figure 1: (a) Unit cell volume from simulation as a function of temperature. The arrows indicating the experimental values at 100 K (pdb: 4p3r) and 273 K (4p3q), respectively. (b) and (c) show the volume of proteins and water, re-

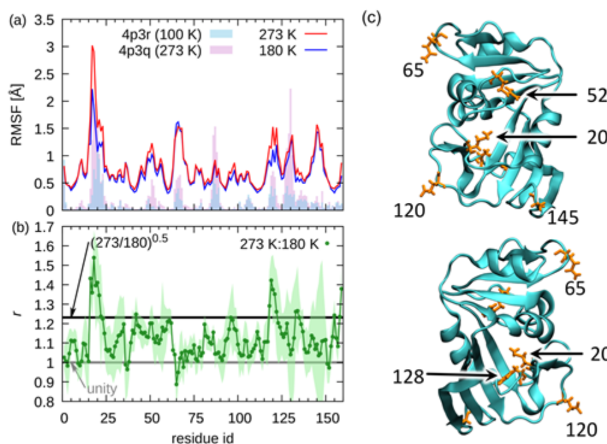

Figure 2: (a) C-RMSF at cryo-temperature and room-temperature, obtained with simulation and experiment. Light blue and pink bars shows experimental data at 100 K and 273 K, while blue and red lines represent the simulation at 180 K and 273 K. (b) Green points show ratio in C RMSF of 273 K to 180 K. The black and gray lines are drawn at the , which is expected for a harmonic energy surface, and unity, which is for ragged surface. In (c), some key residues shown with licorice.

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Diana Monteiro, Rice University

Protein Activation Methods: Photocaging and Microfluidics at Monochromatic Synchrotron Sources

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Henning Tidow (University of Hamburg, Hamburg, Germany)
Martin Trebbin (University of Hamburg, Hamburg, Germany and University at Buffalo, NY, USA)

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Dynamically resolved, or better known time-resolved, structural studies of proteins rely on specific sample environments at both synchrotrons and XFELs due to two main requirements: the continuous delivery of fresh sample to the X-ray beam and the need to trigger the protein activity. The two most widely used methods for protein triggering are the diffusion of actuators by rapid mixing and light activation using laser pulses.

Within collaborative efforts between the Trebbin and Tidow groups and other groups at the University of Hamburg, DESY and ESRF, we have developed sample environments and methods for these complex studies. Specifically, we have recently developed different X-ray compatible devices for serial synchrotron crystallography (SSX) data collection in flow with mixing, including Kapton/metal and 3D-printed microfluidic devices. These devices are capable of delivering ligands to microcrystalline crystal slurries to generate metastable protein species which can then be studied by X-ray diffraction. We have also demonstrated the use of photo-decaying for protein activation for TR-SAXS studies.

These versatile methods can be tailored to the requirements of the protein target characteristics such as time scales of reaction and sample. Therefore, the development of general and user-friendly sample environments is an important step in making these experiments more widely available.

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Keywords: time-resolved, microfluidics, photocaging

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BioXFEL

Sankar Raju Narayanasamy, SUNY at Buffalo

Computational fluid dynamic characterization of liquid sheets suitable for XFEL and synchrotron experiments

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The development of ultrafast X-ray spectroscopic methods has opened new frontiers in ultrafast molecular science to obtain insights into molecular dynamics and interactions [1]. For X-ray spectroscopic use, liquid sheets can be used for sample delivery as they satisfy several requirements, the first of which is optical flatness. The second requirement is that the sheet thickness should be appropriate for spectroscopic studies. And finally, the flat liquid sheets should ideally operate at a low mass flow rate. One sample delivery approach for this application are lithographic microfluidic devices since they allow the production of jets with micrometer and submicron diameter droplets [2, 3, 4] as well as liquid sheets [5,6] using a gas dynamic virtual nozzle (GDVN) geometry. However, characterizing their dynamic behavior experimentally can be complex and time-consuming.

The goal of this work is to use computational fluid dynamic (CFD) methods [7] to study the dynamics of ultrathin liquid sheets that are suitable for time-resolved (TR) absorption spectroscopy and scattering experiments at ultrabright X-ray sources. For this task, we combined high performance computing (HPC) and time dependent numerical analysis with high speed video microscopy to study flat liquid sheets. We use numerical analysis by finite volume approach (FVM) to large eddy simulation (LES) to learn about the underlying physics of complex fluid flow in thin sheet-like micro-liquid jets. This computational methodology allows the prediction of the flow's profile, velocity, temperature, density and its dynamics. We're working on establishing this combined experimental and theoretical approach as a versatile method for the optimization of microfluidic sample delivery devices. The gained insights can help to reduce sample consumption and minimize wasted beamtime due to instability-related clogging-issues in the future. In addition to the spectroscopic applications mentioned above, the use of stable and flat liquid jets offer great potential for XFEL beam diagnostics to monitor pulse repetition rate and beam position.

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Karol Nass, Arizona State University

Long wavelength native SAD phasing serial femtosecond crystallography experiments at the SwissFEL X-ray free-electron laser

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Serial femtosecond crystallography (SFX) is an emerging and highly successful technique for structure determination at X-ray free electron lasers (XFELs) of radiation sensitive samples such as micro-crystals in general and metalloproteins in particular. In addition, the ultra-short duration of XFEL pulses allows access to ultrafast reaction time scales previously not reachable by conventional time-resolved crystallography at synchrotrons. Recently, a collaborative group of scientists from the Paul Scherrer Institute and members of the LeadXpro and Heptares pharmaceutical companies performed the first SFX pilot user experiment at SwissFEL. This successful experiment verified the technical feasibility of SFX at SwissFEL and was an important milestone for the facility before regular user operation that begins in January 2019. During the pilot experiment structure of a membrane protein was determined using native SAD phasing and wavelengths longer than used so far at any other XFEL source for *de novo* structure solution. Additionally, we used a model protein in a systematic study focused on a comparison between the strength of the anomalous signal and the required number of images for *de novo* structure solution. The results suggest a significant decrease of the number of required images at lower photon energies as compared to higher photon energies. The results from the first SFX pilot user experiment confirm that accurate, high-resolution data from protein micro-crystals can be recorded using the JUNGFRAU 16M detector in an efficient manner at SwissFEL by means of the SFX technique. A summary of SwissFEL's parameters, instruments relevant for SFX and results from the first SFX pilot user experiment at SwissFEL will be presented.

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Reza Nazari, Arizona State University

Structural Changes Interpretation in Time-Resolved Solution Scattering with Noise and Real Data 3D Printed Nozzles

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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. High-resolution 3D printing can be used to improve the reproducibility of existing GDVN designs, as well as to rapidly prototype and experiment with new design concepts. Different types of 3D printed nozzles have been developed to achieve low liquid and gas flow rates, high liquid jet velocities, nanodroplets, mixing devices, and more. A double-pulsed nanosecond laser imaging system has been developed to perform Particle Tracking Velocimetry (PTV) in order to rapidly determine jet speeds and diameters, and to quantitatively assess jet stability/reproducibility. Testing results show that some designs can easily achieve stable liquid jets with velocities of more than 80 m/s, which are needed for MHz XFEL sources. 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

Structural Changes Interpretation in Time-Resolved Solution Scattering with Noise and Real Data

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The very recently published work [1] presented interesting method for inverting time-resolved Small Angle X-ray Scattering (SAXS) / Wide Angle X-ray Scattering (WAXS) difference data as it has been thought that there is not enough information to recover a high resolution solution. By extending the concept of the “difference Fourier” method, the work 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.

We here focus on adding Poisson noise to SAXS/WAXS data and determine whether we can successfully reconstruct difference density at given certain noise levels. The effect of noise is significant as SAXS/WAXS intensity falls rapidly at higher q values. The conclusion is that the basic results of our work [1] are applicable when there is an experimental noise. Moreover, we will also try to validate our technique for extracting structural information from experimental time-resolved SAXS/WAXS difference data obtained from [2].

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Nadia Opara, University of Basel

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

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

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

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Valerie Ortiz Gomez, University of Puerto Rico-Rio Piedra

Biophysical characterization of novel biomimetic peptide-polymer conjugate using the properties of antimicrobial peptide Maximin H5

It is estimated that 663 million people still lack improved drinking water sources. Poor sanitation practices lead to biological contamination with viruses, pathogenic bacteria and others microorganisms. Of all the pathogenic bacteria strains present in nature, Escherichia coli and Pseudomonas aeruginosa (both Gram-negative) represent two of the most deleterious and are important indicators of water contamination. The antimicrobial peptides (AMP'S) are active biomolecules using against important multiresistant bacteria. Recent investigations have shown the antimicrobial activity of the peptide Maximin H5 (ILGPVLGLVSDTLDDVLGIL, MH5) which is an integral part of the defense system in the frog Bombina maxima. We evaluate the performance of MH5 as an antimicrobial peptide in order to determine the feasibility of incorporating such peptides onto the structure of water purification membranes. MH5 is compared with hydrophilic and cationic model peptide Tet-20.MH5 is one of these peptides with a unique mechanism, which reduces the growth of waterborne pathogens. This will allow us to discover the appropriate conditions for working with the peptide as well as to obtain information about the viability of the conjugation of Maximin H5 with polymers for the fabrication of the membrane. PEGylation in peptides improve stability, solubility and thermal stability. In this study we present the peptide-polymer conjugation with the polymer Methoxy-PEG-Maleimide (2K, 5K, 20K). For this study, we have performed a SDS-PAGE and Bioanalyzer (capillary electrophoresis) technique for determination of polymer-peptide conjugate. To evaluate the molecular mass of this conjugate MALDI-TOF was employed. NMR and FTIR technique we used for characterization the covalent bond in this reaction. Additionally, we study the antimicrobial activity of this conjugate with Minimal Inhibitory Concentration (MIC) and Growth Curves. Thereby, this peptide-polymer conjugate can use to biomedical applications and in biomaterials such as membranes with water purification approach.

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Suraj Pandey, UW-Milwaukee

Structural basis for light control of cell development revealed by crystal structures of a myxobacterial Phytochrome*

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Phytochromes belong to family of red light photoreceptors that control light responses in various organisms. They were first characterized in plants with homologs in photosynthetic and non-photosynthetic bacteria known as Bacteriophytochromes (BphPs). BphPs change the structures upon absorption of light. We have worked on an unusual Phytochromes from the non-photosynthetic myxobacterium Stigmatella aurantiaca (SaBpHP1). SaBpHP1 lacks a commonly conserved histidine residue near the chromophore which is replaced by a threonine. Although, the Histidine residue is thought to be important for the Pf to Pfr transition, this transition is still observed even with threonine.

In this poster, we demonstrate the cryo- and room-temperature structures of SaBpHP1 and also show their role in fruiting body formation of this photomorphogenic bacterium.

*This work is available in https://doi.org/10.1107/S2052252518010631

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Surya Pulavarti, SUNY Buffalo

X-ray free electron lasers meet NMR: structural enzymology of β-lactamase

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To exemplify the outstanding potential synergy of 'mix-and-inject serial crystallography' (MISC) free electron laser[1] and NMR solution data, we are pursuing a structural-enzymological study of 29 kDa class A β-lactamase (BlaC) from *M. tuberculosis*. Our solution NMR data confirm that the XFEL BlaC structure[1] obtained before substrate (ceftriaxone) binding represents an excellent solution structure model. In contrast, our NMR data strongly suggest that large protein conformational excursions occur during catalysis relative to low free energy states captured in the XFEL structures of intermediate BlaC complexes formed during catalysis. Furthermore, our NMR data reveal pronounced product inhibition of BlaC (see accompanying poster by Yuen *et al.*). These findings pave the way for (i) future solution NMR studies of the BlaC complexes formed during catalysis, (ii) XFEL SAXS solution studies to reconcile XFEL and NMR data, and (iii) the development of dynamic structural-biological models of the enzymatic cycle, which promise to feature unprecedented accuracy.

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See also accompanying poster: Yuen *et al.* "Product inhibition of β-lactamase".

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BioXFEL

Angel Rodrigues, UPR-Mayaguez

Oxy-myoglobin's interaction with hydrogen sulfide: A pathway from compound III to compound 0

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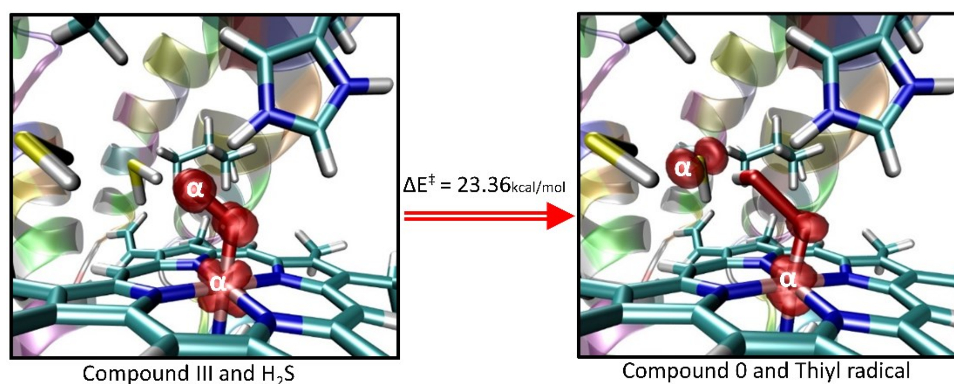
Myoglobin (Mb) binds oxygen with high affinity as a low spin singlet complex and thus functions as an oxygen storage and transport protein that is highly resistant to autoxidative processes. Yet, Density Functional Theory/Molecular Mechanics calculations of oxy-Mb models predict that simply substituting an active-site water molecule for hydrogen sulfide (H_2S) significantly lowers the energy barrier to forming Compound (Cpd) 0 and overwhelmingly favors a Cpd III ground state singlet over the triplet state when a neutral His64 is present. At neutral pH, His64 is predominantly monoprotonated and thus proton donation to progress beyond the Cpd 0 intermediate state is limited. Surprisingly, the calculations predict that the spin state preference is completely reversed at acidic pH (when His64 is doubly protonated) favoring a paramagnetic triplet, the barrier to Cpd 0 is reduced to 23.36 kcal/mol. In both cases the models foresee that the intermediate should be detectable in UV-VIS experiments. In each case, H_2S donates a hydrogen atom to Cpd III forming Cpd 0 and a thiyl radical, a phenomenon not observed with water. Typically, exotic techniques are required to observe Cpd 0 but under the conditions just described, this intermediate is readily detected in UV-Vis spectra at room temperature. The effect is observed as a 2 nm red shift of the Soret band from 414nm to 416nm at an acidic pH, and from 416nm to 418nm at more basic pH levels.



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Jessica Rodriguez-Rios, UPR Rio Peidras

Uncovering DNA binding specificity of cardiac transcription factors complexes of GATA4, NKX2-5 and TBX5

Jessica Rodríguez-Rios[1]; Emili Rosado-Rodríguez [1]; José A. Rodríguez-Martínez [1]

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Transcription factors (TFs) are DNA binding proteins responsible for cellular differentiation and development. Human TFs are notorious for not working alone and they often bind DNA as multimeric complexes to regulate gene expression. GATA4, NKX2-5 and TBX5 are transcription factors that are central components of the gene regulatory network of heart development and function. Recent studies have determined the binding specificity of these transcription factors as monomers. However, the DNA-binding specificity of the cooperative complexes between GATA4, NKX2-5 and TBX5 remain undetermined. Based on this, we want to know the intrinsic DNA binding preferences and the emergent properties that result from these TFs complexes. We used the cell -free protein expression system to produce full-length GATA4, NKX2-5 and TBX5. After this, we successfully validated their DNA-binding activity through an Electrophoretic Mobility Shift Assay (EMSA). We are currently working to confirm their cooperative assembly using EMSA. Subsequently, we will determine the *in vitro* DNA-binding specificity of GATA4, NKX2-5 and TBX5 complexes using Cognate Site Identification by High-Throughput SELEX. The findings of this study will help understand the spatial and temporal gene regulation rules involved in normal heart development.

Grant Acknowledgement: This research was supported by NIH SC1GM127231 and NSF HRD-1400868.

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Alexander Rose, San Diego Supercomputer Center, UC San Diego

Towards web molecular graphics for multi-scale models in space and time

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Serial femtosecond X-ray crystallography and Integrative/hybrid experimental methods are rapidly evolving and emerging methods for determining three-dimensional structures of biomolecules. They provide means for studying multi-scale structures in space and time. These structures typically consist of multiple models depicted using components of varying resolution and length scale (e.g., all atom representations, gaussian shapes). To handle such models, visualization and analysis tools need to be able to deal with integrated data from multiple experimental methods - instead of from a single method - and with heterogeneous ensembles of models - instead of a single model or a homogeneous ensemble.

Web-based visualization and analyses of macromolecular structures and associated data represents a critical step in enabling access and gaining knowledge from these data. By embracing advances in browser technology we can create scalable molecular graphics and analysis tools with near-instant data access. To this end we started Mol* (*/'mol-star/,* molstar.org), a collaborative open-source software development project that will provide a common library for macromolecular visualization and analysis to facilitate building tools and services for the scientific community. Examples include showing experimental/validation related data including I/HM models and multi-model trajectories; displaying annotations like SCOP, PFAM, UniProt; or visualizing results from structural bioinformatics or computer aided drug discovery approaches. Mol* aims for interoperability with existing and future solutions by supporting standard file formats and defining *open* domain specific languages for representing and manipulating macromolecular structure data (e.g., molql.org). Examples from PDB (pdb.org) and PDB-Dev (pdb-dev.org) will be presented.

Support: RCSB PDB (NSF, NIGMS, NCI, DOE) and PDBe (EMBL-EBI, Wellcome Trust, BBSRC, EU, MRC).

Keishla Sanchez Ortix, UPR Mayaguez

Optimization and Characterization of Protein Crystals to Study Molecular Structure using Crystallography Methods

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One third of all enzymes are metalloenzymes, enzyme proteins containing metal ions. The oxidation state of the metal cofactor of these enzymes is often a key factor involved in catalyzing reactions necessary for cellular functions. This project focuses on heme proteins myoglobin and hemoglobin. Both proteins contain a heme prosthetic group that conveys functionality. In the body, myoglobin carries oxygen to muscle tissue and hemoglobin is the oxygen-carrier in blood. The primary method used to study the molecular structures of these enzymes is x-ray crystallography. In this technique, the protein crystal causes a beam of incident x-rays to diffract into a specific pattern. By studying the properties of the spots produced by the diffracted beams, a three-dimensional picture of the atoms within the crystal can be produced. The primary focus was to optimize the crystallization and diffraction processes for these heme proteins. To do that, over a hundred conditions were tested including changes in salt, protein and precipitation concentration. Different post-crystallization treatments like seeding, cross-linking and crystal dehydration were also tested. The best condition to grow small and individual human hemoglobin crystals was high concentrations of salt and precipitant with a low protein concentration. Experiments using UV-Vis spectroscopy were carried out to determine the oxidation state of the iron atom present in the protein crystals. The spectra showed four Q bands meaning the crystals had a mixture of met-hemoglobin and deoxy-hemoglobin. Further studies must be done to optimize the diffraction resolution. The small hemoglobin and myoglobin crystals can be used to run calibration experiments in the beamlines.

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BioXFEL

Lysmarie Santos-Velazquez, University of Puerto Rico

Sulfhemoglobin 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 (OxyHb), with H_2S in an anaerobic UV-Vis cuvette. The UV-Vis spectroscopy was used to monitor the sulfhemoglobin complex. The sample characteristic bands are: 420 nm, 620 nm and 720nm. The results showed a positive correlation between the sulfhemoglobin absorbance at 620nm 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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Manoj Saxena, University of California Davis Medical Center

Diverse applications of reconstituted high-density lipoprotein nanoparticles

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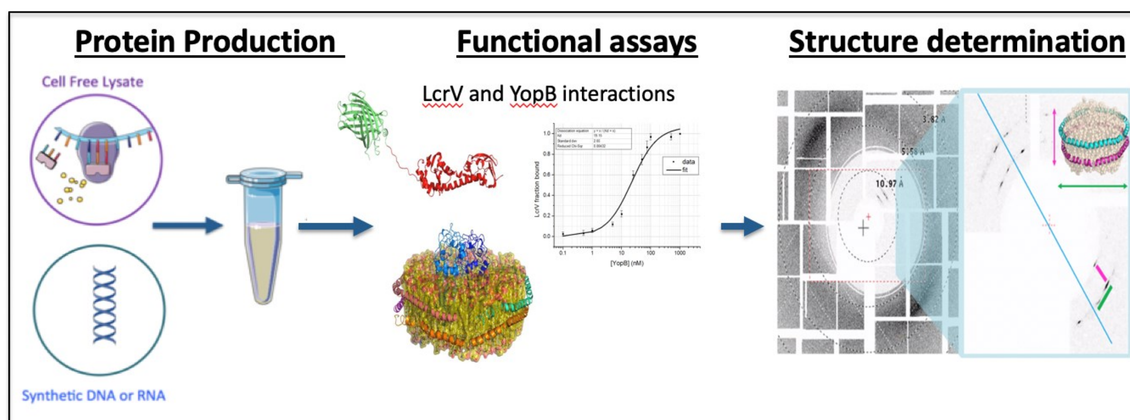
We have developed multiple applications in protein biochemistry utilizing a platform based on High Density Lipid (HDL) particle formation. HDL particles are involved in lipid and cholesterol transport and form proteolipid nanoparticles. These nanolipoprotein particles (NLPs; aka nanodiscs), form 10-25 nm discs and can be reconstituted in vitro. 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 formation. Here, we discuss how our laboratory has used NLPs technologies to produce and support trans-membrane proteins for on-going and future XFEL-based studies. First, we have focused on using NLPs to support functional and structural analysis of membrane proteins. We have produced over three dozen fully functional membrane bound proteins and multi-protein complexes. These include receptors such as rhodopsins, G-protein coupled receptors (GPCRs), kinases, cytokines, antibodies, proteases, outer membrane proteins, and multiple secretion system complexes. Second, the NLPs have shown utility for in vitro and in vivo delivery of functional proteins as well as therapeutics. Correctly folded membrane proteins were shown to fully recapitulate protein signaling in cells. In addition, NLPs have been shown to increase bioavailability and enhanced therapeutic efficacy of molecules complexed with NLPs. Finally, we have demonstrated that NLPs are amenable to multiple structural techniques to include fluorescence correlation spectroscopy (FCS), circular dichroism (CD), electron microscopy (EM), small-angle X-ray as well as neutron scattering (SAXS/SANS) and X-ray diffraction at LCLS and SSRL. These structural techniques have provided a dynamic scale of imaging for understanding HDL particles and NLP-supported membrane proteins. Overall, NLPs represents a unique solution to address multiple bottlenecks in the production and functional and structural characterization of previously difficult to obtain lipid-bound proteins. On-going studies will help refine the function and structure of the aforementioned proteins of interest.



*Contributed equally. Funding was provided by the U.S. Department of Energy under contract number DE-AC52-07NA27344. In addition, work was support by funds from the following: National Institutes of Health: NIGMS grant R01GM117342 and NIAID grant R21AI120925. Funding was also provided by the NASA Translational Research Institute and the NSF BioXFEL Center.

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Robin Schubert, European XFEL

Biological User-support at the European XFEL

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The **European XFEL Sample Environment and Characterization** group provides in-house expertise in sample preparation, delivery and diagnostics methods for the six scientific instruments at European XFEL. The group operates the user sample preparation laboratories and aims to provide excellent support for users coming for beam-time. This includes supporting them with state-of-the-art sample preparation and characterization methods as well as the support and development of novel sample delivery techniques.

The Sample Environment and Characterization group provides large scale instrumentation like X-ray generators, electron microscopes and vacuum test chambers and is involved in the fields of surface science, fluidic systems, cryogenics, magnetism, chemistry, and biology to provide assistance for users preparing their samples and bringing their samples into the beam.

The XBI User Consortium has created and now operates sample preparation and characterization facilities for biological samples at the European XFEL. This purpose built laboratory offers a wide range of biochemical and biophysical characterization techniques and is directly above and connected via a dedicated lift to the SPB/SFX instrument. The Sample Environment and Characterization and XBI and its staff can support users in all steps from gene expression through protein purification, biophysical characterization, and crystallization up to and including sample delivery of both crystalline and non-crystalline samples of a wide range of biological systems at the instruments of the European XFEL.

The poster will present an overview about available equipment in the labs. In addition, possible workflows for sample preparation and characterization are combined and visualized. It serves as a roadmap to optimize samples step by step in order to ensure the best sample quality for experiments at European XFEL.

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Alex Sedlacek, Rigaku Innovative Technologies

Rigaku Innovative Technologies, Inc. provides Meter-Class, Multi-Stripe Coated Mirrors to Stanford Linear Accelerator Facility for use on the Linear Coherent Light Source

Sedlacek, A. [1]

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Rigaku Innovative Technologies, Inc. (RIT) is a provider of precision multilayer and single layer coated optics and crystals for use in industry and scientific research. Using deposition technology developed by Rigaku Innovative Technologies, three (3) 1-meter long mirrors were provided to the Stanford Linear Accelerator Facility (SLAC) for use on the Linear Coherent Light Source (LCLS).

The mirrors feature three different material stripes along the width of the mirrors. The different materials are B_4C, Ni, and a stripe of uncoated Si substrate. This allows researchers the ability to use a wide range of incident angles and energies using a single optic.

RIT's deposition by magnetron sputtering allows for high-density films. Densities, near bulk, allowed for the highest reflectivity possible and maximizing the amount of useful data that can be achieved. The density of the B_4C coating was found to be 2.4 g/cc, with the bulk density of B4C being 2.52 g/cc. The density of the Ni coating was found to be 8.6 g/cc, with the bulk density of Ni being 8.9 g/cc.

Low surface roughness is a vital parameter that must be controlled in the deposition process. High spatial frequency roughness values were measured before and after coating. The intrinsic coating roughness was found to be -0.053 nm RMS for the B_4C, and -0.066 nm RMS for the Ni coating. This means the coating actually smoothed the surface on the high spatial frequency scale.

Utilizing RIT's large optic coating capability (deposition chamber allows up to 1.5 m long substrates to be coated), the mirrors were able to be coated in subsequent deposition runs, allowing for minimal variation between the mirrors.

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MEGAN SHELBY, LAWRENCE LIVERMORE NATIONAL LAB

FIXED TARGET DELIVERY FOR SFX OF WEAKLY-DIFFRACTING OBJECTS

Shelby, M.,[1] Gilbile, D.,[2] Grant, T.,[3] Seuring, C.,[4] Segelke, B.,[1] He W.,[1] Evans A.C.,[1] Pakendorf, T.,[4] Fischer, P.,[4] Hunter, M.S.,[5] Batyuk, A.,[5] Barthelmess M.,[4] Meents, A.,[4] Kuhl, T.,[2] Coleman, M.A.,[1,2], and Frank, M.[1,2]

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The goal of this work is to demonstrate the use of polymer thin films and graphene for sandwiching and support of various weakly diffracting objects on fixed targets as a generally applicable method for high-throughput and high-resolution biological imaging at room temperature. Rapid scanning using the Roadrunner fixed target system has recently been implemented at LCLS (Linac Coherent Light Source), at the MFX endstation in humidified helium and at the CXI endstation in vacuum. Our aim has been to adopt a rapid-scanning approach in the both the vacuum environment of CXI and the humidified environment at MFX using polymer thin-films and graphene as support materials to minimize the background for weakly diffracting samples. Our initial studies focus on 1) 2D crystals of Steptavidin (SA) and 3D microcrystals of 2) Rapid encystment protein (Rep24) to provide a benchmark for polymer/graphene sandwich performance and 3) nanolipoprotein (NLP) particles, or "nanodiscs", weakly diffracting objects for which these constitute novel structural studies.

2D protein crystals of Streptavidin (SA), which were used successfully in our previous 2D X-ray crystallography work,[1] were used as both a test case for feasibility of rapid scanning of 2D crystals in vacuum and to pursue its potential use as an ordered 2D "template" to prepare arrays of single molecules for structure determination. Unlike microcrystalline samples, a continuous, low background surface is needed to support 2D crystals for SFX measurement. The unique feature of having a single molecular layer of the protein makes the opposing side free for interaction without strict conditions for crystal integrity.

Our efforts towards developing supports for microcrystals focus on 1) maintaining sample hydration in vacuum with polymer and graphene encapsulation and establishing methods to perform SFX experiments with minimal amounts of small crystals. The structure of Rep24 was previously solved by our group to 2.0 Å in fixed-target SFX experiments measuring oil-suspended microcrystals at a low repetition rate (10 Hz).[2] Rep24 diffraction was measured at 120 Hz both in the humidified He environment of MFX and enclosed in a polymer thin film/graphene sandwich to characterize the performance of these devices for maintaining sample hydration. NLP microcrystals were similarly studied both in humidified He and in vacuum within a polymer/graphene sandwich. NLPs are discs consisting of a 5 nm thick lipid bi-layer surrounded by a 10-20 nm diameter "belt" of apolipoprotein and are thus membrane-model systems into which membrane proteins can be inserted and thus solubilized. While NLPs provide a scaffold in which membrane proteins can be crystalized in a native lipid environment, the resulting crystal diffraction is highly anisotropic and diffuse, making low background measurements critical.

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, RCSB Protein Data Bank-Rutgers

Incoherent Diffractive Imaging: Simulating Data and Reconstructing a Small Molecule

Andrew S. H. Shevchuk[1], Richard A. Kirian[1], Kevin E. Schmidt[1] and John C. H. Spence[1]

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Classen et al. (2017) proposed incoherent diffractive imaging (IDI), a technique that uses intensity interferometry (i.e., the Hanbury Brown and Twiss effect) between X-ray fluorescent photons emitted by the sample to determine its structure. The effect has advantages over the standard coherent diffractive imaging (CDI) and might be used at XFELs for biomolecular imaging. For example, IDI is element-specific and does not limit the obtainable q-space data to the Ewald sphere like CDI. It utilizes the intensity correlations between pixels on the detector to fill q-space, and this provides the data for a full reconstruction in the limit of many shots captured at a single orientation on a spherical detector.

We show a full forward simulation of this process by generating mock IDI data on a spherical detector from an initial distribution of heavy atoms and then reconstructing the charge density from this data. We examine both the idealistic case of fluorescence generated instantaneously by a delta-function X-ray pulse and the case of a finite pulse duration with realistic fluorescence decay times. As the test case for our methods, we use atoms located at the points of an icosahedron to be suggestive of an icosahedral virus. These simulations were performed on a personal computer, but our methods are scalable to higher-resolution detectors and larger molecules when ported to GPUs on high-performance clusters.

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)

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Jonah Shoemaker, Arizona State University

TBD

Diffractive Stereo Imaging

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In this study, a prospective new method for collecting x-ray diffraction data called diffractive stereo imaging was explored. By placing a tilted mirror behind a sample prior to its diffraction event, a portion of the x-ray beam can be reflected upward before diffracting through the object, and the two resulting diffraction patterns interfere with one another to produce a compound pattern picked up by a detector. This pattern can be used to reconstruct two different 2D projections of the real-space distribution of the object's electrons by using iterative phase retrieval. Since the two diffraction patterns will experience a difference in path length due to the spacing of the object to the mirror, the reflected x-rays arrive out of phase with their counterparts and appear out of focus as a result. Fresnel propagators can be used to bring the reflected image into the same plane as the original before applying the support constraint in the phase retrieval algorithm. In this study, data of a diffraction pattern of three polystyrene spheres with radii of 0.5 microns and their reflections was simulated to test the ability to use the algorithm developed. The simulated diffraction pattern was reconstructed successfully, indicating that the techniques developed in the analysis show promise in regards to use on experimental patterns collected using the stereo imaging setup.

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Abhishek Singharoy, Arizona State University

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

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

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

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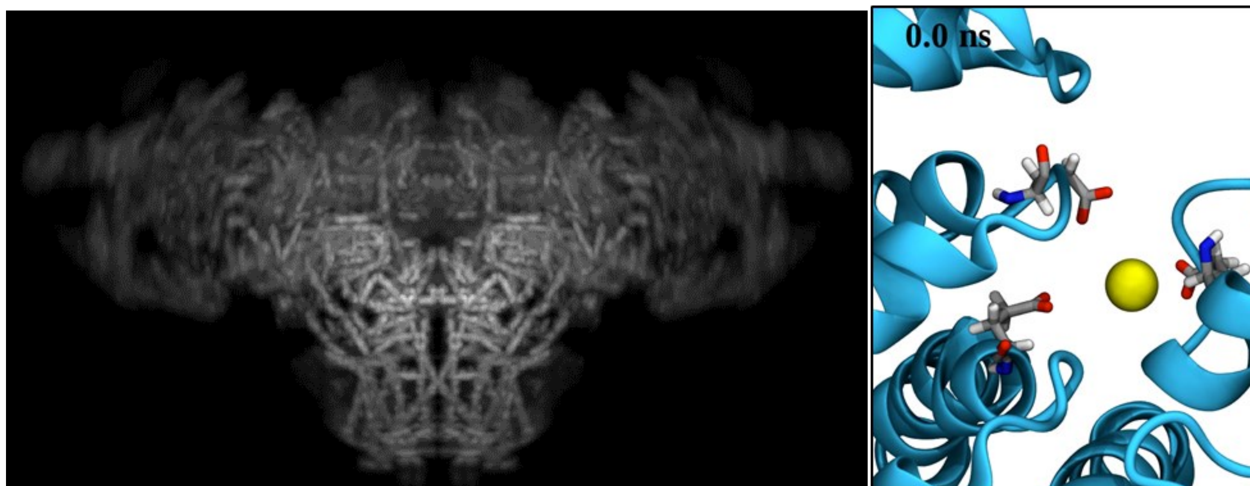

From data to model using molecular dynamics simulations

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TIM STACHOWSKI, HWI-UB

X-RAY RADIATION INDUCED TRANSFORMING GROWTH FACT β1 (TGFβ1)

Stachowski, T., 1,2,3,5 Grant, T., 1,2,3 and Snell, E. 1,3,4

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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 (TGFβ-1) 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 TGFβ-1 is secreted with the 55 kDa dimer, Latency associated peptide (LAP), that renders TGFβ-1 inactive, and together are known as Latent-TGFβ-1 (LTGFβ-1). Dissociation from this arrangement allows the now "activated" TGFβ-1 to bind cognate receptors that initiate signaling pathways and ultimately alter gene expression.

X-ray radiation induced TGFβ-1 activation was first observed in the immuno-histochemical staining of irradiated mammary gland cells [1]. Later work showed that reactive oxygen species (ROS) generated in vitro could also activate TGFβ1 through a non-conserved methionine in LAP [2]. These studies however, overlooked the effect of X-ray generated reductive stress, which could modulate LTGFβ-1 activity through disulfide disruption, and is a phenomenon that has been observed in other proteins by our lab [3]. Therefore, to understand the complete structural pathway of X-ray radiation induced TGFβ-1 activation, we used small angle solution X-ray scattering (SAXS) to simultaneously trigger TGFβ-1 activation and monitor: (1) the dose kinetics of activation, (2) the role of certain amino acids in triggering activation, and (3) to characterize the global structural changes involved in activation.

Our results indicate that X-ray ray radiation serves as a primer for activation, causing a small structural change, where a second step is necessary for TGFβ-1 to fully dissociate from LAP. Additionally, we observed that LAP is more sensitive to radiation when unbound to TGFβ-1 than when bound, suggesting that its ability to rebind TGFβ-1 or to trigger inflammation pathways following irradiation might be hindered. Future experiments aim to investigate the small structural change induced by X-rays using crystallography, hydrogen-deuterium exchange, and circular dichroism. Together, this project aims to not only determine and characterize a novel TGFβ-1 activation mechanism that is relevant for radiation biology, but also to understand how radiation influences protein structure/function.

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NATASHA STANDER, ARIZONA STATE UNIVERSITY

DATVIEW: A GRAPHICAL USER INTERFACE FOR LARGE DATASETS

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DatView is a graphical user interface for plotting multi-parameter datasets, making selections that are synced across all plots, and exporting selections. It was developed for use with CrystFEL indexing results, but can be adapted for any tabulated data with an external xml configuration file. It combines the functionality of two existing programs: cell_explorer[1] and cxiview[2] to provide both synced histograms and a frame-by-frame viewer. Using a preprocessing step to reduce the data into a table index of the original files, it can open large datasets faster. For example, a 71 GB stream file with 94,990 indexed frames that takes around 10 minutes to load in cell_explorer, then around 42 minutes in cxiview, is preprocessed (tabulated) for DatView in around 15 minutes and takes 10 seconds for DatView to open. In this example, the preprocessing step parsed the stream file and extracted additional metadata from the raw diffraction frames (optional). In addition to the features of cell_explorer and cxiview, DatView supports four other plot types, flexible data filtering and sorting tools, and rapid exporting of selections in multiple formats including CrystFEL stream files.

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1. White, T. A. *et al.* Recent developments in CrystFEL. *J. Appl. Cryst* **49,** 680–689 (2015).
2. Barty, A. *et al.* Cheetah: software for high-throughput reduction and analysis of serial femtosecond X-ray diffraction data. *J. Appl. Cryst* **47,** 1118–1141 (2014).

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Shuo Sui, University of Massachusetts Amherst

X-ray compatible microfluidics for advanced room temperature crystallography

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Microfluidic strategy is becoming a powerful approach to enable the crystal growth and subsequent *in situ* serial crystallographic analysis, avoiding the necessity of harvesting micro-crystals and thereof potential physical damages. It has the potential to facilitate the advanced structural characterization that resistant to single-crystal stratedy and to allow room temperature data collection while keeping the sample stability. A microfluidic platform has been established with unique features of incorporated photoresist/graphene films seving as ultra-thin X-ray diffraction window and vapor-diffusion barrier. 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 revealing the dynamics of molecular structures and even simultaneously the analysis of oxygen-sensitive targets because of the low background and barrier properties of the graphene layers, approaching the best balance of sample stability control and X-ray diffraction quality. We are also exploring the feasibilities of utilizing the conductivity of graphene film as an X-ray compatible integrated electrode for the application of an electric field for voltage-jump triggering of protein structural dynamics. The excellently high dielectric strength of photoresist film and the minimized electrode distance of the microfluidic architecture collaboratively allow a remarkably high electric strength applied on the crystal triggering biologically relevant conformational changes. Last but not least, with recently developed fabrication strategy utilizing cleanroom photolithography and nanoimprint lithography, the microfluidic platform can be updated to have high density parallel features and precise feature coordinates, allowing the device manipulations in an automated high throughput fashion. We have been closely collaborating with synchrotron sources to integrate the microfluidic platform with beamline robot arms and sample cassettes to achieve a much accelerated and efficient data collection process.

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Martin Trebbin, SUNY at Buffalo

Microfluidic Reaction Control for Time-Resolved Structure Determination at XFELs and Synchrotrons

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

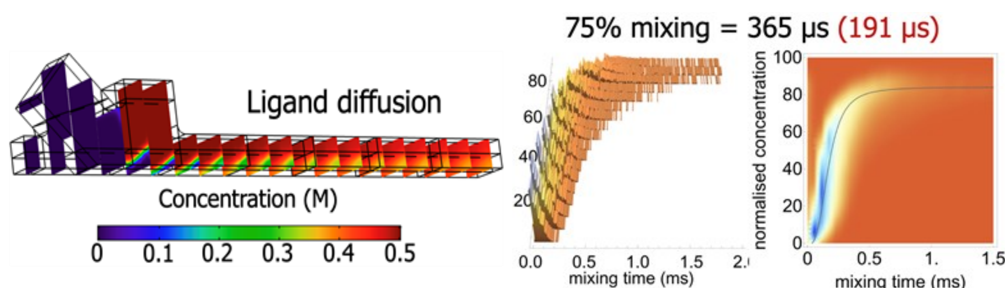

Fig. 1. TR-CFD simulation of microfluidic rapid mixing geometry. (*Top*) 3D-flow simulation of interdiffusion and (*bottom*) the resulting mixing time distribution analysis.

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[2] Schmidt, M., Mix and Inject: Reaction Initiation by Diffusion for Time-Resolved Macromolecular Crystallography, *Adv. Cond. Matter Phys.* 167276 (2013); DOI: 10.1155/2013/167276.

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[4] Monteiro, DCF, Beddard, G., Trebbin M., Defining mixing times, kinetics and time-resolution through statistics: the design of an accurate sub-millisecond rapid-mixing Gas-Dynamic Virtual Nozzle, *in preparation*.

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Jennifer Vargas, UPR-Mayaguez

Hydrogen Sulfide (H2S) Trapment by Hemoglobin I From Lucina pectinata Encapsulated in Sol-gels

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Hydrogen sulfide (H_2S) has been related to be toxic and to have a role in human physiological functions. Therefore, there is a necessity to comprehend ways to scavenger hydrogen sulfide from different media. Here, we used recombinant metaquo-Hemoglobin I (metHbI) from *Lucina pectinate* and metaquo-myoglobin (metMb) encapsulated in tetramethylorthosilicate gel (TMOS), to facilitate the understanding of H_2S transfer toward these metaquo-hemeproteins. In this sol-gel environment, metHbI binds and releases H_2S with rate constants of 5.97 $x10^{-2}$ $M^{-1}s^{-1}$ and 6.67 x 10^{-5} s^{-1}, respectively. This generates a H_2S affinity constant (k_{on}/k_{off}) of 8.9 x 10^2 M^{-1}, which is 10^7 lowers than the analogous constant in solution (6.3 x 10^9 M^{-1}). Although the H_2S k_{off} for the HbI-SH_2 complex are almost similar in both sol-gel and solution. To further understand how the H_2S k_{off} from HbI-SH_2 in solution (5µM) is influence by the protein concentration gradient, metHbI and met-Mb (25µM) encapsulated in TMOS sol-gel. Under these circumstances the H_2S transfer from a solution of the rHbI-SH_2 complex to encapsulated hemeprotein resulted in k_{off} values of 2.50 x 10^{-4} s^{-1} and 2.09 $x10^{-4}$ s^{-1} resulted in the formation of the TMOS rHbI-SH_2 and Mb-SH_2 species, respectively. The results suggest that the: (1) extreme ionic TMOS construct limits the H_2S pathways to reach the hemeprotein active center, (2) possible interaction with met-rHbI hydrophilic forces increases the hydrogen bonding networking and decreases the H_2S association constant, (3) the hemeproteins concentration gradients also influence the reactivity with hydrogen sulfide. The encapsulated met-rHbI reaction with H_2S results were not affected by presence of oxygen or hydrogen peroxide, while met-Mb generated a mixture of met-$MbSH_2$ and sulfmyoglobin (sulfMb) derivative. Consequently, the results show that met-rHbI encapsulated in TMOS is an excellent trap for H_2S from solution or gas media.

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Darex Vera-Rodriguez, University of Puerto Rico

Revealing the mode of action of Ras-Raf inhibitors by molecular dynamics simulations

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Ras/Raf, a protein complex implicated in more than 30% of all human cancers, initiates signaling that can contribute to the development of tumors. Several Monocyte Chemoattractant Proteins (MCP) have been found to inhibit this complex by binding to Ras and thus deactivating Raf gene expression. This work is focused on determining the binding site of the Ras inhibitors using molecular docking and molecular dynamics (MD) simulations. MCP compounds were docked to the Ras crystal structure available in the Protein Data Bank (PDB) database (PDB ID: 4G0N) using SMINA. MD simulations were performed using AMBER between the predicted pose of the MCP compounds and Ras. The results indicate that the C-terminal complexes with the Ras inhibitors via several hydrophobic interactions and hydrogen bonds. These results can lead to the design of more potent Ras inhibitors which could prevent the development of human cancer tumors.

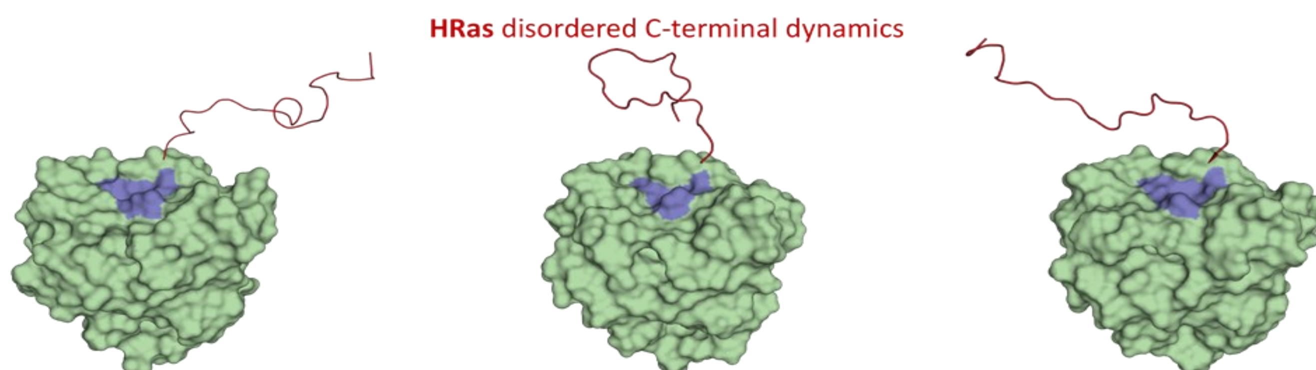

Figure 1. Representation of molecular dynamics simulations on HRas core protein and disordered C-terminal. The red part presented never showed stabilization during the poses obtained**.**

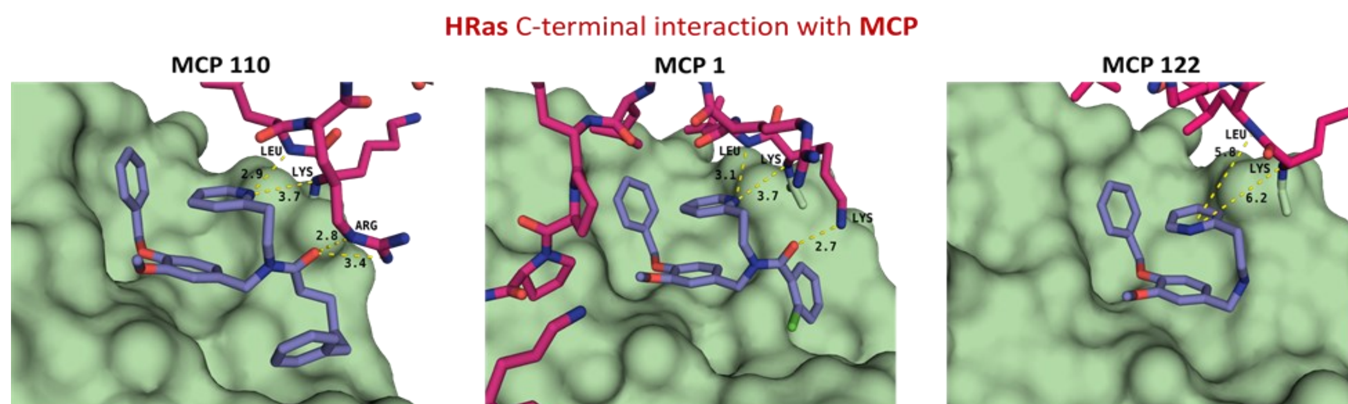

Figure 2. Results on molecular dynamic simulations of MCP compounds interacting with HRas C-terminal. Bonds between the Ras inhibitors and aminoacids are considered stable from measures of 4.0 Å and below.

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Shirley Yuen, University of Puerto Rico

Product inhibition of β-lactamase

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The kinetics of β-lactamase (BlaC) catalyzed cleavage of the antibiotic ceftriaxone (CEF) (Figure 1) was monitored using UV spectroscopy to obtain accurate Michaelis-Menten (MM) parameters in the absence and presence of reaction products. Firstly, MM parameters were measured at 25 °C in three different buffers: (1) 20 mM Tris buffer at pH 7.0 containing 100 mM NaCl (used for NMR resonance assignment; see accompanying poster by Pulavarti et al.), (2) 100 mM MES buffer at pH 6.4 (used for the study by Hugonnet et al.1), and (3) 100 mM sodium acetate buffer at pH 5.0 (used for the MISC XFEL study). The reaction yields two products irreversibly: thiotriazinone (TTA) and CFO (CEF with β-lactam ring hydrolyzed and TTA cleaved off) (Figure 1). Hence, measurements were performed in the presence of product mixture or TTA alone. This revealed for CFO (primarily) competitive inhibition with KI ≈ 20 μM for CFO. In contrast, TTA hardly interacts with BlaC and does not inhibit BlaC significantly.

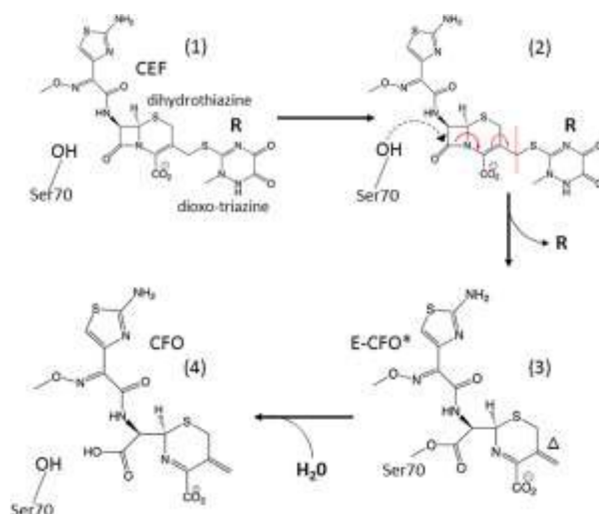

Figure 1 from Olmos *et al.*2 (1) Formation of the enzyme substrate complex by non-covalently binding CEF. (2) Nucleophilic attack of the active site residue Ser 70 results in rearrangement of double bonds and ultimately leads to the opening of the β-lactam ring (blue arrow points to the bond to be cleaved) and the detachment of the leaving group (R). (3) Covalent bond formation between Ser 70 and a shortened species (E-CFO*). Note the double bond Δ. The double bond may react with water to form an alcohol (OH). Evidence for all four intermediate species is found in the experiments of *et al.*2. (4) Species (3) is further hydrolyzed from Ser 70 and leaves the enzyme as product.

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2. Olmos, J. L. *et al.* Enzyme intermediates captured “on the fly” by mix-and-inject serial crystallography. *BMC Biology* **2018,** *16*, 59.

See also accompanying poster: Pulavarti *et. al.* “X-ray free electron lasers meet NMR: Structural enzymology of β-lactamase”.

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Shirley Yuen, University of Puerto Rico

Proteins and enzymes: How many single-particle snapshots do we need?

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XFEL (X-ray Free Electron Laser) sources can be used to determine 3D structure of biomolecules and their complexes using crystallographic methods. These methods require protein crystals. However, crystals are very often difficult to grow, in particular if the molecules are flexible. Single particle imaging (SPI) at near atomic resolution could be an effective way to determine structures and conformations of proteins without the use of crystals. SPI has been applied to investigate large viruses. Based on simulations, we find conditions under which these experiments are also applicable for smaller proteins and enzymes with molecular weights on the order of 200 KDa. In particular, we estimate the required number of diffraction patterns for sufficiently sampling the 3D Fourier space for structural retrievement by iterative phasing. Given realistic X-ray fluences, we estimate how many diffraction patterns are needed to determine the 3D-structure of bacterial phytochrome as a function of resolution.

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Iris Young, University of California-San Francisco

Water oxidation reaction in photosystem II studied by X-ray spectroscopy and crystallography

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The development of XFELs has opened up opportunities for studying the dynamics of catalysis and biological enzymes. Intense XFEL pulses enable us to apply both X-ray diffraction and X-ray spectroscopic techniques to dilute systems or small protein crystals. By taking advantage of ultra-bright femtosecond X-ray pulses, one can also collect the data under functional conditions of temperature and pressure, in a time-resolved manner, after initiating reactions, and follow the chemical dynamics during catalytic reactions and electron transfer.

We have developed spectroscopy and diffraction techniques necessary to fully utilize the capability of the XFEL X-rays for a wide variety of metalloenzymes, and to study their chemistry under functional conditions. One of such methods is simultaneous data collection for X-ray crystallography and X-ray spectroscopy, to look at the overall structural changes of proteins and the chemical changes at metal catalytic sites. The sample is photochemically or chemically activated at various time delays to capture reaction intermediates with crystallography and spectroscopy.

We have used the above techniques to study photochemical activation of the water oxidation reaction of the Photosystem II (PSII) multi subunit protein complex, in which the Mn_4CaO_5 cluster catalyzes the reaction. We report the light-induced structure and electronic state changes of the intermediates during the catalysis. The current status of this research and the mechanistic understanding of this metalloenzyme based on the X-ray techniques is presented (*Kern et al., Nature, 192, 087*).

Grants: This project is supported by the Director, Office of Science, Office of Basic Energy Sciences (OBES), Division of Chemical Sciences, Geosciences, and Biosciences (CSGB), Department of Energy (DOE) and NIH (GM055302, GM110501, GM126289, GM117126).

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Liz Santiago, University of Puerto Rico

Novel biomimetic membrane for water filtration purposes using Lipidic Cubic Phases

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The inadequacy of wastewater infrastructure has led to the development of alternative and more efficient and sustainable purification methods. Biomimetic membranes are a type of interface which is being considered for a wide range of applications, including water remediation. This increased interest is due primarily to their high permeability and selectivity. Lipid Cubic Phases (LCP) are nano-compartmentalized biomaterials composed of lipids that form highly organized structures in all dimensions. These materials are advantageous because they provide stability and are excellent membrane candidates as a material for water separation applications because they form aqueous channels that enable continuous diffusion throughout the membrane.

In the water remediation field, the degradation of small contaminants such as urea has become an increasing issue because it is an uncharged and small molecule that is difficult to reject by size or charge. Various studies have reported the use of LCP's with incorporated enzymes while assessing the residual activity of the enzyme. However, this is the first study that seeks to understand the interactions that occur between LCP's and Urease. Enzymatic assay and Enzyme quantification were performed at different wavelengths between 630nm & 670 nm to determine if the urease activity is affected by encapsulation and to establish a quantitative comparison between free and encapsulated enzyme. Scanning electron microscope images were taken of the membrane material support to help determine pore size and observe rugosity of the material. The membrane support combined with a polymer/doping material was exposed to the FO system and compared to the commercial membrane (HTI) and NF270. Moreover, X-ray measurements are to be employed in order to determine pore size. Achievement of the desired selectivity and specificity could lead to more cost-effective purification system.

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BioXFEL

Jessika Pazol, University of Puerto Rico

Using electrospray deposition technique for enzyme adsorption onto Di-block copolymer self-assembled nano films for water treatment applications

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The proposed study seeks to understand the fundamental aspects related to the packing density of a porous polymeric active nano layer. It is important to create novel membranes for sustainable water treatment as an area of research particularly to mitigate contamination from bacterial origin. In pursuit of that vision, we foster path-breaking scientific investigations via working with bio reactive layers in membrane separation sciences. The porous nano layer results from the self-assembly characteristics of polystyrene-b- poly(4 vinyl pyridine) (PS-b- P4VP) block copolymer (BCP) to obtain cylindrical domains. The PS-b-P4VP forms supramolecular systems that had been previously studied in the bulk [1]. More recently, the complexes of this system to produce porous layers has been investigated via phase inversion and as thin films via spin coating technique [2]. Moreover, we look forward to adsorb enzymes onto the polymeric matrix prepared from the aforementioned method to add functional activities to the active layer of the nano membrane. This present work aims to understand the design of ultra-thins functionalized polymeric nano layers using enzymes. The prepared films will serve as a model to develop further reactive layer membranes for water purification application. Enzymes were physically adsorbed using the electrospray technique which is an emergence investigation area for this type of film preparation. The details for this investigation regarding the methods and analytical techniques will be presented. This work is supported by the NIH RISE program (Grant # 5R25GM061151-16).

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