BioXFEL

A National Science Foundation Science and Technology Center

8th International Conference

February 2-4, 2021

POSTER COMPETITION ABSTRACTS

POSTER SESSION

TUESDAY 4:30-5:30 PM
WEDNESDAY 5:00-6:00 PM

JUDGING COMMITTEE: Sabine Botha (ASU), Bill Bauer (HWI), Darya Marchany-Rivera (UPR), Sarah Perry (UMass), Marc Messerschmidt (ASU)

POSTER #	FIRST NAME	LAST NAME	TITLE
1	Wester	Aldarondo Torres	High-speed Phasing of Single Particle XFEL Diffraction Patterns of Viruses
2	Roberto	Alvarez	Evaluation of Droplet on Demand System for XFEL Sample Delivery
3	Emmanuel	Amoah	Synthesis of Photocaged β2-Adrenocepter Ligands for Time-Resolved Studies of Structure and Dynamics of G-Protein Coupled Receptors (GPCRs)
4	Adil	Ansari	Aerosol beam profile manipulation to improve particle density
5	Lea	Brings	Biological sample preparation and characterization at the European XFEL's XBI laboratory
6	Joe	Chen	Towards phasing using crystal defects.
8	Natalia	Crespo-Rosado	Multi-Modal Imaging for the Location and Detection of Protein Micro and Nanocrystals
10	Connie	Darmanin	Analysis of Multi-Hit Crystals in Serial Synchrotron Crystallography Experiments Using High-Viscosity Injectors
11	Diandra	Doppler	Electronically Stimulated Hybridized Segmented Flow Microfluidics for Reduced Sample Consumption During Serial Femtosecond Crystallography
12	Kara	Zielinski	Mix-and-Inject Serial Crystallography: Routine Even During Remote Operations
13	Mario	Reiser	Tracking equilibrium protein dynamics in solution using MHz X-ray Photon Correlation Spectroscopy
14	Deepshika	Gilbile	Versatile cyclic olefin copolymer (COC) fixed-targets for hydrated, room-temperature serial protein crystallography.
15	Thomas	Grant	Ultrafast time-resolved solution X-ray scattering reveals femtosecond structural dynamics in visual rhodopsin

POSTER SESSION, continued

POSTER #	FIRST NAME	LAST NAME	TITLE
16	Bill	Graves	
17	Frances	Heredia	A novel artificial intelligence-based approach for identification of deoxynucleotide aptamers
18	Rebecca	Jernigan	Structural and Functional Studies of Prophylactic Drug Target, NendoU from SARS-CoV-2 Protease at the Linac Coherent Light Source
19	Konstantinos	Karpos	Multiscale Time-Resolved Solution X-ray Scattering Experiments with Visual Rhodopsin
20	Gabriela	Diaz Figueroa	A Route to Sulfheme Biomarker: Methemoglobin Reactions with Hydrogen Sulfide
21	Abigael	Kosgei	Structural Interrogation of Enzymes Involved in the Biosynthesis of Enediyne Natural Products
22	Dan Bi	Lee	Observation of water dynamics in the Mg2+ coordination at the active site in hen egg white lysozyme
23	Haiguang	Liu	Early-stage dynamics of chloride ion pumping rhodopsins revealed by femtosecond X-ray laser
24	Juan	Lopez-Garriga	Aerobic Met-Myoglobin Single Crystal and Its Hydrogen Sulfide Reaction Products
25	Tek Narsingh	Malla	Pushing the resolution of mix-and-inject serial crystallography to the limit
26	Abhik	Manna	Microfluidic Devices for Membrane Protein Structure Determination and Space Travel Application
27	Dushyant Kr	Srivastava	Structural and functional characterization of histone chaperone Anti-silencing function 1 (PfAsf1) from Plasmodium falciparum
28	Andrew	Shevchuk	Maximizing Visibility in Incoherent Diffractive Imaging
29	Mitchell	Miller	Experience with Molecular Replacement phasing using predicted models
30	Rainier	Mobley	A Metropolis Monte Carlo Algorithm for Merging Single-Particle Diffraction Intensities

POSTER SESSION, continued

POSTER #	FIRST NAME	LAST NAME	TITLE
31	Reza	Nazari	3D Printed Gas Dynamic Virtual Nozzles to Synchronize Droplets with the XFEL (X-Ray Free Electron) Pulses
32	Jessika	Pazol	Understanding how the initial water condition containing calcium ions (Ca2+) affects mutant bacterial lipopolysaccharides (Ra LPS) structure using BioSAXs.
33	Israel	Rodriguez	Elucidating ribonucleotide reductase inhibition by compounds of titanium(IV) chemical transferrin mimics as an anticancer drug strategy
34	Sarthak	Saha	X-ray Compatible Centrifugal Device for Protein Crystallography
35	Ana Sofía	Santiago-Russe	Watch SARS-CoV-2's MTase Kinetic & Dynamic Functions at the XFEL
36	Manoj	Saxena	Cryo-EM structure of the human Lin28b nucleosome reveals likely pioneer factor binding sites.
37	Megan	Shelby	Crystallization of ApoA1 and ApoE4 nanolipoprotein particles and initial XFEL-based structural studies
38	Emina	Stojkovic	High-resolution Crystal Structures of Transient Intermediates in the Phytochrome Photocycle
39	Victoria	Mazalova	Commissioning of a compact X-ray emission spectrometer for the AXSIS facility. Application for manganese-based catalysis
40	Juan Carlos	Villalobos	Expression and Characterization of the Human a4b2 Nicotinic Acetylcholine Receptor for *In Meso* Crystallization
41	Stephanie	Wankowicz	Assessing how side chain conformational heterogeneity changes upon ligand binding
42	Jennifer	Wierman	No Title
43	Lena	Worbs	Optimized nanoparticle injectors for single-particle diffractive imaging experiments
44	Iris	Young	qPTxM: a Tool for Quantifying [Evidence in a Density Map for] Post-Transcriptional Modifications

POSTER #1

Wester Aldarondo-Torres

Northeastern Illinois University

RETURN TO INDEX

High-speed Phasing of Single Particle XFEL Diffraction Patterns of Viruses

Aldarondo-Torres, Wester[1]; Schwander, Peter[2]

1 Department of Computer Science and Engineering, University of Puerto Rico, Mayagüez Campus, Mayagüez, P.R.
2 Department of Physics, University of Wisconsin-Milwaukee, Milwaukee, WI.

In single particle diffractive imaging, differently orientated single particles are beamed with an XFEL. During the imaging, the single particle's phases needed to reconstruct an image are lost; however, phase retrieval recovers these phases by measuring the pixel intensities inside the diffraction pattern. This is customarily done by performing a generalization of the Gerchberg-Saxton algorithm, also known as the error reduction algorithm, via a Central Processing Unit (CPU). A CPU's cores allow the performance of a variety of simultaneous calculations; however, a CPU is not optimal for handling operations with big data, such as large matrix multiplications or Fourier Transforms due to its memory limitations among other things. This means there exists a possibility for the optimization of the iterative error reduction algorithm by simply performing time costly operations, such as Fourier transforms and large matrix multiplications via a Graphics Processing Unit (GPU) which uses its thousands of smaller and efficient GPU cores allowing for the performance of specialized functions on large data sets quickly. Benchmarking tests written in MATLAB were performed using a dummy diffraction pattern obtained from a Covid-19 google image. With this, the error reduction algorithm performs 35 times faster when using the GPU. Additionally, other variations of the error reduction algorithm that use the shrink wrap and beam stop techniques, which assist in generating higher quality images, also experienced the same decrease in computing time. Phase retrieval can now be completed in seconds notably accelerating the phasing of diffraction patterns and the single particle imaging process.

Acknowledgment: This project was funded by BioXFEL National Science Foundation Science & Technology Center NSF STC Award - 1231306

POSTER #2

Roberto Alvarez

Arizona State University, Tempe

RETURN TO INDEX

Evaluation of Droplet on Demand System for XFEL Sample Delivery

Alvarez, R. C.,[1] Karpos, K.,[1] Botha, S.,[1] Cantor, J.,[2] Challa, M.,[2] Nezari, R.,[1,3] Kirian, R.,[1] Sierra, R.,[4] and Hunter, M.[4]

1. Department of Physics, Arizona State University, Tempe, AZ 85287, USA;
2. Scienion US Inc., Tempe, AZ 85281, USA;
3. School for Engineering of Matter, Transport and Energy, Arizona State University, Tempe, AZ 85287, USA;
4. SLAC National Accelerator Laboratory, Menlo Park, CA 94025, USA.

Time-resolved studies of protein dynamics at X-ray Free-Electron Lasers (XFELs) are often limited by the quantity of sample necessary to collect a complete dataset. For some studies, this is a severe problem: for example, with Gas-Dynamic Virtual Nozzle (GDVN), a time-resolved solution scattering dataset from the irreversible photoreaction of visual rhodopsin samples can require a gram or more of purified protein. Likewise, scanning fixed-target schemes are not ideal due to the additional background caused by the substrate and efficient viscous-extrusion injectors cannot be used with such samples. Replacing the GDVN with a synchronized stream of droplets (~50-micrometer in diameter, collecting at 120 Hz) could improve the sample delivery efficiency (photons scattered per sample volume) by a factor of 1000, while simultaneously increasing the data rate (average number of photons scattered per time) by a factor of 10. Despite these prospects, synchronized droplets are not routinely used at XFEL beamlines, often due to the difficulty of reliably operating such systems. We present investigations that show the exceptional promise of delivering XFEL samples with a fully automated drop-on-demand system produced by Scienion US Inc. The system includes live droplet analysis feedback, humidity control, temperature-controlled 96-well sample plates, automated sample infusion and suspension, and automated positioning/swapping of several piezo-driven droplet injectors. If integrated into an XFEL beamline, a drop-on-demand system could vastly improve the prospects for time-resolved protein dynamics studies. Similarly, such a system could enable studies to be conducted at the Compact X-ray Light Source, soon to be commissioned at Arizona State University.

This material is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02-76SF00515 and NSF Awards 1231306, 1943448, 1817862.

POSTER #3

Emmanuel Amoah

Hauptman-Woodward Medical Research Institute

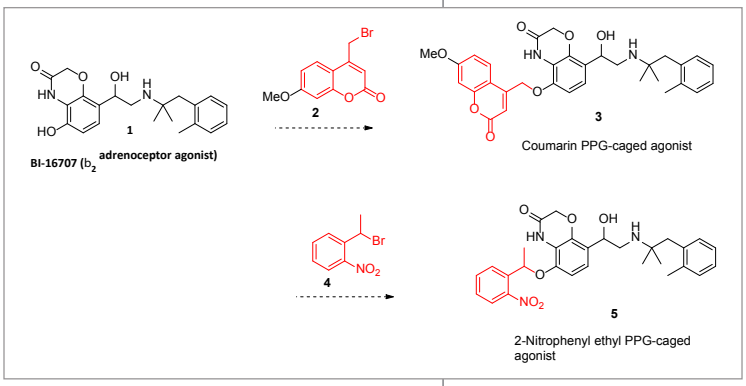
RETURN TO INDEX

Synthesis of Photocaged β2-Adrenocepter Ligands for Time-Resolved Studies of Structure and Dynamics of G-Protein Coupled Receptors (GPCRs)

Emmanuel Amoah[a], Diana Monteiro[a], Petra Fromme[b], Alexandra Ros[b], Wei Liu[b], Michele Zacks[b], and Lan Zhu[b]

a Hauptman-Woodward Medical Research Institute, 700 Ellicott St. Buffalo, NY 14203;
b Biodesign institute, 1001 S McAllister Ave, Tempe, AZ 85281

The role of membrane proteins in processes such as vision, taste, respiration, smell, and nerve function, makes them prime targets for drug design to fight human diseases. Their presence in the cell membranes allow them to control cell-cell interactions, including recognition of bacteria and viruses that enter the cell by interfacing and binding to human cell receptors. The understanding of the complex processes catalyzed by membrane proteins requires the elucidation of their structure, dynamics, and functions. Although G-Protein Coupled Receptors (GPCRs) are very dynamic, most of present images of them are static pictures. Time-Resolved Serial femtosecond crystallography (TR-SFX) provides opportunities for membrane proteins in biological processes to be observed in real time at the molecular level to create molecular movies[1a-c] by triggering their "photocaged" agonists and antagonists with UV laser flashes.



Currently, synthesis[2a-c] of the β2-adrenergic receptor (b2AR) agonist, **1**, has been completed and will be coupled with the photocleavable coumarin, **2**, and 1-(2-nitrophenyl) ethyl, **4**, type protecting groups to produce the "caged" agonists **3**, and **5**. Higher quantum yields (Φ), and high decay constants of the 1-(2-nitrophenyl) ethyl and coumarin PPGs make them suitable for rapid release of the ligand to bind the GPCRs.

References

1. a) Aquila, A.; Hunter, M., S.; Doak, R. B.; Kirian, R., A.; Fromme, P.; White, T., A.; Andreasson, J.; Arnlund, D.; Bajt, S.; Barends, T., R., M. *Opt. Express,* 2012, 20, 3, 2706-2716.;
   b) Tenboer, Jason; Basu, Shibom; Zatsepin, Nadia; Pande, Kanupriya; Milathianaki, Despina; Frank, Matthias; Hunter, Mark; Boutet, Sebastien; Williams, Garth J.; Koglin, Jason E. *Science,* 2014, 346, 6214, 1242-1246.;
   c) Kupitz, C.; Basu, S.; Grotjohann, I.; Fromme, R.; Zatsepin, N., A.; Rendek, K., N.; Hunter, M., S.; Shoeman, R., L.; White, T., A.; Wang, D. *Nature*, 2014. 513, 7517, 261-265.
2. a) Sousa, Ceu M.; Berthet, Jerome; Delbaere, Stephanie; Coelho, Paulo J. *J. Org. Chem.* 2012, 77, 8, 3959–3968,
   b) Kim, Young-Ah; Ramirez, Daniel M. Carter; Costain, Willard J.; Johnston, Linda J.; Bittman, Robert. *Chem. Comm.* 2011,47,9236-9238,
   c) Lu, Yi; Wang, Dong-Hui; Engle, Keary M.; Yu, Jin-Quan. *J. Am. Chem. Soc.* 2010, 132, 5916-5921.

POSTER #4

Adil Ansari

Arizona State Univeristy

RETURN TO INDEX

Aerosol beam profile manipulation to improve particle density

Charged droplets can be used to manipulate the trajectory of an aerosolized particle beam. In this simulation, the Uppsala injector particle beam trajectory is optimized for improving the SNR of the diffraction pattern for XFEL single-particle imaging. Data of droplet size, frequency, position, and velocity distribution are ascertained in Bielecki et. al., 2019 and Hankte et. al., 2018 using Rayleigh scattering microscopy. The aerosolized particle trajectories are integrated from its forces and particle count is aggregated on a bin to generate a discrete map of the particle density profile. This profile is optimized by adding an electric field outside the accelerating nozzle of the Aerodynamic Lens Stack (ALS). As such the acceleration of the particles is a function of aerodynamic drag, electric field forces from the charged plate as well as coulomb repulsion. Under such fields, the slowdown of particles increases the particle density at a point dependent on the strength of the electric field by almost 10x in simulation..

POSTER #5

Lea Brings

European XFEL, Schenefeld, Germany

RETURN TO INDEX

Biological sample preparation and characterization at the European XFEL's XBI laboratory

Huijong Hana[b], Ekaterina Round[c], Robin Schuberta[d,e], Yasmin Gül[c], Jana Makroczyová[a,f], Christina Schmidt[a], Anaïs Chretien[a], Lea Brings[a], Domingo Meza[g], Philipp Heuser[c], Martin Aepfelbacher[h], Imrich Barák[f], Christian Betzeld[e], Petra Fromme[g], Inari Kursulab[i], Poul Nissen[j], Elena Tereschenko[k], Joachim Schulz[a], Charlotte Uetrechta[l,] Jozef Ulický[m], Matthias Wilmanns[c], Janos Hajdun[o]*, Victor S. Lamzin[c]* and Kristina Lorenzen[a]*

a European XFEL GmbH, Holzkoppel 4, 22869 Schenefeld, Germany,
b Biocenter Oulu and Faculty of Biochemistry and Molecular Medicine, University of Oulu, Aapistie 7, 90220 Oulu, Finland,
c European Molecular Biology Laboratory, Notkestrasse 85, 22607 Hamburg, Germany,
d Institute of Biochemistry and Molecular Biology, Laboratory for Structural Biology of Infection and Inflammation, University of Hamburg, c/o DESY, Building 22a, Notkestrasse 85, 22603 Hamburg, Germany,
e The Hamburg Centre for Ultrafast Imaging (CUI), Luruper Chaussee 149, 22761 Hamburg, Germany,
f Institute of Molecular Biology, Slovak Academy of Sciences, Dúbravská cesta 21, 845 51 Bratislava, Slovak Republic,
g Biodesign Center for Applied Structural Discovery and School of Molecular Sciences, Arizona State University, Tempe, AZ, USA,
h Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany,
i Department of Biomedicine, University of Bergen, Jonas Lies vei 91, 5009 Bergen, Norway,
j DANDRITE, Nordic EMBL Partnership for Molecular Medicine, Aarhus University, Department of Molecular Biology and Genetics, Gustav Wieds Vej 10C, DK – 8000 Aarhus C, Denmark,
k Institute of Crystallography, Russian Academy of Sciences, 59 Leninsky prospekt, Moscow, 117333, Russian Federation,
l Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Martinistrasse 52, 20251 Hamburg, Germany,
m Department of Biophysics, Institute of Physics, Faculty of Science, P. J. Šafárik University, Jesenná 5, 04154 Košice, Slovak Republic,
n The European Extreme Light Infrastructure, Institute of Physics, Academy of Sciences of the Czech Republic, Za Radnici 835, 25241 Dolní Břežany, Czech Republic, and
o Laboratory of Molecular Biophysics, Department of Cell and Molecular Biology, Uppsala University, Husargatan 3 (Box 596), SE-751 24 Uppsala, Sweden

The science of X-ray free-electron lasers (XFELs) critically depends on the performance of the X-ray laser and on the quality of the samples placed into the X-ray beam. The stability of biological samples is limited and key biomolecular transformations occur on short timescales. Experiments in biology require a support laboratory in the immediate vicinity of the beamlines. The XBI BioLab of the European XFEL (XBI denotes XFEL Biology Infrastructure) is an integrated user facility connected to the beamlines for supporting a wide range of biological experiments. Arranged around a central wet laboratory, the XBI BioLab provides facilities for sample preparation and scoring, laboratories for growing prokaryotic and eukaryotic cells, a Bio Safety Level 2 laboratory, sample purification and characterization facilities, a crystallization laboratory, an anaerobic laboratory, an aerosol laboratory, a vacuum laboratory for injector tests, and laboratories for optical microscopy, atomic force microscopy and electron microscopy. Here, an overview of the XBI facility is given.

a The laboratory was financed and built by a collaboration between the European XFEL and the XBI User Consortium, whose members come from Finland, Germany, the Slovak Republic, Sweden and the USA, with observers from Denmark and the Russian Federation.

POSTER #6

Joe Chen

Arizona State University, Tempe

RETURN TO INDEX

Towards phasing using crystal defects

J. P. J. Chen[1], J. J. Donatelli[2,4], K. Pande[3,4], K. E. Schmidt[1] and R. A. Kirian[1]

1 Department of Physics, Arizona State University, Tempe, AZ, 85287, USA
2 Department of Applied Mathematics, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA
3 Molecular Biophysics and Integrated Bio-imaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA
4 Center for Advanced Mathematics for Energy Research Applications, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA

Inter-Bragg intensities arising from arrangements of molecules deviating from perfect, infinite lattices can yield an increased information content that allows for model-free structure determination[1-7]. We have previously developed an occupancy formalism that is able to describe the diffraction from finite, arbitrarily truncated crystals, and an associated phase retrieval algorithm that successfully phases such diffraction[8]. Here we show that the same approach can be applied to phase inter-Bragg intensities arising from crystal defects such as substitutional disorder and certain kinds of stacking faults.

Acknowledgments

RAK, KES and JPJC acknowledge support from NSF Awards DBI-1231306 and DBI-1565180. KP acknowledges support from LBNL Laboratory Directed Research and Development Program FY2020. JJD acknowledges support from the Advanced Scientific Computing Research and the Basic Energy Sciences programs, which are supported by the Office of Science of the US Department of energy under Contract DE-AC02-05CH11231.

References

1 D. Sayre, *Acta Cryst.*, 5, 843 (1952).
2 H.N. Chapman *et al., Nature*, 470, 73-78 (2011).
3 J.C.H. Spence *et al., Opt. Express,* 19, 2866-2873 (2011).
4 V. Elser, *Acta Cryst. A,* 69, 559–569 (2013).
5 J.P.J. Chen and R.P. Millane, *J. Opt. Soc. Am.* A, 31, 1730–1737 (2014).
6 R.A. Kirian *et al., Phil. Trans. R. Soc. B,* 369, 20130331 (2014).
7 R.A. Kirian *et al., Phys. Rev. X,* 5, 011015 (2015).
8 J.P.J. Chen *et al., Acta Cryst. A,* 75, 239–259 (2019).

POSTER #8

Natalia Crespo-Rosado

State University of New York at Buffalo

RETURN TO INDEX

Multi-Modal Imaging for the Location and Detection of Protein Micro and Nanocrystals

Natalia Crespo-Rosado[1,3], Dr. Miranda Lynch[2], Dr. Edward Snell[1,2,3]

1 Department of Material Design and Innovation, SUNY Buffalo, Buffalo, New York 14203 USA;
2 Hauptman-Woodward Medical Research Institute, Buffalo, New York 14203, USA;
3 BioXFEL Center, Buffalo, New York 142036, USA

There has been an increased interest in membrane proteins due to their role in human disease. Known difficulties to crystallize gave rise to high throughput experiments where numerous crystallization conditions can be tested in a single experiment. This approach results in a high volume of output images.

MARCO implements a convolutional neural network to calculate the probability of that image belonging to a particular category in an attempt to standardize scoring. Where, despite reported accuracies being over 90%, this advance comes at the cost of increased false positives in the two major categories: crystals and precipitate.

In this study, we look at additional imaging modalities as potential sources of additional information that could be used to further identify conditions either missed or misidentified by MARCO. Given that case, we explore what image analysis tools could help implement this added data towards addressing false positive and false negatives.

POSTER #10

Connie Darmanin

La Trobe University, Bundoora, Austraila

RETURN TO INDEX

Analysis of Multi-Hit Crystals in Serial Synchrotron Crystallography Experiments Using High-Viscosity Injectors

Marjan Hadian-Jazi[1,2,3], Peter Berntsen[1], Hugh Marman[1], Brian Abbey[1], and Connie Darmanin[1]

1 Australian Research Council (ARC) Centre of Excellence in Advanced Molecular Imaging, Department of Chemistry and Physics, La Trobe Institute for Molecular Sciences, La Trobe University, Bundoora, VIC 3086, Australia;
2 Australian Nuclear Science and Technology Organisation (ANSTO), NSW 2234, Australia
3 European XFEL GmbH, Holzkoppel 4, 22869, Schenefeld, Germany

Serial Synchrotron Crystallography (SSX) is rapidly emerging as a promising technique for collecting data for time-resolved structural studies or for performing room temperature micro-crystallography measurements using micro-focused beamlines. SSX is often performed using high frame rate detectors in combination with continuous sample scanning or high-viscous or liquid jet injectors. When performed using ultra-bright X-ray Free Electron Laser (XFEL) sources serial crystallography typically involves a process known as 'diffract-and-destroy' where each crystal is measured just once before it is destroyed by the intense XFEL pulse. In SSX, however, particularly when using high-viscous injectors (HVIs) such as Lipidico, the crystal can be intercepted multiple times by the X-ray beam prior to exiting the interaction region. This has a number of important consequences for SSX including whether these multiple-hits can be incorporated into the data analysis or whether they need to be excluded due to the potential impact of radiation damage. Here we investigate the occurrence and characteristics of multiple hits on single crystals using SSX with a high viscosity injector

POSTER #11

Diandra Doppler

Arizona State University, Tempe

RETURN TO INDEX

Electronically Stimulated Hybridized Segmented Flow Microfluidics for Reduced Sample Consumption During Serial Femtosecond Crystallography

Diandra Doppler[1,2], Mukul Sonker[1,2], Ana Egatz-Gomez[1,2], Dai Hyun Kim[1,2], Jorvani Cruz Villarreal[1,2], Reza Nazari[2,3], Sahba Zaare[2,3], Darren Thifault[2,3], Raimond Fromme[2,3], Sabine Botha[2,3], Thomas Grant[4], Petra Fromme[1,2], Richard Kirian[2,3], and Alexandra Ros[1,2]

1 School of Molecular Sciences, Arizona State University, Tempe, Arizona 85287-1604, USA.
2 Center for Applied Structural Discovery, The Biodesign Institute, Arizona State University, Tempe, Arizona 85281, USA
3 Department of Physics, Arizona State University, Tempe, Arizona 85287-1504, USA.
4 Department of Structural Biology, Jacobs School of Medicine and Biomedical Sciences, SUNY University at Buffalo, 955 Main St, Buffalo, NY 14203, USA.

With recent advances of X-ray free electron lasers (XFEL), serial femtosecond crystallography (SFX) has enabled the structure determination of challenging proteins such as membrane protein complexes and their reaction dynamics. In SFX experiments with XFELs, the crystal experiences destruction after a single XFEL pulse, and therefore thousands of new crystals must be reintroduced into the X-ray beam to complete a full data set needed to construct an electron density map of the corresponding protein. Because of the serial nature of any SFX experiment, up to 99% of the sample delivered to the X-ray beam during its 'off-time' is wasted due to the intrinsic pulsed nature of all current XFELs. To solve this major problem of large sample consumption, we report a revolutionary sample saving method which is compatible with all current XFELs.

Previously, we had developed a device capable of generating and electronically stimulating aqueous crystal droplets segmented by an immiscible oil phase.[1,2] The device creates crystal laden droplets segmented by an immiscible oil, and allows injection with liquid jets. The phase and frequency of the droplets is optically detected and then shifted, through electronic stimulation, to match the phase of the XFEL. In the most recent triggering device, there are three primary components: a droplet generator and electrical stimulator that can be integrated with an upstream mixer, an optical droplet detector, and the nozzle. These pieces can either be capillary coupled or designed to fit together in a LEGO block style. Once the droplets are generated, the base frequency of the droplets can be detected through the differences in the refractive index by optical fibers situated less than 2mm from the generation site. Previously, the base frequency of the droplets was compared to that of the XFEL it was employed at, and an electrical stimulation was given to retard the generation and change the phase of the droplets. In our most recent experiment, droplets at a frequency lower than the base frequency were generated, then issued continuous electronic stimulations at a set duration, phase, and frequency. This continuous stimulation increased the generation frequency to that of the XFEL and the phase of the droplet could then be shifted to align with the pulses of the laser.

A feedback mechanism for the droplet generation frequency and phase compared to the XFEL was developed by monitoring with LabChart software and custom software controlling delay generator and signal amplifier through a Raspberry Pi. The modular "LEGO" devices for sample delivery along with its intricate Raspberry Pi data processing was deployed at the LCLS in September of 2020. Crystal laden droplets we successfully generated, detected, and stimulated at the Macromolecular Femtosecond Crystallography (MFX) beamline with lysozyme and KDO8PS crystals in varying buffers. Future work includes more in-depth verification of the droplet feedback mechanism as well as studying the nature of the droplets after it leaves the nozzle in the jet.

References

1 Kim, D.; et al. Electric Triggering for Enhanced Control of Droplet Generation. Anal. Chem. 2019, 91 (15), 9792–9799.
2 Echelmeier, A.; et al. Segmented Flow Generator for Serial Crystallography at the European X-Ray Free Electron Laser. Nat. Commun. 2020, 11 (1), 1–10

POSTER #12

Kara Zielinski

Cornell University, Ithaca, New York

RETURN TO INDEX

Mix-and-Inject Serial Crystallography: Routine Even During Remote Operations

Zielinski, K.A.,[1] and Pollack, L.[1]

1. School of Applied and Engineering Physics, Cornell University, 254 Clark Hall, Ithaca, New York 14853, USA

Serial Femtosecond Crystallography (SFX) at X-ray Free Electron Lasers (XFELs) determines room temperature, damage-free, protein structures. Gas Dynamic Virtual Nozzles (GDVNs) rapidly introduce fresh crystals for each X-ray pulse by exploiting a helium sheath to thin the sample stream down to a high-speed jet just before the liquid exits the nozzle aperture. By introducing a microfluidic mixer upstream of the GDVN, Mix-and-Inject Serial Crystallography (MISC) can be performed (Olmos et al., 2018; Calvey et al., 2016; Calvey et al., 2019). A robust design that is modular in nature uses triaxial capillaries to couple a hydrodynamic focusing mixer to a GDVN (Calvey et al., 2019). This device supports stable jets with sufficient speeds for high repetition rates, has wide sample channels (50-100 µm) to reduce the risk of clogging, uses relatively low sample flow rates (3-12 µL/min), and has the flexibility to access timepoints ranging from 3 ms-2000 ms. Additionally, in the event of a clog, each component of the device can be separated and recovered. These injectors have been used successfully at many beamtimes and are compatible with different XFEL facilities, including remote operations during the Covid-19 pandemic (Olmos et al., 2018; Pandey et al., 2020). For remote beamtimes, a set of 16 pre-assembled and pre-characterized nozzles were sent to the XFEL where beamline scientists successfully operated the devices with only virtual assistance. The first remote experiment was at EuropeanXFEL in March 2020, which resulted in five datasets, including one with a time delay as short as 5 ms (Pandey et al., 2020). The second remote experiment was at LCLS MFX in October 2020, which resulted in seven datasets and data analysis is still on-going. The success of these two remote experiments demonstrate that MISC is becoming routine and this is in part due to the ease of use of these nozzles.

POSTER #13

Mario Reiser

Stockholm University, Sweden

RETURN TO INDEX

Tracking equilibrium protein dynamics in solution using MHz X-ray Photon Correlation Spectroscopy

M. Reiser[1*], A. Girelli[2], A. Ragulskaya[2], S. Das[1], S. Berkowicz[1], M. Bin[1], M. Ladd Parada[1], H. Poggemann[1], M. S. Akhundzadeh[3], A. Al-Masoodi[3], S. Timmermann[3], L. Randolph[3], Y. Chushkin[4], T. Seydel[5], U. Bösenberg[6], J. Hallmann[6], A. Madsen[6], J. Möller[6], A. Rodriguez-Fernandez[6], R. Rosca[6], R. Schaffer[6], M. Scholz[6], R. Shayduk[6], A. Zozulya[6], F. Schreiber[2], F. Zhang[2], F. Perakis[1] and C. Gutt[3]

1 Department of Physics, Stockholm University, Sweden
2 Institut für Angewandte Physik, Universtitat Tübingen, Germany
3 Department of Physics, University of Siegen, Germany
4 European Synchrotron Radiation Facility, Grenoble, France
5 Institut Laue-Langevin, Grenoble, France
6 European XFEL, Schenefeld, Germany

Proteins are subjected to thermal fluctuations at physiological temperatures, which is essential for their function. The dynamics of proteins in solution include a variety of processes, such as backbone and side-chain fluctuations, interdomain motions, as well as global rotational and translational (i.e. center of mass) diffusion. So far, XFEL experiments have focused on crystallized proteins aiming either for static structure determination or for ultrafast (fs-ps) laser excited state dynamics. However, the biological relevant dynamics of proteins take place in their native aqueous environment and depend on parameters such as local charges, temperature, concentration, etc. This is especially true for collective dynamics in concentrated protein systems on time scales from microseconds to seconds and in particular with respect to dynamics and kinetics under various conditions.

For instance, it is still an open question to which extent concepts like crystallization, phase separation, and glass transition can be applied to describe non-equilibrium processes like Liquid-Liquid Phase Separation (LLPS) where proteins condense into a dense phase that resembles liquid droplets and a dilute phase[1-4]. Equilibrium processes like dynamic cluster formation in concentrated protein solutions have attracted much interest in the last decade as well due to their relevance for pharmaceutical protein formulations, disease related protein aggregations, drug delivery and formation of organelles in the interior of the cell[5-8]. These are just two examples where a deeper understanding of the microscopic protein dynamics may shed light on the fundamental phenomena and their driving forces.

Investigating these phenomena on the relevant time and length scales is possible by Megahertz X-ray photon correlation spectroscopy (MHz-XPCS)[9] although very challenging and hampered mostly by radiation damage. We present the first step towards systematic studies of complex phenomena in concentrated protein solutions at an XFEL. The measurements were conducted at the Materials Imaging and Dynamics instrument (MID) at European XFEL with highly concentrated solutions of Gamma-Globulin with added PEG (IgPEG). Our findings indicate that it is possible to measure equilibrium dynamics in a short window before the X-rays alter the sample dynamics. This is supported by the shape of the two-time correlation functions and the dispersion relations both pointing to Brownian motion of the protein molecules. After a certain dose threshold has been surpassed, the sample dynamics speed up rapidly. This is accompanied by ultrafast structural changes visible in the azimuthally integrated intensity. Both effects might be due X-ray induced modifications of the local sample chemistry that result in a reduced local viscosity.

References

* mario.reiser@fysik.su.se
1 L. Tang, Nat. Methods , 16 (2019): 18.
2 A. Ianiro et al., Nat. Chem. 11 (2019): 320.
3 S. Alberti, A. Gladfelter, and T. Mittag, Cell 176.3 (2019): 419.
4 P. J. Lu, E. Zaccarelli, F. Ciulla, A. B. Schofield, F. Sciortino, and D. A. Weitz, Nature 453 (2008): 499.
5 N. E. Valadez-Pérez, Y. Liu, and R. Castañeda-Priego, Phys. Rev. Lett. 120 (2018): 248004.
6 K. P. Johnston et al., ACS Nano 6 (2012): 1357.
7 S. von Bülow, M. Siggel, M. Linke, and G. Hummer, Proc. Natl. Acad. Sci . 116 (2019): 9843.
8 F. Roosen-Runge et al., Proc. Natl. Acad. Sci . 108 , (2011): 11815.
9 F. Perakis and C. Gutt, Phys. Chem. Chem. Phys. 22 (2020): 19443.

POSTER #14

Deepshika Gilbile

University of California at Davis

RETURN TO INDEX

Versatile cyclic olefin copolymer (COC) fixed-targets for hydrated, room-temperature serial protein crystallography.

Gilbile, D.[1], Shelby, M.L.[2] Lyubimov, A.[3] Ridgway, E.[1] Wierman, J.L.[3] Cohen, A.E.[3] Russi, S.[3] Coleman, M.A.[2] Frank, M.[2], and Kuhl, T.L.[1]

1 Department of Chemical Engineering, University of California at Davis, Davis, CA 95616, USA;
2 Biosciences and Biotechnology Division, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA.
3 Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, Menlo Park, CA 94025.

One of the key challenges with serial femtosecond crystallography has been efficient, continuous delivery of hydrated protein crystal sample to the X-ray beam while maintaining a low scatter background. Fixed-targets offer several advantages over the widely adopted jet-based sample delivery techniques, like- clog-free delivery, significantly lower sample consumption, and the ability to control crystal sample distribution on-chip using surface-functionalization strategies. The modular, enclosed fixed-target approach enables one to maintain long-term crystal hydration, while tuning sample layer and support film thicknesses to maximize signal-to-noise. Moreover, fixed-targets can be integrated with microfluidic elements to deliver ligands, caged reactants, or electric fields, paving the way for time-resolved experiments.

This work demonstrates the development of versatile, hot-embossed cyclic olefin copolymer (COC) enclosed fixed-targets with ultra-thin, tunable water barrier films made from COC and/or graphene. These supports are inexpensive to fabricate and maintain protein crystal hydration over several days. The supporting polymer layers contribute minimally to the X-ray scatter background while circumventing obstacles arising from beam damage as observed with Si supports. The enclosing COC thin films can be functionalized and patterned using UV-initiated photografting to create surface charge or binding-affinity driven protein-rich interfacial domains that act as “universal” protein crystal nucleants, thereby enabling precise control of sample distribution on chip.

Initial pseudo-serial crystallography measurements at a synchrotron light source (SSRL 12-1 beamline) using model proteins Lysozyme and Thaumatin indicate that high-resolution, high-completeness (no preferential orientation) data collection is possible using these targets with minimal background contribution from the chip components. Our on-chip crystallization approach allows for sample preparation and crystal screening ahead of beamtime, with the aim of maximizing crystal hit-rates and sample throughput. Additionally, surface-interaction driven specific crystal nucleation provides an alternative strategy for protein crystallization, particularly for proteins that are difficult to express in large quantities and crystallize using traditional approaches.

Acknowledgment

This work was performed, in part, under the auspices of the U.S. DOE by LLNL under Contract DE-AC52-07NA27344. This work was also supported by National Science Foundation (NSF) BioXFEL STC Grant 1231306, 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.

POSTER #15

Thomas Grant

State University of New York at Buffalo

RETURN TO INDEX

Ultrafast time-resolved solution X-ray scattering reveals femtosecond structural dynamics in visual rhodopsin

Thomas D. Grant[1], Leslie Salas-Estrada[2], Suchithranga M.D.C. Perera[3], Andrey V. Struts[3], Sarah Chamberlain[4], Xiaolin Xu[5], Steven D.E. Fried[3], Nipuna Weerasinghe[3], Udeep Chawla[3], Roberto Alvarez[6], Jesse Coe[7], Raimund Fromme[7], Konstantinos Karpos[6], Stella Lisova[6], Domingo Meza[7], Reza Nazari[6], Abishek Singharoy[7], Sahba Zaare[6], Nadia A. Zatsepin[8], Fivos Perakis[9], Sergio Carbajo[10], Mark S. Hunter[10], Mengning Liang[10], Matthew D. Seaberg[10], Sébastien Boutet[10], Alan Grossfield[2], Derek Mendez[11], Petra Fromme[7,12], Richard A. Kirian[6], and Michael F. Brown[3,5]

1 Department of Structural Biology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, New York 14203, USA
2 Department of Biochemistry and Biophysics, University of Rochester Medical Center, Rochester, New York 14642, USA
3 Department of Chemistry and Biochemistry, University of Arizona, Tucson, Arizona 85721, USA
4 Roswell Park Comprehensive Cancer Center, University at Buffalo, Buffalo, NY 14203, USA
5 Department of Physics, University of Arizona, Tucson, Arizona 85721, USA
6 Department of Physics, Arizona State University, Tempe, Arizona 85287, USA
7 School of Molecular Sciences, Arizona State University, Tempe, Arizona 85281, USA
8 Department of Chemistry and Physics, La Trobe University, Victoria 3086, Australia
9 Department of Physics, AlbaNova University Center, Stockholm University, SE-10691 Stockholm, Sweden
10 Linac Coherent Light Source, SLAC National Accelerator Laboratory, Menlo Park, California 94025, USA
11 Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
12 Center for Free Electron Laser Science, Deutsches Elektronen-Synchrotron DESY, D-22607 Hamburg, Germany

The recent development of ultrafast X-ray free electron lasers (XFELs) provides exciting new opportunities for investigating the functional dynamics of membrane proteins. For G-protein-coupled receptors (GPCRs) like rhodopsin, an important question is how the rapid local dynamics of the ligand correspond to functional protein transitions. Here we show the structural transitions of bovine visual rhodopsin in solution during the first 10 picoseconds immediately following the photoactivation of retinal using ultrafast pump-probe X-ray scattering and molecular dynamics (MD) simulations. Analysis of the time-resolved scattering profiles and MD simulations shows two dominant states present in solution, likely corresponding to the photorhodopsin and bathorhodopsin intermediates. X-ray scattering data show ultrafast structural changes begin immediately following excitation and are complete within the first five hundred femtoseconds. MD simulations reveal that these changes are local structural motions corresponding to the cis-trans isomerization of retinal and the binding pocket. These local structural changes are followed by slower global protein motions including the expansion of the protein within ~2-3 picoseconds. Comparison of the MD simulations with the scattering data shows that the picosecond global structural expansion is dominated by the movement of the seven transmembrane helices within the first 1-2 picoseconds. The MD simulations reveal that the structural changes propagate outward from the cofactor binding pocket at the speed of sound to drive the global protein motions. Our findings demonstrate how solution scattering with an XFEL is able to inform the functional dynamics of membrane proteins with visual rhodopsin as an important archetype.

POSTER #16

Bill Graves

RETURN TO INDEX

POSTER #17

Frances L. Heredia

University of Puerto Rico

RETURN TO INDEX

A novel artificial intelligence-based approach for identification of deoxynucleotide aptamers

Frances L. Heredia[1], Abiel Roche-Lima[2], Elsie I. Parés-Matos[1]

1 Department of Chemistry, University of Puerto Rico at Mayagüez, Mayagüez, Puerto Rico, United States of America

2 Center for Collaborative Research in Health Disparities, University of Puerto Rico-Medical Sciences Campus, San Juan, Puerto Rico, United States of America

The selection of a DNA aptamer through the Systematic Evolution of Ligands by EXponential enrichment (SELEX) method involves multiple binding steps, in which a target and a library of randomized DNA sequences are mixed for selection of a single, nucleotide-specific molecule. Usually, 10 to 20 steps are required for SELEX to be completed. Throughout this process it is necessary to discriminate between true DNA aptamers and unspecified DNA-binding sequences. Thus, a novel machine learning-based approach was developed to support and simplify the early steps of the SELEX process, to help discriminate binding between DNA aptamers from those unspecified targets of DNA-binding sequences. An Artificial Intelligence (AI) approach to identify aptamers were implemented based on Natural Language Processing (NLP) and Machine Learning (ML). NLP method was used to extract information from the nucleotide sequences. Four ML algorithms (Logistic Regression, Decision Tree, Gaussian Naïve Bayes, Support Vector Machines (SVM)) were trained using data from the NLP method along with sequence information. The best performing model was SVM because it had the best ability to discriminate between positive classes and negative classes. In this model the Accuracy (A) represents the fraction of samples that the model correctly classified, was 0.995 and the Area Under the Receiving Operating Curve (AUROC) represents the degree by which a model is capable of distinguishing between classes, was 0.998. The AI approach developed is useful to identify potential DNA aptamers to reduce the amount of rounds in a SELEX selection. This new approach could be applied in the design of DNA libraries and result in a more efficient and faster process for DNA aptamers to be chosen during SELEX.

Keywords: Artificial Intelligence; DNA aptamers; Machine Learning Approach; Natural Language Processing; SELEX

POSTER #18

Rebecca Jernigan

Arizona State University, Tempe

RETURN TO INDEX

Structural and Functional Studies of Prophylactic Drug Target, NendoU from SARS-CoV-2 Protease at the Linac Coherent Light Source

Rebecca Jernigan[1], Dhenugen Logeswaran[1], Sabine Botha[1], Nirupa Nagaratnam[1], Jay-How Yang[1], Diandra Doplar[1], Mokul Sonker[1], Michele Sheik[1], Gihan Hetawala[1], Megan Shelby[2], Mimi Cho Young[2], Matthew Coleman[2], Julius Chen[1], Matthew Coleman[2], Emily Kaschner[1], Sebastien Boutet[3], Mark Hunter[3], Meng Liang[3], Ray Sierra[3], Raimund Fromme[1], Michelle Zacks[1], Matthias Frank[2], Jose Martin Garcia[1,4], Marius Schmidt[4], Alexandra Ros[1], Debra Hansen[1], Julius Chen[1], Petra Fromme[1]

1 School of Molecular Science and Biodesign Center for Applied Structural Discovery, Arizona State University, Tempe, Az, 85287-1604
2 Lawrence Livermore National Laboratory, 7000 East Avenue, Livermore, CA 94550, USA
3 LCLS SLAC National Accelerator Laboratory
4 Institute Physical-Chemistry Rocasolano, Spanish National Research Council, Serrano street 109, 28006, Madrid, Spain
5 Physics Department, University of Wisconsin-Milwaukee, 3135 N. Maryland Ave, Milwaukee, Wisconsin 53211, USA

NendoU is an endoribonuclease from the SARS-CoV-2 virus (causative agent of the COVID-19 pandemic) that aides in the evasion of host immune system sensors. The negative strand of messenger RNA from the virus contains a repeated sequence of uracil nucleotides identified as a polyuridine (poly-U) tail. This poly-U tail is identified by the immune system as unique to RNA viruses and signals an immune system response. The recently discovered function of NendoU is to cleave the poly-U of negative-stranded RNA, helping hide the presence of SARS-CoV-2 from the immune system. The goal of the project is to determine the first snapshots of NendoU's catalytic mechanism by time resolve serial femtosecond (fs) crystallography (TR-SFX). Purified NendoU protein was shown to be active in solution and in crystallization conditions. *In this poster, we present the first preliminary results of the active NendoU/citrate structure solved to 2.6 Å at room temperature an x-ray free electron laser source.* The structure was refined to an R_{work}/R_{free} of 0.30/0.36. NendoU was allotted 7 shift beamtime at LCLS at MFX (Aug 28th –Sept 1st, 2020 & October 9th – 10th, 2020) where additional data sets were also collected for NendoU apo and mix-and-inject conditions with cleavable RNAs under cleaving and non-cleaving conditions and with inhibitors. *Understanding the structure and catalytic mechanism of NendoU could lead to optimization of existing drugs and novel drug development for those infected or exposed to the SARS-CoV-2 virus.*

Acknowledgment

The project was funded by the National Science Foundation, RAPID funding for COVID research.

POSTER #19

Konstantinos Karpos

Arizona State University, Tempe

RETURN TO INDEX

Multiscale Time-Resolved Solution X-ray Scattering Experiments with Visual Rhodopsin

Konstantinos Karpos[6], C. Swathi K. Menon[3], Roberto Alvarez[6], Leslie Salas-Estrada[2], Suchithranga M.D.C. Perera[3], Andrey V. Struts[3], Sarah Chamberlain[4], Xiaolin Xu[5], Steven D.E. Fried[3], Nipuna Weerasinghe[3], Udeep Chawla[3], Jesse Coe[7], Raimund Fromme[7], Stella Lisova[6], Domingo Meza[7], Reza Nazari[6], Abishek Singharoy[7], Sahba Zaare[6], Nadia A. Zatsepin[8], Fivos Perakis[9], Sergio Carbajo[10], Mark S. Hunter[10], Mengning Liang[10], Matthew D. Seaberg[10], Sébastien Boutet[10], Alan Grossfield[2], Derek Mendez[11], Petra Fromme[7,12], Richard A. Kirian[6], Thomas D. Grant[1], and Michael F. Brown[3,5]

1 Department of Structural Biology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, New York 14203, USA
2 Department of Biochemistry and Biophysics, University of Rochester Medical Center, Rochester, New York 14642, USA
3 Department of Chemistry and Biochemistry, University of Arizona, Tucson, Arizona 85721, USA
4 Roswell Park Comprehensive Cancer Center, University at Buffalo, Buffalo, NY 14203, USA
5 Department of Physics, University of Arizona, Tucson, Arizona 85721, USA
6 Department of Physics, Arizona State University, Tempe, Arizona 85287, USA
7 School of Molecular Sciences, Arizona State University, Tempe, Arizona 85281, USA
8 Department of Chemistry and Physics, La Trobe University, Victoria 3086, Australia
9 Department of Physics, AlbaNova University Center, Stockholm University, SE-10691 Stockholm, Sweden
10 Linac Coherent Light Source, SLAC National Accelerator Laboratory, Menlo Park, California 94025, USA
11 Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
12 Center for Free Electron Laser Science, Deutsches Elektronen-Synchrotron DESY, D-22607 Hamburg, Germany

Our findings based on previous studies of the Visual Rhodopsin GPCR at the Linac Coherent Light Source (LCLS) demonstrate how time-resolved solution scattering (TR-SS) with an XFEL can enable structural studies of functional dynamics of membrane proteins down to sub-picosecond timescales, particularly time-resolved structural detection of cis-trans isomerization of retinal in its binding pocket (see poster by T. Grant, et al.). However, a complete understanding of the activation mechanism of visual rhodopsin requires the extension of this work to longer timescales and the inclusion of additional control studies such as variations in optical excitation wavelength, detergent micelle environment, solvent temperature, dye-mediated heating, and more. Here we describe our ongoing efforts to extend previous work on visual rhodopsin to include multi-timescale small- and wide-angle TR-SS within a broader range of time delays (fs→ms), and with multiple control methods that will help identify the effects of bulk solvent heating. Data collected at the Advanced Photon Source (APS), Linac Coherent Light Source (LCLS) and Spring-8 Angstrom Compact FEL (SACLA) are presented and discussed. In addition to the development of improved control measurement methods, we are also developing a droplet delivery system for TR-SS that will drastically reduce sample consumption and data collection times (see poster by R. Alvarez, et al.).

POSTER #20

Gabriela Díaz-Figuero

University of Puerto Rico

RETURN TO INDEX

A Route to Sulfheme Biomarker: Methemoglobin Reactions with Hydrogen Sulfide

Díaz-Figueroa, G., López-Garriga, J., Santos-Velázquez, L.

University of Puerto Rico, Mayagüez Campus, Mayagüez P.R. 00680

Exogenous hydrogen sulfide (H2S), at a concentration higher than 300 µM, is a highly toxic gas. However, at the same time, H2S is produced endogenously in mammals from the cysteine substrate by four different enzymes in concentrations ranging nearby from 50 µM to 300 µM. Also, increases or decreases in hydrogen sulfide concentrations have been related to different diseases and immunology incidences. For example, H2S decrease in concentration near 100 µM has been associated with inducing fibrosis. At low concentrations, H2S is implied to have potential therapeutic effects, attributing its involvement in different physiological processes as a signaling gas (N. Yang et al., 2020). In the presence of dioxygen (O2) or hydrogen peroxide (H2O2) and H2S, both Hemoglobin (Hb) and Myoglobin (Mb) proteins generate Sulfhemoglobin (sulfHb) and Sulfmyoglobin (sulfMb), respectively. As a result, these complexes could potentially function as biological markers for human diseases.

References

Arbelo-Lópezlópez, H. D., Rodriguez-Mackenzie, A. D., Roman-Morales, E. M., Wymore, T., Lópezlópez

Garriga, J., Mayagü Ez Campus, R., Ez, M., & Rico, P. (2018). Charge Transfer and π to π* Transitions in the Visible Spectra of Sulfheme Met Isomeric Structures. J. Phys. Chem. B, 122, 51. https://doi.org/10.1021/acs.jpcb.7b12393

Paganga, G., Rice-Evans, C., Andrews, B., & Leake, D. (1992, November). Oxidised low density lipoproteins convert oxyhaemoglobin from ruptured erythrocytes to reactive ferryl forms, from https://www.ncbi.nlm.nih.gov/pubmed/148699

Yang, N., Yang, A., Liu, Y., College, M., Li, T., Administration, O., & Tuo, Q. (2020, January 30). Role of Hydrogen Sulfide in Chronic Diseases. Retrieved December, 2020, from https://www.liebertpub.com/doi/abs/10.1089/dna.2019.5067

POSTER #21

Abigael Kosgei

Rice University, Houston, Texas

RETURN TO INDEX

Structural Interrogation of Enzymes Involved in the Biosynthesis of Enediyne Natural Products

Kosgei A[1], Miller M[1], Xu D[1], Phillips G[1,2]

1 Department of Biosciences, Rice University, Houston, TX 77005
2 Department of Chemistry, Rice University, School of Natural Sciences, Houston, TX 77005, USA.

Corresponding author: Abigael J Kosgei, Department of Biosciences, Rice University, 6100 Main St., Houston, TX 77005, ajk15@rice.edu

The need for novel drugs to treat diseases such as cancer and to curb antibiotic-resistance has prompted scientists to use natural products that are a great resource for discovery of drugs, especially small molecule drugs. Most natural products owe their existence to the enzymatic machinery that runs inside microbes and plants. The biosynthetic machinery is made of enzymes that work in a specific fashion to render a complex natural product framework. The structural and functional diversity generated by these natural architects has stimulated interest in elucidating their underlying enzymology. Through understanding the natural products biosynthetic pathway, we can exploit the catalytic ability of enzymes and use them as biocatalysts for structural diversification, activity optimization, and generation of previously unobserved natural products with novel antitumor functions. The enediynes natural products show great potential as anticancer antibiotics. They bind to the minor groove of DNA in such a way that they can abstract two hydrogen atoms from the sugars of the opposite strands causing double-stranded DNA cleavage. The study of the genes in the biosynthetic gene cluster of the 10-membered ring enediynes, calicheamicin (CAL) and dynemicin (DYN), will help us understand the role each enzyme plays in their respective biosynthetic pathways. X-ray crystallography was used to perform structural analysis of proteins CalU17 and DynF. Dimeric structure of DynF was solved to a 1.6 angstroms resolution. Palmitic acid was bound within the dimeric beta barrel suggesting that DynF may be involved in binding the precursor polyene, Heptaene, required for the biosynthesis of dynemicin core. Further biochemical characterization will be done to ascertain the role of DynF in the biosynthetic gene cluster of dynemicin. The His-tag on and tag-off structures of CalU17 were solved to ~2.5 angstroms. CalU17 may have a conserved Ca2+ binding site and hypothesized to catalyze the oxidation of peptidyl cysteine to formylglycine (fGly). Further characterization of CalU17 and DynF, and determination of their functions will expand our abilities to modify and generate a library of novel small molecules that will be used for drug screening.

Acknowledgment

I would like to acknowledge BioXFEL and the National Institute of Health (NIH) Grant # RK745 for providing funds for this project and our collaborators, Thorson and Van Lanen Research groups from the University of Kentucky.

POSTER #22

Dan Bi Lee

Korea University, Sejong, Korea

RETURN TO INDEX

Observation of water dynamics in the Mg2+ coordination at the active site in hen egg white lysozyme

Dan Bi Lee1, Eun Jeong Kim1, Ki Hyun Nam2, Ji-Hye Lee1, Jae Deok Jo1, Jaehyun Park2, Mark S. Hunter3, Sebastien Boutet4, Mi Sook Chung5, and Kyung Hyun Kim1**

1 Department of Biotechnology & Bioinformatics, Korea University, Sejong, Korea;
2 Pohang Accelerator Laboratory, Pohang University of Science and Technology, Pohang, Korea;
3 Physics Division, Lawrence Livermore National Laboratory, Livermore, CA, USA;
4 Linac Coherent Light Source, SLAC National Accelerator Laboratory, Menlo Park, CA, USA;
5 Department Food and Nutrition, Duksung Women's University, Seoul 01369, Korea

Hen egg white lysozyme (HEWL) crystallized readily in the presence of salts is chosen as a model protein to demonstrate the application of serial femtosecond crystallography (SFX) experiments in many XFEL facilities. The optimal activity of HEWL is at pH 5–6.5 with a significant drop in stability at <pH 3 or at >pH 6 in the presence of a reducing agent, 1,4-dithiothreitol (DTT). We collected diffraction data at pH 4.0, pH 5.5, pH 7.0, and pH 7.0 in the presence of DTT using XFEL and synchrotron sources. The SFX structures of HEWL revealed differential binding of Mg+2 to Asp52 and water molecules at the active site, depending on pH, unlike the synchrotron structures. At pH 7.0, the Mg+2 ion shows a tight octahedral geometry with a cluster of five or six water molecules in the first coordination shell and nine water molecules as the second shell. In contrast, loss of a water molecule and simultaneous perturbation of the cation-water interaction is observed at pH 5.5, resulting in loose coordination of the hydrated Mg2+ ion, whereas Mg2+ is no longer present at pH 4.0. Unique structural rearrangements of the hydrated Mg2+ bound by a carboxyl group, observed by SFX, provide a remarkable opportunity to understand water dynamics in metal ion coordination.

POSTER #23

Haiguang Liu

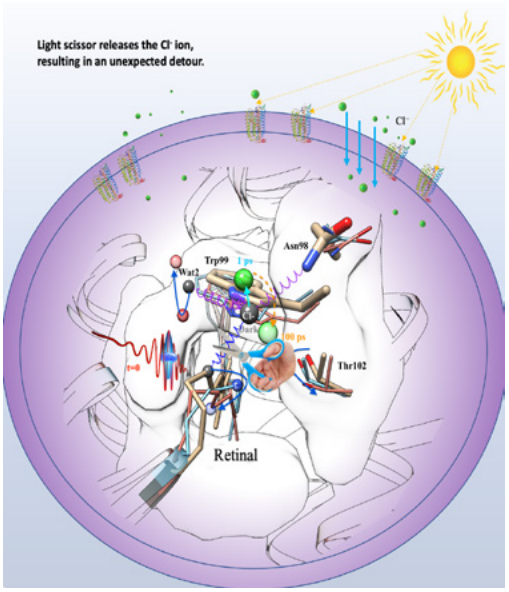
Beijing Computational Science Research Center

RETURN TO INDEX

Early-stage dynamics of chloride ion pumping rhodopsins revealed by femtosecond X-ray laser

Ji-Hye Yun[1†], Xuanxuan Li[2,3†], Jianing Yue[4], Jae-Hyun Park[1], Zeyu Jin[1], Chufeng Li[5], Hao Hu[5], Yingchen Shi[2,3], Suraj Pandey[6], Sergio Carbajo[7], Sébastien Boutet[7], Mark S. Hunter[7], Mengning Liang[7], Raymond G. Sierra[7], Thomas J. Lane[7], Liang Zhou[4], Uwe Weierstall[5], Nadia A Zatsepin[5,8], Mio Ohki[9], Jeremy R. H. Tame[9], Sam-Yong Park[9], John C. H. Spence[5], Wenkai Zhang[4], Marius Schmidt[6*], Weontae Lee[1*] and Haiguang Liu[2,4*]

1 Department of Biochemistry, College of Life Sciences & Biotechnology, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul, 120-749, South Korea
2 Complex Systems Division, Beijing Computational Science Research Center, 8 E Xibeiwang Rd, Haidian, Beijing, 100193, People's Republic of China
3 Department of Engineering Physics, Tsinghua University, Beijing, 100086, People's Republic of China
4 Physics Department, Beijing Normal University, No. 19 Xinjiekouwai St., Haidian, Beijing, 100875, People's Republic of China
5 Department of Physics, Arizona State University, Box 871504, Tempe, AZ, 85287, USA
6 Physics Department, University of Wisconsin, Milwaukee, 3135 North Maryland Ave, Milwaukee, Wisconsin, 53201, USA
7 Linac Coherent Light Source, SLAC National Accelerator Laboratory, 2575 Sand Hill Road, Menlo Park, CA, 94025, United States
8 ARC Centre of Excellence in Advanced Molecular Imaging, Department of Chemistry and Physics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Victoria 3086, Australia
9 Drug Design Laboratory, Graduated School of Medical Life Science, Yokohama City University, Suehiro 1-7-29 Tsurumi-ku, Yokohama 230-0045, Japan



Chloride ion pumping rhodopsin (ClR) in some marine bacteria utilizes light energy to actively transport Cl– into cells. How the ClR initiates the transport is elusive. Here we show the dynamics of ion transport for the first time observed with time-resolved serial femtosecond (fs) crystallography using the Linac Coherent Light Source (LCLS). X-ray pulses captured structural changes in ClR upon illumination with a 550 nm fs-pumping laser. High-resolution structures for five states (dark to 100 ps after illumination) reveal complex and coordinated dynamics comprising retinal isomerization, water molecule rearrangement, and conformational changes of various residues. Combining data from time-resolved spectroscopy experiments and molecular dynamics simulations, this study reveals that the chloride ion close to the retinal chromophore undergoes a dissociation-diffusion process in the early stage of transport.

POSTER #24

Juan Lopez-Garriga

University of Puerto Rico

RETURN TO INDEX

Aerobic Met-Myoglobin Single Crystal and Its Hydrogen Sulfide Reaction Products

Lopez-Garriga, J., Marchany-Rivera, D., Sanchez-Ortiz, K.

University of Puerto Rico, Mayagüez Campus, Mayagüez P.R. 00680

Hydrogen sulfide (H2S) is produced in humans from the amino acid cysteine and four different enzymes. Variations in its concentrations from 50 µM to 150µM have been associated with relevant physiological diseases.[1] Biologically, H2S targets hemeproteins, such as myoglobin (Mb) and hemoglobin (Hb). However, despite extensive efforts, there is a need to comprehend the species resulting from the reaction between met-aquo-myoglobin, H2O-MbFe(III), with H2S in the presence of molecular dioxygen (O2) or hydrogen peroxide environments. Insight into the reaction will lead further to comprehend the H2S role in oxygen storage and delivery systems. A three-minute aerobic reaction between met-aquo-Mb single crystal and H2S (Figure 1) shows electronic transitions at 406 nm, 428 nm, 542 nm, 578 nm, 623 nm, and 644 nm, respectively. Even though there is a mixture of H2O-MbFe(III) and other heme derivatives, they were cryo-trapped, and X-ray information collection was possible. Data processing showed partially undefined density in the iron vicinity with the possible presence of multiple heme species. Water or H2S used as the six heme ligand did not help define the unaccounted heme electron density and its planarity distortion. However, taking into account the formation of sulfMb (a sulfur atom insertion into the heme pyrrole B) derivative and H2S as the heme six coordinating ligand, a better definition of the heme electron density was obtained. The results suggest that the sulfMb derivative formation causes the heme distortion, as previously observed for the analogous reaction between H2O-MbFe(III), hydrogen peroxide, and H2S. A 20 minutes H2O-MbFe(III) reaction with H2S resulted in the reduced Mb derivate, supporting the met-aquo-Mb solution's reaction. The observations allow concluding that met-aquo-Mb in the presence of an H2S excess, generates a deoxy heme species, which with dioxygen generates the oxy-heme-Fe(II) derivatives. Then, these species in the presence of H2S produces sulfMb characterized by the 623 nm transitions.[2] However, a heme partial negative density and the explanation for the unassigned 644 nm transition leaves the situation for further exploration.

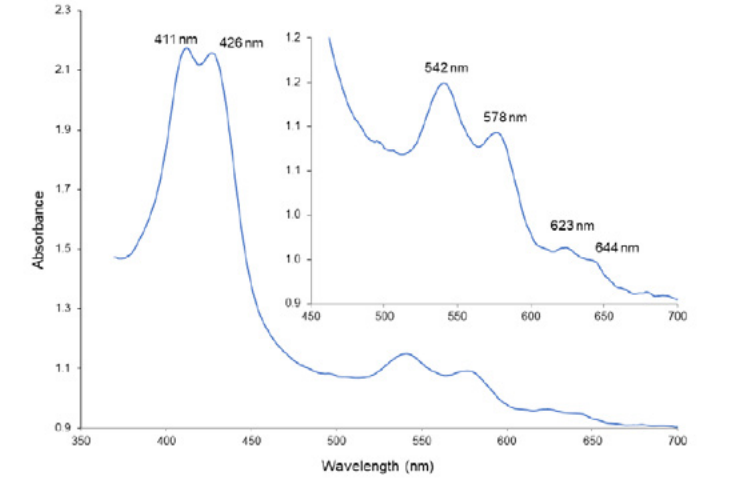


Figure 1. UV-vis spectrum of 3 minutes reaction between H_2O-MbFeIII single crystal with H_2S in a molecular dioxygen (O_2) environment

References

1 Whitfield, N.L., et al., Reappraisal of H2S/sulfide concentration in vertebrate blood and its potential significance in ischemic preconditioning and vascular signaling *Am. J. Physiol. Regul. Integr. Comp. Physiol.* (2008), 294: R1930–R1937,

2 Arbelo-Lopez, H., et al., Sulfheme π to dπ Charge Transfer and π to π* Transitions Leads to Its Like Deoxy and Met Isomeric Structures Visible Spectra. J. Chem. Phys. B. (2018), 122, 4947– 4955..

Acknowledgment

Part of this work was completed at the Stanford Synchrotron Radiation Light source.

POSTER #25

Tek Narsingh Malla

RETURN TO INDEX

Pushing the resolution of mix-and-inject serial crystallography to the limit

Names here?

University of Wisconsin-Milwaukee; Northeastern Illinois University; Cornell University; Rice University; Arizona State University; LCLS

Structural enzymology is now possible at X-ray free electron lasers (XFELs) using mix-and-inject serial crystallography (MISC) technology. In time resolved (TR) MISC, reaction in enzymatic crystals is triggered by mixing with a substrate, and the resulting structural changes are probed by X-ray pulses. We have been studying *Mycobacterium tuberculosis* β-lactamase (BlaC) as a model system. Previous experiments using ceftriaxone (CEF) as substrate solution demonstrated the robust case for routine (TR) MISC at XFELs. Time resolution, however, has been diffusion limited due to large CEF molecule that has to diffuse into enzyme crystals. With recent experience of binding study with sulbactam (SUB), a 3 times smaller molecule than CEF, we planned to push the time resolution of the MISC to the limit. Besides, SUB, in addition, is an inhibitor that irreversibly binds to BlaC. Here we present the results of lowest time resolution ever achieved with MISC, and the enzymatic reaction of BlaC with SUB.

POSTER #26

Abhik Manna

Arizona State University, Tempe

RETURN TO INDEX

Microfluidic Devices for Membrane Protein Structure Determination and Space Travel Application

Abhik Manna,[1,2] Mukul Sonker,[1,2] Sarah Kessans,[3] Alexandra Ros[1,2]

1 School of Molecular Sciences, Arizona State University, Tempe, AZ 85287, United States
2 Center for Applied Structural Discovery, The Biodesign Institute, Arizona State University, Tempe, AZ 85287, United States
3 School of Biological Sciences, University of Canterbury, Canterbury 8041, New Zealand

Membrane proteins are major targets for drug delivery as they play an important role in transport across the cell membranes. Drug molecules can be specifically developed to target the membrane protein of interest if the binding site structure and conformation is known at atomic resolution. The major bottleneck for resolving a natural membrane protein structure via X-ray crystallography is the difficulty in stabilizing these proteins outside the cell membrane to enable crystallization. Lipidic cubic phase (LCP) provides the membrane proteins with their native like environment and can be used to stabilize these proteins outside the cell membrane. To conserve precious proteins, microfluidic devices requiring minimal protein sample can be designed to screen for protein crystals and further deliver protein crystals to state-of-the-art X-ray sources for structure determination.

We have previously designed a microfluidic device with 207 nanowells separated from one another by vacuum-actuated valves which establish and preserve unique crystallization conditions in each well.[1] These devices were previously fabricated with polydimethylsiloxane (PDMS) requiring only 5µL of protein for screening crystallization conditions.[1] Here, we report devices fabricated with the thermoplastic materials cyclic olefin copolymer (COC) and cyclic olefin polymer (COP) with a similar design and functionality facilitated through flexible PDMS membranes. Due to use of highly X-ray transparent material like COC and COP, these devices can be used as fixed-targets to deliver crystals to X-ray light sources for structure determination, in addition to screening for numerous crystallization conditions. The different layers of the device have been imprinted successfully in the thermoplastic material by hot embossing. Water contact angle measurement of thermoplastic surfaces revealed that hydrophilicity increases after 5 seconds of oxygen plasma exposure, which only marginally changed over the course of several days when preserved in 100% humidity. The thermoplastic control layer therefore plasma oxidized prior to bonding with a plasma oxidized PDMS membrane, spin coated on a glass slide. Another thermoplastic fluid layer was aligned with the PDMS containing control layer to fabricate the hybrid device. The optimized assembly conditions resulted in a functioning hybrid device as demonstrated by valve deflection upon applying vacuum to the control layer. To avoid the extremely high viscosity of the traditional LCP, sponge phase with larger aqueous pore sizes and reduced viscosity will be employed to promote crystallization in future crystallization trials with G protein-coupled receptors (GPCRs). The device will be used as fixed-target in the newly developed Compact X-ray Light Source at Arizona State University.

Another two-layer robust version of thermoplastic device was fabricated with COC and COP to study protein crystallization in space. The device also consisted of 207 nanowells but connected via channels not exhibiting valves. The design was imprinted on a 1 mm thick thermoplastic layer by hot embossing and fabrication was completed by thermally bonding the imprinted layer with another 1 mm thick coversheet at 160° C under pressure for 25 min. The infrastructure for mounting and imaging the devices

inside a CubeSat are being optimized at the University of Canterbury for first expected space travel in June 2021.

References:

Abdallah, B. G.; Roy-Chowdhury, S.; Fromme, R.; Fromme, P.; Ros, A. Protein Crystallization in an Actuated Microfluidic Nanowell Device. *Cryst. Growth Des.* 2016, *16* (4), 2074–2082.

POSTER #27

Dushyant Kumar Srivastava

CSIR-Indian Institute of Chemical Biology, Kolkata, India

RETURN TO INDEX

Structural and functional characterization of histone chaperone Anti-silencing function 1 (PfAsf1) from Plasmodium falciparum

Dushyant Kumar Srivastava[1], Sarika Gunjan[2], Chandrima Das[3], Vasudevan Seshadri[2], Siddhartha Roy[1,*]

1 Structural Biology & Bioinformatics Division, CSIR-Indian Institute of Chemical Biology, Kolkata, West Bengal, India
2 National Centre for Cell Science, Pune, Maharashtra, India
3 Saha Institute of Nuclear Physics, Kolkata, West Bengal, India
*correspondence: roysiddhartha@iicb.res.in

Nucleosome assembly and disassembly is tightly governed by histone chaperone proteins such as NAP1, Asf1 and FACT complex. In this study we have done the structural characterization and molecular identification of Asf1 from Plasmodium falciparum (PfAsf1), which is the causative agent of severe form of malaria in humans. We have solved the high resolution crystal structure of histone chaperone domain of PfAsf1 protein. Although the core histone chaperone domain structure is conserved, however, surface charge distribution pattern of PfAsf1 shows distinct features. Two monomeric units of PfAsf1 molecule was observed in the asymmetric unit of the crystal structure of PfAsf1 indicating the protein might exists in a dimeric state which is different in comparison to its yeast and human homologues. This observation was further substantiated by in solution cross-linking experiments. Further, PfAsf1 interacted with Plasmodium specific histone H3 and H4 in our in-vitro protein interaction assay. Identification of critical residues mediating the interaction of PfAsf1 and histone H3-H4 has been done and validated by site directed mutagenesis. Biophysical characterization of the PfAsf1-histone interaction was done by surface plasmon resonance wherein high affinity interaction of PfAsf1 with histone H3-H4 was observed. Histone deposition assay revealed PfAsf1 deposits histone H3-H4 dimer onto DNA template resulting into enhanced disome formation. Our result shows the distinct structural attributes of PfAsf1 and establishes its role as a histone H3-H4 specific chaperone.

POSTER #28

Andrew Shevchuk

Arizona State University, Tempe

RETURN TO INDEX

Maximizing Visibility in Incoherent Diffractive Imaging

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

1. Department of Physics, Arizona State University, PO Box 871504, Tempe, AZ 85287, USA.

The first generation of compact X-ray free-electron lasers (XFELs) is currently under construction and will have the capability to produce much shorter X-ray pulses than large XFELs, albeit also with much lower intensity. This presents a challenging question: What techniques are well-suited to imaging biomolecules with shorter yet weaker X-ray pulses? We analyze the viability of a new technique known as incoherent diffractive imaging (IDI) under these conditions. IDI applies the principles of intensity interferometry (famously known in quantum optics via the Hanbury Brown and Twiss effect) to image biomolecules using their coherent X-ray fluorescence and has some advantages over the standard technique of coherent diffractive imaging, including elemental specificity and 3D structural information in a single diffraction pattern. We study IDI through theory and simulations with a model of inner-shell fluorescence generated by semiclassical dipole radiators (i.e., excited high-Z atoms). Our results suggest that the interferometric visibility in IDI can be maximized by placing the fluorescence detector near the forward direction and by minimizing the pulse duration to be no longer than the coherence time. Compact XFELs make both of these requirements possible, despite their much smaller pulse fluence compared to large XFELs.

This work is supported by BioXFEL award 1231306.

POSTER #29

Mitchell Miller

Rice University, Houston, Texas

RETURN TO INDEX

Experience with Molecular Replacement phasing using predicted models

Mitchell D. Miller,[1] Shikai Jin,[1,2] Peter Wolynes,[2,3,4] George N. Phillips, Jr.[1,3]

1 Department of Biosciences,
2 Center for Theoretical Biological Physics,
3 Department of Chemistry, and
4 Department of Physics, Rice University, Houston, TX 77005, USA.

Once diffraction data are collected, phasing the X-ray amplitudes is the next bottleneck in the structure determination process. Molecular Replacement (MR) has emerged as the dominate phasing technique for PDB depositions. However, MR phasing requires the knowledge of a template structure that is sufficiently similar to the new structure to be able to correctly place it in the new target's unit cell. Such homologous structures are not always available. Here we report on our experience with MR using computationally-efficient, coarse-grained models derived from Associative memory, Water-mediated, Structure and Energy Model (AWSEM) molecular dynamics runs (Jin et al., 2020. IUCrJ 7, 1168). The AWSEM produced models successfully phased a number of structures where the available low sequence identity homologs were unsuccessful in MR phasing directly. The method was found to produce models that were complementary to some derived from more computationally-expensive pipelines. The recent success of AlphaFold2 in the CASP14 experiment (Callaway, 2020. Nature 588, 203) suggests that predicted models will become more useful in improving and extending MR going forward.

An online server implementing the AWSEM-suite algorithm is available at https://awsem.rice.edu.

Acknowledgment

This material is based upon work supported in part by the STC Program of the National Science Foundation (NSF) through BioXFEL under Agreement No. 1231306, the Center for Theoretical Biological Physics and NSF grant (PHY-2019745).

POSTER #30

Rainier Mobley

Arizona State University, Tempe

RETURN TO INDEX

A Metropolis Monte Carlo Algorithm for Merging Single-Particle Diffraction Intensities

B.R. Mobley, R. A. Kirian, J. P. J. Chen, and K. E. Schmidt

Department of Physics, Arizona State University, Tempe, AZ, 85287, USA

Exhaustive approaches to merging single particle diffraction intensities such as those employed by the standard expand-maximize-compress (EMC) algorithms, are computationally limited by the number of degrees of freedom of the target. An alternative technique based on Metropolis Monte Carlo sampling is presented. Using simulated data, the new technique is compared to the standard algorithm, and the higher dimensional cases of mixed species targets and variable x-ray fluence are explored.

References

Loh, Physical Review E, 80, 1550-2376 .(2011)

POSTER #31

Reza Nazari

Arizona State University, Tempe

RETURN TO INDEX

3D Printed Gas Dynamic Virtual Nozzles to Synchronize Droplets with the XFEL (X-Ray Free Electron) Pulses

Reza Nazari1[2], Adil Ansari[1,2], Konstantinos Karpos[1], Sahba Zaare[1], Roberto Alvarez[1,3], John C.H. Spence[1], Uwe Weierstall[1], Ronald J. Adrian[2], Richard A. Kirian[1]

1 Department of Physics, Arizona State University, Tempe, Arizona 85287, USA
2 School for Engineering of Matter, Transport and Energy, Arizona State University, Tempe, AZ 85287, USA
3 School of Mathematics and Statistical Sciences, Arizona State University, Tempe, AZ 85287, USA

Continuous liquid microjets have been widely used in time-resolved solution scattering (TR-SS) experiments at X-Ray Free Electron Lasers (XFELs) to deliver samples to the intense focus of the x-ray beam. When conducting experiments at XFELs with pulse repetition rates on the order of100 Hz, these continuous jets waste the vast majority of injected sample between shots, which is a major problem particularly in cases of irreversible reactions that do not allow for sample recycling. This is a major problem for expensive or difficult-to-produce samples and as such, triggering periodic droplets or jets under vacuum is a highly desired improvement for solution scattering experiments. Here we develop and test 3D-printed gas dynamic virtual nozzles (GDVNs) that are designed to produce periodic droplets that may be synchronized with XFEL pulses.

POSTER #32

Jessika Pazol

University of Puerto Rico, San Juan

RETURN TO INDEX

Understanding how the initial water condition containing calcium ions (Ca2+) affects mutant bacterial lipopolysaccharides (Ra LPS) structure using BioSAXs.

Pazol, J.[1,2] and Nicolau, E.[1,2]

1 Department of Chemistry, University of Puerto Rico at Rio Piedras Campus, San Juan, Puerto Rico 00931 USA;
2 Molecular Science Research Center (MSRC), San Juan, Puerto Rico, 00931, USA.

Lipopolysaccharides (LPS) belongs to one of the strongest pathogens groups that can stimulate the human immune system.[1] This molecule also known as endotoxin comes from the outer membrane of Gram-negative bacteria cell wall. Gram-negative bacteria shed-out LPS in the form of blebs[2] from its outer membrane particularly after biofilms successfully mature in events where stagnated waters occur. Despite its pathophysiological toxicity mechanism are well-established, little is known about its interaction in water sources that ultimately reach people. Furthermore, understanding the physical and chemical characteristics of LPS in water media will help to elucidate its initial physical and chemical condition to design specific inhibiting treatments. LPS are amphipathic molecules that can self-aggregates into different sizes and structures.[3] Removing LPS from water systems had been a challenge because of its high variability in size and its high resistance to temperature or pH changes.[4] In this study, the interactions of LPS and calcium ions (Ca^{2+}) had been studied and characterized to fundamentally understand its biochemical interaction when in aqueous media. Results have demonstrated different structural responses that will serve as the groundwork principle to develop further technology for water purification. The observed interaction had been characterized via dynamic light scattering (DLS), surface charge, Fourier-transform infrared spectroscopy (FTIR), and x-ray diffraction. The details for this investigation methods and analytical techniques will be presented. This work is supported by the NIH RISE program (Grant # 5R25GM061151-19).

References

1 Heinbockel, L.; Weindl, G.; Martinez-de-Tejada, G.; Correa, W.; Sanchez-Gomez, S.; Bárcena-Varela, S.; Goldmann, T.; Garidel, P.; Gutsmann, T.; Brandenburg, K. Inhibition of lipopolysaccharide-and lipoprotein-induced inflammation by antitoxin peptide Pep19-2.5. Frontiers in immunology 2018, 9, 1704.

2 Munford, R.; Hall, C. Purification of acyloxyacyl hydrolase, a leukocyte enzyme that removes secondary acyl chains from bacterial lipopolysaccharides. Journal of Biological Chemistry 1989, 264 (26), 15613-15619.

3 Santos, N. C.; Silva, A. C.; Castanho, M. A.; Martins-Silva, J.; Saldanha, C. Evaluation of lipopolysaccharide aggregation by light scattering spectroscopy. ChemBioChem 2003, 4 (1), 96-100.

4 Batista, P. d. O. M. D.; Lopes, A. M.; Mazzola, P. G.; Yagui, C. d. O. R.; Penna, T. C. V.; Pessoa Júnior, A. Methods of endotoxin removal from biological preparations: a review. 2007.

POSTER #33

Israel Rodriguez

University of Puerto Rico, San Juan

RETURN TO INDEX

Elucidating ribonucleotide reductase inhibition by compounds of titanium(IV) chemical transferrin mimics as an anticancer drug strategy

Israel Rodriguez Rodriguez, Kavita Gaur and Arthur D. Tinoco*

Department of Chemistry, University of Puerto Rico-Río Piedras Campus, San Juan, Puerto Rico 00936.
*arthur.tinoco@upr.edu

Iron, although it is essential element, it plays a crucial role in the growth and proliferation of cancer cells.[1] Two major iron-based cancer cell pathways found in cancer cells are the overexpression of the transferrin receptor (TfR), which increases both iron uptake and the intracellular labile iron pool used for cellular function, and the overexpression of the iron-dependent ribonucleotide reductase (RNR), an enzyme responsible for producing the building blocks for DNA replication.[2,3] Titanium(IV), a chemical mimic of iron(III), has shown the capacity to form stable structures with high affinity iron chelators belonging to the chemical transferrin mimetic (cTfm) family of chelators. These complexes have shown to be effective at inhibiting the bioavailability of intracellular labile Fe(III) and induce cell death via apoptosis.[4,5] They operate intracellularly to decrease the levels of Fe by reacting with the labile iron pool via transmetalation. In this process, the chelators bind Fe(III) to form highly stable Fe(III) complexes leaving Fe functionally inert. Simultaneously, the Ti(IV) is released into the cytosol and is capable of binding to Fe free biomolecular sites including the RNR enzyme. The RNR R2 subunit contains a diiron binding site and depends on the redox activity of Fe to become activated. As a redox inert metal, Ti(IV) binding at the diiron site can prevent RNR activation and potentially block Fe binding. This work demonstrates that the Ti(IV)-cTfm complexes can attenuate the activity of the RNR enzyme a process that leads to cellular antiproliferation and, subsequently, potent cytotoxicity. Fe(III) binding at the diiron cofactor site of the RNR protein domain leads to a critical activation event, which produces a tyrosyl radical. This radical can be observed by whole cell electron paramagnetic resonance. Treatment of Jurkat leukemia cells with the Ti(IV)-cTfm compounds resulted in significant loss of the tyrosyl radical signal, indicative of decreased RNR activity. Considering the mechanistic route for the Ti(IV) compounds they were combined with the DNA targeting drug, cisplatin to explore whether they may operate synergistically via their own specific targets. The cytotoxic activity from the cotreatment performed against the Jurkat cells revealed both synergistic and additive behaviors. Additionally, molecular docking experiments were performed and revealed that these compounds have the capacity to bind different sites of the RNR R2 subunit.

References

1 Torti, S. V.; Torti, F. M. *National Reviewa Cancer* 2013, *13,* 342.
2 Corcé, V.; Gouin, S. G.; Renaud, S.; Gaboriau, F.; Deniaud, D. *Bioorganic & Medicinal Chemistry Letters* 2016, *26,* 251.
3 Aird, K. M.; Li, H.; Xin, F.; Konstantinopoulos, P. A.; Zhang, R. G. *Cell Cycle* 2014, *13,* 199.
4 Loza-Rosas, S. A.; Vazquez, A. M.; Rivero, K. I.; L.J., N.; Delgado, Y.; Benjamin-Rivera, J. A.; Vazquez-Maldonado, A. L.; Parks, T. B.; Munet-Colon, C.; Tinoco, A. D. *Inorganic Chemistry* 2017, *56,* 7788.
5 Tinoco, A. D.; Incarvito, C. D.; Valentine, A. M. *Journal of the American Chemical Society* 2007, *129,* 3444.

Acknowledgment

I.R. was supported by the NIH RISE 5R25GM061151-17 grant and NSF STC BioXFEL 1231306 grant; K.G. and A.D.T. were supported by the NIH 5SC1CA190504 and NIH R21CA240997-01A1.

POSTER #34

Sarthak Saha

University of Massachusetts Amherst

RETURN TO INDEX

X-ray Compatible Centrifugal Device for Protein Crystallography

Saha, S. and Perry, S.L.

Department of Chemical Engineering, University of Massachusetts Amherst, MA, 01003, USA.

Sample mounting and handling of fragile protein crystals are the major bottleneck in the process of crystallography. Microfluidics and microfabricated devices are a powerful strategy to enable crystal growth and subsequent serial crystallography, thus avoiding the need to handle microcrystals and potential damage. In particular, our efforts are focused on fabricating polymer-based devices that allow low background scattering while circumventing the issue of stray diffraction caused by crystalline materials. The use of cleanroom photolithography enables need-based custom tailoring of these devices and easy integration into beamlines and XFELs. In addition, we focus on introducing liquid handling capabilities into our microfluidic devices. We take advantage of the capillary forces that are dominant in the microfluidic domain to create “valves” that would be activated when applied a centrifugal force. Such valving helps us to control the movement of each liquid in the device, permitting better control over the crystallization process. This same fluid handling system can also be harnessed to trigger enzymatic reactions for time-resolved structural studies through the addition of small molecules without the need for complicated fluidic connections.

POSTER #35

Ana Sofia Santiago-Russe

Rice University, Houston, Texas

RETURN TO INDEX

Watch SARS-CoV-2's MTase Kinetic & Dynamic Functions at the XFEL

Ana Sofía Santiago-Russe[1,2,3], Jayashree Soman[1], Abigael Kosgei[1], George Phillips[1], William Bauer[2]

1 Phillips' Lab, Rice University, 6100 Main St, Houston, TX 77005, United States;
2 BioXFEL Science and Technology Center, 700 Ellicott Street Buffalo;
3 University of Puerto Rico- Mayagüez, New York 14203, PR-108, Mayagüez, 00682

The Severe Acute Respiratory Syndrome (SARS-CoV-2) is a type of common virus that can infect human's respiratory system. Coronaviruses have a general genome replication and transcription mechanism. Following the assembly of the replication & transcription complex (RTC), it encodes two S-adenosyl-L-methionine (SAM)-dependent methyltransferases (MTases) that modify the 5'-end of the viral RNA. This process is done by sequentially methylating the RNA cap at guanosine-N7 and ribose 2'-O positions. The MTases in study are Nsp14 N7-Mtase and Nsp16 2'-O Mtase, respectively. The cap structure that is created, primarily by these methyltransferases-, is required for mRNA stability and protein translation. For this reason, the RNA cap methylation process is vital for the coronaviral replication process, as it is responsible for disguising the viral mRNA from innate immunity sensors and thus enabling it to enter the host cell. Bringing down the cap methylation genes in SARS-CoV-2 be detrimental for the virus replication, possibly inhibiting the widespread infection of the virus.

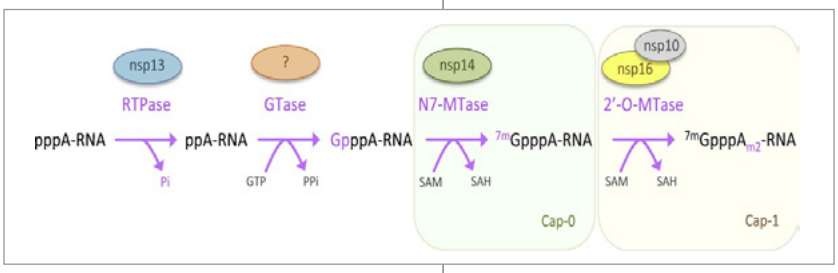


Figure 1: SARS-CoV encodes two S-adenosyl-L-methionine (SAM)-dependent methyltransferases (Mtase) that modify the 5'-end of the viral RNA by sequentially methylating the RNA cap at guanosine-N7 and ribose 2'-O positions : Nsp14 N7-Mtase and Nsp16 2'-O Mt

SARS-CoV replicates in the cytoplasm, without access to the nuclear host-cell machinery for mRNA capping. This means, it has to produce its own RNA- capping enzymes such as NSP14, NSP15 and the N7-MTase. The primary role of NSP10 is to promote NSP16 to bind to capped RNA substrate and the methyl donor SAM. It also stabilizes the SAM-binding pocket and extends the capped RNA-binding groove. Nsp14 generates to Cap-0 with its N7-Mtase activity and Nsp16 further modifies the coronavirus mRNA to have a Cap-1. These recurrent findings not only provide insights into the mechanism of SARS-CoV 29-O-methylation process, but also facilitates the design and development of highly specific antiviral drugs targeting the cap structure formation steps in which these NSPs are directly involved in (Figure 1). Although various vaccines have been approved for the immunization of individuals at high-risk, antiviral drugs are not yet available for the treatment of individuals already infected with SARS-CoV-2. In this historically relevant pandemic, identifying new targets and uses for already approved drugs has a possibility of shortening the development time and reduce the cost of new compounds targeting one or several of the viral proteins. Very few attempts have been made to restart the pharmacological defense of the coronaviral MTases. Our goal is to perform a mix- and -inject crystallography experiment which will help us understand the mechanism of methylation process and thus help us develop the inhibitors against corona virus methyltransferases. The results will expectantly reveal, in near atomic detail, the methyltransferase process and inactivation on the millisecond to second time scales including the crossover from transition state kinetics to steady-state kinetics.

POSTER #36

Manoj Saxena

Birkbeck College, London, UK

RETURN TO INDEX

Cryo-EM structure of the human Lin28b nucleosome reveals likely pioneer factor binding sites.

Saxena, M[1,2], Soufi, A.[3], and Robinson, P.J.J[1,2]

1 Birkbeck College, Department of Biological Science, Malet Street, London, U.K.;
2 Institute of Structural and Molecular Biology, Malet Street, London, U.K.;
3 Centre for Regenerative Medicine, Institute for Regeneration and Repair, University of Edinburgh, U.K.

Human DNA is tightly wrapped around the core histones in the form of nucleosomes. One of the ways transcription is regulated in humans is by controlling the accessibility of the DNA to transcription factors. However, a group of transcription factors known as pioneering factors can access nucleosomal DNA by binding to surface-exposed cognate sites. Such binding is a crucial step for transcription, allowing the recruitment of chromatin remodelling complexes and other transcription factors which are unable to bind nucleosomal DNA unaided.

To understand how pioneering factors can access nucleosomal DNA, we have solved the structure of the Lin28b nucleosome using single particle cryo-EM. The Lin28b nucleosome is a known site for the binding of multiple pioneering factors such as Oct4 and Sox2. However, the majority of related nucleosomal structures known so far are based on artificial sequences. By solving the human native Lin28b nucleosome we can, for the first time, model the binding of the pioneering factor Oct4 at developmentally important genomic loci.

POSTER #37

Megan Shelby

Lawrence Livermore National Laboratory

RETURN TO INDEX

Crystallization of ApoA1 and ApoE4 nanolipoprotein particles and initial XFEL-based structural studies

M.L. Shelby,[1] D. Gilbile,[2] Grant, T.D.,[3,4] Bauer, W.J.[4] Segelke, B.,[1] He W.,[1] Evans A.C.,[1,2] Crespo, N., [3,4] Fischer, P.,[5] Pakendorf, T.,[5] Hennicke, V.,[5] Hunter, M.S.,[6] Batyuk, A.,[6] Barthelmess M.,[5] Meents, A.,[5] Kuhl, T.L.,[2] Frank M.,[1,2,*] and Coleman, M.A.,[1,2*]

1 Biosciences and Biotechnology Division, Lawrence Livermore National Laboratory, Livermore, CA, USA.
2 Department of Chemical Engineering, University of California at Davis, Davis, CA, USA.
3 Department of Structural Biology, Jacobs School of Medicine and Biomedical Sciences, SUNY University at Buffalo, Buffalo, NY, USA
4 Hauptman-Woodward Medical Research Institute, Buffalo, NY, USA.
5 Center for Free-Electron Laser Science, Hamburg, Germany.
6 Linac Coherent Light Source, SLAC National Accelerator Laboratory, Menlo Park, California, USA.

Serial femtosecond crystallography (SFX) at X-ray free electron lasers (XFELs) has proven a powerful tool for structural characterization of membrane proteins, which are traditionally difficult to crystallize as large well-diffracting single crystals due to solubility and stability obstacles during expression and purification. Nanolipoprotein particles (NLPs), also called “nanodiscs”, are discoidal membrane mimetic particles corralled by apolipoproteins that possess both utility as membrane-model systems into which membrane proteins can be inserted and solubilized and a physiological role in lipid and cholesterol transport via HDL and LDL maturation. While conventional assembly of membrane protein-NLP complexes involves the detergent solubilization and purification of the membrane protein of interest prior to assembly, we have implemented a cell-free expression approach which facilitates self-assembly of the complex, eliminating of the need for detergent.

To investigate the role of two apolipoprotein/lipid complexes, ApoA1 and ApoE4, in lipid binding and to develop new SFX methods involving NLP membrane protein encapsulation, we have prepared and crystallized ApoA1 and ApoE4 NLPs. Crystallization of empty NLPs yields pseudo-crystalline semi-ordered objects that yield highly anisotropic and diffuse X-ray diffraction, similar in characteristics to fiber diffraction. Thus, low-background, sample conservative methods of delivery are critical. Here we implemented a fixed target sample delivery scheme utilizing the Roadrunner fast-scanning system and ultra-thin polymer/graphene support films, providing a low-volume, low-background approach to membrane protein SFX. Several unit cell parameters were approximately determined for both NLPs from these measurements. This study represents initial steps in obtaining structural information for ApoA1 and ApoE4 NLPs and developing this system as a supporting scaffold for future structural studies of membrane proteins crystalized in a native lipid environment.

POSTER #38

Emina Stojkovic

Northeastern Illinois University, Chicago

RETURN TO INDEX

High-resolution Crystal Structures of Transient Intermediates in the Phytochrome Photocycle

Emina A. Stojković[a], Melissa Carrillo[a#], Suraj Pandey[b#], Juan Sanchez[a], Moraima Noda[a], Ishwor Poudyal[b], Luis Aldama[a], Tek Narsingh Malla[b], Elin Claesson[c], Weixiao Yuan Wahlgren[c], Denisse Feliz[a], Vukica Šrajer[d], Michal Maj[c], Leticia Castillon[c], So Iwata[e,f], Eriko Nango[e,g], Rie Tanaka[e,f], Tomoyuki Tanaka[e,f], Luo Fangjia[e,f], Kensuke Tono[e,h], Shigeki Owada[e,h], Sebastian Westenhoff[c] and Marius Schmidt[b]

a Department of Biology, Northeastern Illinois University, Chicago, IL, USA
b Physics Department, University of Wisconsin-Milwaukee, Milwaukee, WI, USA
c Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden
d The University of Chicago, Center for Advanced Radiation Sources, Argonne, IL, USA
e Department of Cell Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan
f RIKEN SPring-8 Center, Hyogo, Japan
g Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Sendai, Japan
h Japan Synchrotron Radiation Research Institute, Hyogo, Japan
# contributed equally

Phytochromes are red/far-red light photoreceptor enzymes in bacteria to plants, which elicit a variety of important physiological responses. They display a reversible photocycle between the resting (dark) Pr state and the light-activated Pfr state. The light signals are transduced as structural change through the entire protein to modulate the enzymatic activity. It is unknown how the Pr-to-Pfr interconversion occurs as the structure of intermediates remain elusive. Here, we present crystal structures of the bacteriophytochrome from myxobacterium *Stigmatella aurantiaca* captured by the Japanese X-ray Free Electron Laser Spring-8 Angstrom Compact free electron LAser (SACLA) 5 ns and 33 ms after light illumination of the Pr state. We observe large structural displacements of the covalently bound bilin chromophore, which trigger a bifurcated signaling pathway. The snapshots show with atomic precision how the signal progresses from the chromophore towards the output domains, explaining how plants, bacteria and fungi sense red light.

Acknowledgment

Funded by NSF-MCB-EAGER 1839513 and NIH T34GM 105549 to E.A.S. and NSF STC BioXFEL center sub-award 6227 to E.A.S. and M.S.

POSTER #39

Victoria Mazalova

Center for Free-Electron Laser Science,
Hamburg, Germany

RETURN TO INDEX

qPTxM: a Tool for Quantifying [Evidence in a Density Map for] Post-Transcriptional Modifications

Mazalova, V.[1], and Fromme, P.[2]

1. Center for Free Electron Laser Science CFEL, DESY, Hamburg 22607, Germany;
2. Department of Chemistry and Biochemistry, Arizona State University, PO Box 871604, Tempe, AZ 85281, USA.

The AXSIS project, which is currently hosted by CFEL (DESY)[1], is devoted to studying the water oxidation reaction in the native PSII complex by time-resolved attosecond X-ray nano-crystallography in combination with X-ray emission (XES), X-ray absorption (XAS) and 2D optical spectroscopies. The combination of these techniques will give an unprecedented insight not only into the structure of PSII, but also into the dynamics that leads to the conversion from light to chemical energy.

A compact and versatile XES spectrometer has been developed for the AXSIS beamline. The spectrometer is based on the von Hamos dispersion geometry. It consists of eight cylindrically bent crystals (their number can be increased to further increase the spectrometer efficiency) for a single-shot collection of energy-resolved photoelectrons scattered or emitted by the sample after its interaction with X-rays. The spectrometer's relatively short working distance of 25 cm provides high efficiency and can be used for non-resonant and resonant XES as well as X-ray Raman scattering (XRS) experiments. The spectrometer weights 14 kg and measures 250(L) x 215(W) x 165(H) mm; it can be installed both vertically or horizontally and integrated into any beamline or laboratory. It can be used to simultaneously collect data with X-ray diffraction or X-ray scattering experiments.

The first commissioning experiment on Mn-based compounds was performed during the R&D beamline at the PAL XFEL XSS beamline in November 2020. A charge transfer mechanism induced by optical laser pulses and probed by x-ray free electron laser pulses has been studied using transient XAS, non-resonant and resonant XES techniques.

A dispersive X-ray absorption spectrometer operating in transmission geometry has been developed to, together with XES, provide access to both electronic and atomic structure in a single-shot mode.

XES and XAS spectrometers will be installed at the AXSIS beamline to perform simultaneous pump/probe XES, X-ray diffraction (or XAS) and 2D optical spectroscopy experiments. The combination of these methods can be used to follow the fast excitation dynamics in Photosystem-II.

References

1 F.X. Kärtner et al., Nuclear Instruments and Methods in Physics Research A 829, 24–29 (2016)

Acknowledgment

The work on excitation energy transfer is supported by the European Research Council, synergy grant "Frontiers in Attosecond X-ray Science: Imaging and Spectroscopy" (AXSIS) (grant no 609920).

POSTER #40

Juan Carlos Villalobos

University of Puerto Rico

RETURN TO INDEX

Expression and Characterization of the Human a4b2 Nicotinic Acetylcholine Receptor for *In Meso* Crystallization

Juan C. Villalobos-Santos[1,2], José A. Lasalde-Dominicci[1,2]

1 University of Puerto Rico-Río Piedras Campus
2 University of Puerto Rico-Molecular Sciences Research Center

Nicotinic acetylcholine receptors (nAChR) are widely known for their role in fast cellular responses. Particularly, the a4b2 nAChR is the most abundant nAChR in the human brain, and has been directly linked to nicotine addiction and other common neurodegenerative conditions.[1] This makes the a4b2 nAChR an ideal model for the development of novel structure-based drugs that may alleviate related clinical illnesses. However, structure-based drug design requires the structural resolution of the target protein to be at least of 2.5 – 3 Angstroms, to accurately place amino acids that interact with molecules that trigger structural changes.[2] Recent developments in single particle Cryo-EM have managed to solve membrane protein (MP) structures at the 3A resolution range,[3] yet X-ray crystallography is still the favored method to yield high resolution structures.[4] In the case of ion-channels, it has been a monumental bottleneck to produce protein single crystals due to their dependence on specific annular phospholipids to remain stable in aqueous crystallization set ups, and the added disruptive effects of detergent solubilization.[5] Phospholipid analog detergents (PADs) have shown to be favorable in producing functional muscle-type nAChRs-detergent complexes, which is attributed to their structural similarity to the most abundant phospholipids known to stabilize nAChRs.[6] For the purposes of crystallization screenings, the ***in meso*** crystallization method provides an appropriate environment for pure samples of the a4b2 nAChR to nucleate and produce diffractable protein crystals.[7] Here we express the human a4b2 nAChR by recombinant baculovirus transduction using HEK GnTI- cells, with the goal of producing milligram amounts of pure protein for crystallization trials. The solubilized crude membranes of transduced HEK GnTI- cells will be screened through fluorescent-size exclusion chromatography to monitor the presence of a4b2 nAChR assembled pentamers prior to purification.[8] Pure samples are used in crystallization trials following the *in meso* method by mixing the pure a4b2 nAChR samples with monoolein (1-oleoyl-rac-glycerol). The resulting crystals were subjected to X-ray diffraction at the Argonne National Laboratory-Advanced Photon Source GM/CA CAT, beamline 23-ID-B.

Acknowledgment

This research was supported by the NIH-NIGMS grants 1R01GM098343 (JALD); COBRE-NIEF 1P20GM103642 (M.M. and JAL-D) and the RISE program 5R25GM061151-19 (J.V-S).

References

1. Barrantes, F. J., Borroni, V. & Vallés, S. Neuronal nicotinic acetylcholine receptor – cholesterol crosstalk in Alzheimer ' s disease. *FEBS Lett.* 584, 1856–1863 (2010).
2. Subramaniam, S., Earl, L. A., Falconieri, V., Milne, J. L. & Egelman, E. H. Resolution advances in cryo-EM enable application to drug discovery. *Curr. Opin. Struct. Biol.* 41, 194–202 (2016).
3. Walsh, R. M. *et al.* Structural principles of distinct assemblies of the human α4β2 nicotinic receptor. *Nature* (2018). doi:10.1038/s41586-018-0081-7
4. Morales-perez, C. L., Noviello, C. M. & Hibbs, R. E. X-ray structure of the human α4β2 nicotinic receptor. *Nat. Publ. Gr.* (2016). doi:10.1038/nature19785
5. Quesada, O. *et al.* Uncovering the lipidic basis for the preparation of functional nicotinic acetylcholine receptor detergent complexes for structural studies. *Sci. Rep.* 6, 1–12 (2016).
6. Padilla-Morales, L. F., Colón-Sáez, J. O., González-Nieves, J. E., Quesada-González, O. & Lasalde-Dominicci, J. A. Functionality and stability data of detergent purified nAChR from Torpedo using lipidic matrixes and macroscopic electrophysiology. *Data Br.* 6, 433–437 (2016).
7. Cherezov, V., Liu, J., Griffith, M., Hanson, M. A. & Stevens, R. C. LCP-FRAP Assay for Pre-Screening Membrane Proteins for in Meso Crystallization. 8, 4307–4315 (2009).
8. Kawate, T. & Gouaux, E. Fluorescence-Detection Size-Exclusion Chromatography for Precrystallization Screening of Integral Membrane Proteins. 673–681 (2006). doi:10.1016/j.str.2006.01.013

POSTER #41

Stephanie Wankowicz

RETURN TO INDEX

Assessing how side chain conformational heterogeneity changes upon ligand binding

Stephanie A. Wankowicz, Saulo H. P. de Oliveira, Gydo van Zundert, Daniel Hogan, Henry van den Bedem, James S. Fraser

Most proteins are modeled and represented as static structures. However, protein dynamics play an important role in many aspects of function, including ligand binding. The ability to quantify changes in conformational dynamics would have many benefits, including enabling the study of the entropic contribution of the receptor for the free energy of binding. Although full access to the conformational ensemble is difficult with any experimental technique, alternative conformations modeled based on evidence from electron density maps calculated by X-ray crystallography provide an initial proxy. Here, we quantified conformational heterogeneity in 728 matched pairs (with and without ligands) of high resolution (>2 angstrom) X-ray crystallography structures. We ran qFit, an automated and unbiased method to model multiconformer protein structures. We then measured the order parameters of the side chains to compare the dynamics. When comparing order parameters close versus far from the ligand, we observe that bound structures less dynamic around the ligand tend to get more dynamic further away from the ligand. This trend may support the idea of entropic compensation during ligand binding. Additionally, proteins that bind ligands with more rotatable bonds and with larger molecular weight tend to be more dynamic around the ligand binding site. This dataset provides important insights into protein dynamic considerations in interpreting ligand binding events.

POSTER #42

Jennifer Wierman

RETURN TO INDEX

To obtain a more quantitative and deeper understanding of the protein structure-function relationship, a combination of several complimentary methods in the study of a single sample can facilitate substantial scientific discovery. This level of understanding must be achieved through observing reactions and identifying the transient chemical species at a variety of time scales. Adapting to this new paradigm is an important strategic direction of the Structural Molecular Biology group (SMB) at the Stanford Synchrotron Radiation Lightsource. To this end, the SMB group has developed new crystallography beamline capabilities that include a fully automated (and remote-access) in-situ UV-Vis microspectrometer system optimized for single crystal measurements. Further, SMB opened BL12-1 for user operations, a state-of-the-art microfocus beamline optimized for micro-crystal data collection. BL12-1 offers advanced equipment for serial and time-resolved experiments at ambient temperatures and controlled humidity, with new algorithms for automated analysis of data from the fast frame-rate EIGER detector. Further BL12-1 is a sister beamline to LCLS MFX, where the SMB group operates a similar setup to conduct serial XFEL diffraction experiments. Together, these new technologies open up a wide range of pioneering research, including time-resolved experiments to follow enzymatic reactions *in crystallo*, monitoring specific radiation damage within crystals, and rapid fragment screening experiments to facilitate drug development, including therapeutics to combat COVID-19.

POSTER #43

Lena Worbs

Center for Free-Electron Laser Science,
Hamburg, Germany

RETURN TO INDEX

Optimized nanoparticle injectors for single-particle diffractive imaging experiments

Lena Worbs[1,2], Jannik Lübke[1,2,3], Simon Welker[1], Muhamed Amin[1], Armando D. Estillore[1], Amit K. Samanta[1], Jochen Küpper[1,2,3]

1 Center for Free-Electron Laser Science, Deutsches Elektronen Synchrotron DESY, 22607 Hamburg, Germany
2 Department of Physics, Universität Hamburg, 22761 Hamburg, Germany
3 Center for Ultrafast Imaging, Universität Hamburg, 22761 Hamburg, Germany

Coherent diffractive imaging at x-ray free-electron lasers (XFELs) promises the imaging and reconstruction of the three-dimensional molecular structure of isolated (biological) molecules and nanoparticles at atomic resolution[1]. However, so far these experiments are limited by low signal-to-noise ratios and, therefore, require the collection of a very large number of diffraction patterns[2]. The ideal sample delivery method for single-particle coherent diffractive imaging experiments delivers exactly one new and preferably identical sample particle into every x-ray pulse.

Here, we present techniques for the control of gas-phase nanoparticles and biomolecules to render such imaging experiments feasible: optimized aerodynamic lens stacks for the generation of focused particle beams[3], their optimization for a certain particle species and a low-temperature (4 K) buffer-gas cell to rapidly freeze biological nanoparticles to cryogenic temperatures and to produce cold and controlled beams of such samples[4]. Using our simulation framework, we are able to optimize the injector-geometry and experimental parameters to produce the highest density particle beams for a specific sample[3], especially important when moving to smaller samples like proteins where the amount of scattering signal and the general transmission of the sample decreases.

We characterize our developed injectors in our aerosol beam characterization setup, using novel laser-based particle and gas-density imaging schemes[5,6].

References

1 M. M. Seibert, et al., *Nature* 470, 78 (2011)
2 K. Ayyer, *Optica* 7, 593-601 (2020)
3 N. Roth, S. Awel, D. A. Horke, J. Küpper, J. *Aerosol Sci.* 124, 17-29 (2018), arxiv:1712.01785 [physics]; N. Roth, et al., *submitted* (2020)
4 A. K. Samanta, M. Amin, A. D. Estillore, N. Roth, L. Worbs, D. A. Horke, J. Küpper, Struct. Dyn. 7, m024304 (2020), arxiv:1910.12606 [physics] (2019)
5 L. Worbs, J. Lübke, N. Roth, A. K. Samanta, D. A. Horke, J. Küpper, *Opt. Express* 27, 25 36580-36586 (2019), arxiv:1909.08922 [physics]
6 D. A. Horke, N. Roth, L. Worbs and J. Küpper, *J. Appl. Phys.* 121, 123106 (2017), arxiv:1609.09020 [physics]

POSTER #44

Iris Young

University of California San Francisco

RETURN TO INDEX

qPTxM: a Tool for Quantifying [Evidence in a Density Map for] Post-Transcriptional Modifications

Young, I.D.[1], Stojković, V.[2], Myasnikov, A.G.[3], Frost, A.[3,4], Fujimori, D.G.[2,3,5], Fraser, J.S.[1,4]

1 Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, CA 94158, USA; 2 Department of Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, CA 94158, USA; 3 Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco, CA 94158, USA; 4 Quantitative Biosciences Institute, University of California San Francisco, San Francisco, CA 94158, USA; 5 Department of Pharmaceutical Chemistry, University of California San Francisco, 600 16th St, MC2280 San Francisco, CA 94158, USA.

X-ray crystallography and cryo-electron microscopy (cryoEM) are complementary techniques for macromolecular structure solution that produce electron density and electrostatic potential maps, respectively. Particularly at the stages of map and model refinement, many tools are applicable to both techniques, leading to potential confusion when the two types of maps do not behave the same way. One such stress point is map quality. Defined as the smallest distance by which two features can be separated and still independently resolvable, the metric of resolution is conceptually water-tight but in practice highly dependent on how it is measured, and whereas resolution is a global (at worst, anisotropic) metric for crystallographic maps, it is a local feature of cryoEM maps that is strongly influenced by processing steps such as masking. As cryoEM maps make up an increasing proportion of near-atomic-resolution macromolecular structures, this makes comparisons across maps quite difficult.

Here we present a technique-agnostic computational tool for assessing map quality by the proxy of discernability of known features (github.com/fraser-lab/qptm, Stojković et al 2020). The profile of densities composing a feature (say, a C5-methylation) can be predicted from the atomic positions, map type, and resolution, and by comparing a predicted density profile to an experimental one, we may assign some measure of believability to the feature. In a structure with many such features, we may set a threshold for "believable enough" and sort our features into detectable and undetectable groups, generating true positives, true negatives, false positives and false negatives. We are then able to compute sensitivity, selectivity, and overall accuracy, which track with the overall quality of the map.

In addition, cryoEM of biological macromolecules has now breached the atomic resolution barrier, making *de novo* detection of features tenable, and crystallographic structures are routinely high enough resolution for this exploration. qPTxM can alternatively be used to identify promising sites in a structure where modifications might be present even in the absence of biochemical (e.g. MS) data. We also provide a plugin for the graphics program Coot (Emsley et al 2010) to allow a researcher to jump directly to these sites of possible modifications.

References

Stojković V, Myasnikov AG, Young ID, Frost A, Fraser JS, Fujimori DG. Assessment of the nucleotide modifications in the high-resolution cryo-electron microscopy structure of the *Escheria coli* 50S subunit. *Nucleic Acids Research,* 2020, *48:* 2723-2732.

Emsley P, Lohkamp B, Scott WG, Cowtan K. Features and development of *Coot. Acta Cryst.,* 2010, *D66:* 486-501.

Acknowledgment

This work is supported by NIH/NIGMS fellowship F32GM133129.