Poster Competition Abstracts

A National Science Foundation Science and Technology Center

Poster Blitz

Tuesday 5:00— 5:30 PM

Chair: Petra Fromme, Arizona State University

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Poster Session

Tuesday 7:00—9:00 PM

Judging committee: Alexandra Ros (ASU), Elspeth Garman (Oxford), Martin Trebbin (UB)

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3	Kaytlin	Alzugaray	Regulation of Oncogenic Stress Response in Gastric Cancer
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6	Ryan	Boyd	PURIFICATION AND BIOPHYSICAL CHARACTERIZATION OF HUMAN SYX RHOGEF FOR STRUCTURAL STUDIES AND DRUG ASSAY DESIGN
7	Elsie	Pares-Matos	Molecular modelling and crystallographic studies of the cytoplasmic domain of Wsc1p
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9	Melissa	Carrillo	Time Resolved SFX on a Classical Phytochrome *from S. aurantiaca*
10	Cecilia	Casadei	Recent developments in computational methods for two-dimensional serial femto-second crystallography: paving the way to the time-resolved study of large-scale movements in membrane proteins
11	Gabriela	Diaz Figueroa	Evaluating the stability of cyanophage ferredoxin
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13	Diandra	Doppler	Electronically Stimulated Crystal Droplets for Reduced Sample Consumption during Serial Femtosecond Crystallography
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30	Surya V. S. R. K	Pulavarti	SAXS / NMR measurements suggest large conformational excursions of β-lactamase during substrate turnover
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37	Lysmarie	Santos Velazquez	Oxyhemoglobin Profile in the Presence of Hydrogen Sulfide
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52	Guillermo	Calero	Transcription with a Laser: Towards a Molecular Movie of Nucleotide Addition
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55	Eduardo	Cruz-cho	High-Throughput Single-Particle Identification of XFEL Images using Nyström Extension
56	Rebbeca	Jernigan	New Structural Insights Into the Function of the Active Full Length Human Taspase1: A Novel Anticancer Target
57	Jose Julian	Del Toro	Subcloning of a 10x histidine tag in the neuronal nicotinic receptor α4β2 for structural studies
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Luis Aldama, Northeastern Illinois University

High-resolution crystal structures of a red-light photoreceptor from the non-photosynthetic myxobacterium *Stigmatella aurantiaca*

Luis Aldama [1], Juan Sanchez [1], Melissa Carrillo[1], Suraj Pandey[2], Moraima Noda[1], Denisse Feliz[1], Gregory C. Tracy [1], Phu Duong[1], Angela Nugent[1], Andrew Field[1], Elin Claesson[3], Weixiao Yuan Wahlgren[3], Sebastian Westenhoff[3], Marius Schmidt[2] and Emina A. Stojković[1]

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Phytochromes (PHYs) are light-regulated enzymes, first found in plants with homologs in photosynthetic and non-photosynthetic bacteria denoted as BphPs (bacteriophytochromes). Recently, BphPs have been engineered as biofluorescent tags for deep tissue imaging due to their fluorescence in the infrared range. Upon photo-excitation, BphPs can reversibly switch between two conformational states: red light Pr (λ max 700 nm) and far -red light Pfr (λ max 750 nm) absorbing states. Structurally, BphPs are homodimeric proteins composed of a photosensory core module (PCM), and an enzymatic domain, usually a histidine kinase. The PCM consists of 3 domains (PAS, GAF, and PHY), and it houses a covalently bound biliverdin (BV) chromophore, which is an open-chain tetrapyrrole. Absorption of light by BV results in a Z to E isomerization of the C15=C16 double bond. We report crystal structures of the wild-type and mutant (His275Thr) forms of the canonical BphP from the non-photosynthetic myxobacterium *Stigmatella aurantiaca* (*Sa*BphP2) in the Pr state. Structures were determined at 1.65 Å and 2.22 Å (respectively), which is the highest resolution of any PCM construct to date. The comparison of the *Sa*BphP2 structure with homologs found in other bacteria highlight and confirm important amino acids near the chromophore that play a role in Pr-Pfr photoconversion and signal transduction into the PHY domain.

Funded by. NSF STC-1231306. E.A.S. was supported by NSF-MCB-RUI 1413360, NSF-MCB-EAGER Grant No. 1839513, and NSF STC BioXFEL center award 6227. M.N. and L.A. training was supported in part by the National Institutes of Health (NIH) Maximizing Access to Research Careers (MARC)- T34 GM105549 grant.

Nadia Ali, Virginia Commonwealth University

Examining the structure of the immature human astrovirus (HAstV) capsid protein

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Human astrovirus (HAstV) is a highly mutagenic, single-stranded, positive-sense RNA virus. This pathogen currently has no vaccine and is known to cause severe gastroenteritis. However, humans have shown to develop an adaptive immune response to the virus after exposure to the mature, infectious particle. This indicates a potential for developing a vaccine against the capsid protein. Rational vaccine design requires an understanding of the epitope and paratope interaction, or more generally, a biological structure of the capsid and the related antibody. We aimed to study the protein structure of HAstV VP90 dimer (residues 71-782), the precursor, immature capsid protein.

To examine the structure of the VP90 dimer, we had to first produce highly concentrated samples of the protein. We produced recombinant VP90 protein containing a 6xHis affinity tag and a SUMO solubility tag by transforming the VP90 plasmid onto Rosetta DE3 competent cells. The protein was purified by affinity chromatography on a nickel resin column. The dimeric state of the protein was isolated using size exclusion chromatography. Crystal trays were set up under various conditions to test crystal formation of the dimer protein. Any crystals produced would be used in X-ray diffraction in order to capture images of the protein that reveal its specific structure.

The VP90 dimer was successfully purified and used to conduct crystal screenings. No significant crystals have been produced, but has potential for future studies. Structuring this protein is vital in understanding the life cycle of the virus and possibly creating vaccines against the viral capsid.

BioXFEL is a National Science Foundation Science and Technology Center supported by NSF STC #1231306.

Kaytlin Alzugaray, Florida International University

Regulation of oncogenic stress response in gastric cancer

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Gastric cancer remains a major worldwide health problem and is considered the second most common cause of cancer death in the world. Despite numerous multimodal treatment options (i.e. chemotherapy, radiation therapy, targeted surgery, etc.), this malignancy is considered to be poorly treatable and carry significant morbidity and mortality rates. In order to better treat and prevent this disease, it is extremely important to thoroughly understand the factors involved in gastric cancer development. A fundamental, innate response to oncogenic transformation is enhanced cellular stress (i.e. metabolic, ER stress, oxidative, DNA damage). Oncogenic stress response is a powerful and persistent antiproliferative response brought on by oncogenic signaling due to the mutation of an oncogene, or the inactivation of a tumor-suppressor gene (i.e. p53). In order to accurately understand the specific metabolic pathways affected and proteins involved in gastric tumorigenesis, a model system was created. Through the cloning process, the TetOne-kRas vector grants a perfect opportunity to understand how oncogenic stress response functions.

In order to observe oncogenic stress response, a model system was created through the standard cloning procedure. The TetOne vector was chosen because it contains all the components needed to induce mutated kRas expression (i.e. Tet-On 3G Transactivator Protein, PTRE3GS Inducible Promoter, etc.). Previous research studies have reported that mutated kRas cause tumor initiation and maintenance, which is the perfect reason as to why it was selected as our insert. For future directions, this model system will be utilized for transfection in mammalian cells and investigated for oncogenic stress response function. With the creation of this model system, it will now be possible to easily detect which metabolic pathways are affected and what key proteins are involved in the regulation of oncogenic stress. Future studies will be conducted with this model system to understand gastric tumorigenesis and help identify potential targets in the prevention of gastric cancer. Overall, the main goal of this research is to prevent and cure one of the most lethal cancers in the world, gastric cancer.

Josue Benjamin, University of Puerto Rico

Studies to determining the transportation of Ti(IV) via serum transferrinin presence of Fe(III)

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Serum Transferrin (sTf) is a bilobal, 80 kDa glycoprotein with its N and C lobes divided into two subdomains form two Fe(III) binding sites[1]. The concentration of sTf is the blood is proximally 37 mM in bloodstream. In a normal plasma, only a 30 of transferrin is bound to Fe(III) ions . Considering the speciation of Fe(III)-sTf in blood (39% apo-sTf, 11.2% Fe_c-sTf, 22.9% Fe_n-sTf, 26.7% Fe_2-sTf).[2] STf having these free binding sites it will interact with other metals in the bloodstream. In 2011, a laboratory in Illinois studies the importance of the Fe(III) in the transportation and metal uptake pathway of Pu(IV).[3] This publication on plutonium(IV) transport suggested that a mixed Fe(III)/Pu(IV) metalation of sTf can result in higher levels of Pu(IV) uptake in cells and that this higher uptake could be involved in its toxicity.[3]Using the distribution of the Fe(III) and sTf theres is a change that protein have two different metal in each biding sites because have aproximaly 30% in the monoferric sTf form. To establish relevant models that involves a direct role that Fe(III) might play in Ti(IV) transport increasing the metal update same way as Pu(IV) . It has been proposed by others although not experimentally demonstrated that Ti(IV) might bind to sTf in a mixed Fe(III)/Ti(IV) metalation form.[4] Herein, we propose that binding Ti to monoferric sTf, would produce a conformational change conducive to a higher uptake of Ti in cells. We performed differential scanning calorimetry and urea gel electrophoresis to examine the afforded conformational and stability changes from mixed metalation of sTf. Results from the DSC demonstrate that Ti, when bound to Fe_c-sTf, demonstrates a stability intermediate between mono and diferric sTf although electrophoresis suggests a stability closer to the monoferric species. Furthermore, we perceived that Fe has more an affinity for the N-site than that of the C-site of sTf. Also, to confirm the conformation of the protein with the mix metals we want to crystalize the protein to see the 3D structure using X-ray Data Collection.

References:

1. Tinoco, A. D.; Saxena, M.; Sharma, S.; Noinaj, N.; Delgado, Y.; Quinones Gonzalez, E. P.; Conklin, S. E.; Zambrana, N.; Loza-Rosas, S. A.; Parks, T. B., Unusual Synergism of Transferrin and Citrate in the Regulation of Ti(IV) Speciation, Transport, and Toxicity. *J Am Chem Soc* **2016,** *138* (17), 5659-65.

2. Williams, J.; Moreton, K., Distribution of iron between the metal-binding sites of transferrin in human-serum. *Biochem. J.* **1980,** *185* (2), 483-488.

3. Jensen, M. P.; Gorman-Lewis, D.; Aryal, B.; Paunesku, T.; Vogt, S.; Rickert, P. G.; Seifert, S.; Lai, B.; Woloschak, G. E.; Soderholm, L., An iron-dependent and transferrin-mediated cellular uptake pathway for plutonium. *Nat. Chem. Biol.* **2011,** *7* (8), 560-565.

4. Aarabi, M.; Mirhashemi, S.; Ani, M.; Moshtaghie, A. J. A. J. B., Comparative binding studies of titanium and iron to human serum transferrin. **2011,** *6*, 191-199.

Acknowledgment

This work was funded by Rise Program 5R25GM061151-18 and National Institutes of Health Grant SC1(5SC1CA190504-02) and BioXFEL Scholar - BioXFEL STC Grant Number 1231306

Sarah Bowman, Hauptman Woodward Institute

Nanocrystal detection methods at the High-Throughput Crystallization Screening Center

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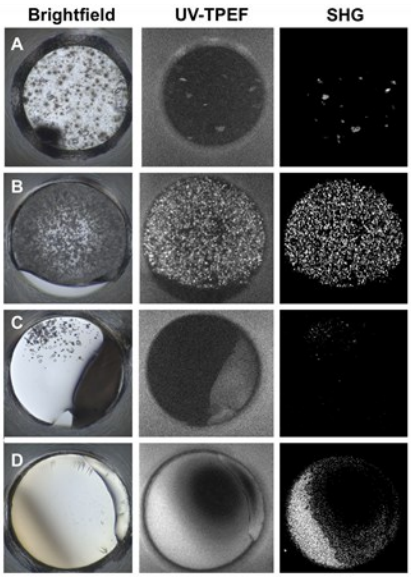
The High-Throughput Crystallization Screening Center (HTCSC) at Hauptman-Woodward Medical Research Institute (HWI) provides high-throughput experimental screens to identify conditions in which biomolecules will crystallize. We use multiple imaging platforms and are developing new methods to enhance detection of microcrystals for use in X-ray free electron laser, serial crystallography and microcrystal electron diffraction experiments. The HTCSC is the only crystallization resource available to external users with a Formulatrix Rock Imager 1000 with SONICC (Second Order Nonlinear Imaging of Chiral Crystals), which uses Second Harmonic Generation (SHG) and UV-Two Photon Excited Fluorescence (UV-TPEF) microcopy to facilitate detection of protein crystals. Quantitative methods to fully exploit the advanced imaging using SHG and UV-TPEF for crystallography have not been developed and one goal of our work is development of new algorithms and computational methods to integrate the information in the multiple imaging modalities. Brightfield imaging can lead to a number of pathologies; crystals can be too small to see or hidden under precipitate, resulting in false negatives, or detected crystals can be composed of salt from the crystallization buffer, and not protein at all, resulting in false positive signals. Figure 1 shows examples of images from crystal screening experiments using brightfield, UV-TPEF and SHG imaging modalities generated at the HTCSC. Each row contains images of a single well at a single time point. In row A, precipitate obscures the protein crystals in the brightfield image, but the large bright spots in the UV-TPEF and SHG images reveal their presence. The precipitate in Row B is shown to be small microcrystals in the UV-TPEF and SHG images. The brightfield image in Row C shows clear crystals, but the lack of UV-TPEF and presence of SHG signal indicate that these crystals are salt, and not protein-containing. Row D brightfield image have no discernible crystals visible, but the UV-TPEF and SHG reveal positive nanocrystal protein hits. From these images, it is clear that additional imaging capabilities enhance the interpretation and quality of information about protein crystals. Utilizing this information currently relies on the same method that the reader just used in examining the images: manual inspection of each image. Even with robotic systems to set up screening experiments, most assessment of the vast number of images generated occurs manually by human examination. One of our goals is to develop new algorithms for enhanced detection of nano- and microcrystals using our imaging capabilities. We will present results from this ongoing work on image synthesis that makes use of the multiple image modalities.



Funding: NIH 5R24GM124135, Seymour H. Knox Foundation

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Ryan Boyd, Arizona State University

Purification and biophysical characterization of hyman Syx RhoGEF for structural studies and drug assay design

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Currently, **patients diagnosed with glioblastoma multiforme (GBM) survive approximately 1 year**, with only 5% surviving longer than 5 years. This dismal prognosis is due to the diffuse nature of these tumors, which makes eradication of all tumor cells with surgery and radiotherapy difficult. RhoA is a small Ras-family GTPase that is cyclically activated by Rho Guanine Nucleotide Exchange Factors (RhoGEFs). Dysregulated **RhoA signaling has been linked to multiple hallmarks of cancer** including cell growth, proliferation, migration, and metastasis. GEFs catalyze the exchange of GDP for GTP on Rho, causing it to change from its inactive conformation to its active conformation, which can activate numerous downstream pathways. The RhoGEF PLEKHG5, also known as Synectin binding RhoA exchange factor **(Syx) associates with membrane complexes where it mediates localized RhoA activation at the apical edge of cells, and stimulate their movement and metastasis.** Inhibition of Syx in GBM mouse xenograft models results in increased mouse survival. We have engineered *E. coli* & insect cell expression construct which facilitated successful production and purification of catalytic fragments of Syx and are **in the process of crystallography experiments and biophysical characterization in the hopes of generating a structure and activity assay to guide development of therapeutics which will inhibit Syx in humans.**

Julio Candanedo, Arizona State University

Dynamics of water irradiated by UED beams

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The remarkable success of the X-ray free-electron lasers (XFELs), and their ability to image biological macromolecules while minimizing secondary radiation damage due photoelectrons when using femtosecond pulses raises the question of whether this can also be achieved using pulsed Ultrafast Electron Diffraction (UED) beams. The limiting factor for both Cryo-EM and x-ray sources, e.g. XFEL, for imaging or capturing a diffraction pattern is radiation damage. Radiation damage is caused by undesirable inelastic excitations by the beam on the sample. In this paper we use excited state molecular dynamics simulations to investigate time resolved radiation damage mechanisms in soft matter, as a function of pulse parameters (such as emittance, duration, and brightness).

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Melissa Carrillo, Northeastern Illinois University

Time Resolved SFX on a Classical Phytochrome from S. aurantiaca

Melissa Carrillo[1], Suraj Pandey[2], Luis Aldama[1], Juan Sanchez[1], Moraima Noda[1], Denisse Feliz[1], Elin Claesson[3], Weixiao Yuan Walhlgren[3], Sebastian Westenhoff[3], Emina A. Stojković[1] and Marius Schmidt[2]

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Bacteriophytochromes (BphPs) are red light photoreceptors found in photosynthetic and non-photosynthetic bacteria. They are dimeric proteins that consist of a photo-sensory core module (PCM) and an enzymatic domain, which is often a histidine kinase. The PCM consists of the Per/Arnt/Sim (PAS) domain, the guanosine monophosphate phosphodiesterase / adenylyl cyclase / FhlA (GAF) domain, and a phytochrome-specific (PHY) domain. The GAF domain contains a covalently bound-chromophore called biliverdin (BV) which is a heme-derived, open chain tetrapyrrole. The BphPs reversibly interconvert between the red-light absorbing Pr state and the far-red light absorbing Pfr state. Recently, we determined the high-resolution crystal structure of the wild-type SaBphP2 PCM found in the non-photosynthetic myxobacterium *Stigmatella aurantiaca*. *Sa*BphP2 PCM crystallizes in the space group ($P2_1$) in the Pr state and diffracts to 1.65 Ångstrom resolution, the highest of any PCM construct to date. Through our recent developments of highly-diffracting *Sa*BphP2 microcrystals, we have successfully been able to use *Sa*BphP2 as a suitable model for investigations of the BphP photocycle and respective intermediates on pico to nanosecond timescales at free electron lasers using Time-Resolved Serial Femtosecond X-ray crystallography (TR-SFX). Our latest experiments at the Japanese X-ray free electron laser Spring-8 Angstrom Compact free electron LAser (SACLA) resulted in a 2.1 Å room temperature structure for the wild type *Sa*BphP2 PCM that is nearly identical to our cryo 1.65 Å structure. More importantly, we report the first successful identification of a 5 ns intermediate during the Pr to Pfr transition of the classical BphP with respective amino acid and BV chromophore rearrangements in the GAF and PHY domains.

Funded by NSF MCB RUI 1413360 and NSF MCB EAGER 1839513 Research Grants to E.A.S., NSF STC "BioXFEL" (1231306) to M.S. and NIH T34 GM105549 to M.N. and L.A.

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Casadei Cecilia, Paul Scherrer Institute

Recent developments in computational methods for two-dimensional Serial femtosecond crystallography: paving the way to the time-resolved Study of large-scale movements in membrane proteins

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

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

1. Casadei, C. M. *et al.* (2019) *IUCrJ,* **6**, 34-45.
2. Casadei, C. M. *et al.* (2018) *IUCrJ,* **5**, 103-117.

Gabriela Diaz Figueroa

Evaluating the stability of cyanophage ferredoxin

Gabriela Diaz Figueroa[1], Ian Campbell [2] and Joff Silberg[3]

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Climate change has increased scientific interest in global carbon and oxygen cycles. Cyanobacteria, photosynthetic organisms abundant in most of Earth's oceans, contribute significantly to both cycles by synthesizing oxygen as a product of their metabolism and using fixed carbon to generate biomass. Cyanophages regularly hijack cyanobacteria metabolism, thereby affecting host cellular processes and their impact on oxygen and carbon cycles. This hijacking is thought to occur because the cyanophage causes the cells to express a more stable version of the bacteria photosynthetic machinery. One of the phage proteins that supports this hijacking is a Ferredoxin (Fd), an iron-sulfur (Fe-S) cluster-containing proteins that functions as an electron carrier for Photosystem I. We will describe our efforts to overexpress Prochlorococcus phage T4-like (PSSM2) Fd in Escherichia coli to purify by anion exchange and size exclusion chromatography, and to characterize its stability using equilibrium unfolding experiments. Additionally, we will describe our efforts comparing the electron transfer activity phage Fd with host Fds using cellular growth assays. These studies will improve our fundamental understanding of cellular controls over electron flow using protein electron carriers.

BioXFEL is a National Science Foundation Science and Technology Center supported by NSF STC #1231306.

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Diandra Doppler , Arizona State University

3D printed Co-flow devices for reduced sample consumption at the European XFEL

[1,2]Diandra Doppler, [1,2]Austin Echelmeier, [1,2]Daihyun Kim, [1,2]Jorvani Cruz Villarreal, [1,2]Sahir Gandhi, [1,2]Alexandra Ros

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Serial femtosecond crystallography (SFX) with X-ray free electron lasers (XFELs) is an emerging and growing field in structural discovery; one of its principal achievements is the ability to resolve the structure of intricate membrane proteins. Serial injection in a liquid jet for this new technique requires high jet speeds and large sample volumes, which is one of its major challenges. At the European XFEL (EuXFEL) for example, the pulse structure is very unique: It delivers 10 Hz trains of X-ray pulses, with an intra-train MHz repetition frequency, requiring jet velocities ≥ 35 m/s to replenish the crystal suspension stream before the next pulse. The gas dynamic virtual nozzle (GDVN) can achieve these high velocities; however, between pulse trains, the continuously injected crystal sample is wasted. Some alternative injectors, like the double flow focusing nozzle or the piezoelectric droplet ejector, have been designed to conserve sample, however, they cannot achieve the required fast sample replenishing between the MHz pulses within the EuXFEL pulse train.

To reduce the volume of sample wasted during a SFX experiment at the EuXFEL, we propose a 3D printed Y-junction device with an integrated GDVN that generates a simultaneous flow of sample with an immiscible liquid prior to being injected into the XFEL beam. The simultaneously flowing liquid, or co- flow, is generated as a parallel flow of protein crystal suspension and immiscible fluorinated oil expelling from the device concurrently, thus minimizing the amount of analyte solution needed while maintaining high jet velocities and high enough total flow rates to maintain jets. This injector is capable of injecting both lower viscosity aqueous liquid samples as well as higher viscosity samples.

An earlier version of the co-flow device was utilized at the EuXFEL SPB/SFX instrument (The Single Particles, Clusters, and Biomolecules & Serial Femtosecond Crystallography) which allowed crystal delivery with reduced clogging for a time-resolved experiment, however, the parallel co-flow of sample with immiscible oil could not be maintained until the nozzle due to the long delivery capillary before the GDVN. Therefore, an integrated co-flow generator/nozzle hybrid device was designed, which is capable of delivering the co-flow until the nozzle tip. The co-flow stability and jetting parameters of the device have been demonstrated with three buffers of varying viscosities and with a suspension containing photosystem II crystals. The mass flow rates of helium used in all experiments ranges from 10 mg/min to as high as 50 mg/min, with pressures up to 700psi for the liquid flow rates; these parameters were then used to determine the velocities at which the jet is expelled from the nozzle as well as the different experimental regimes in which co-flow will be ideal for a given analyte sample. The co-flow can be achieved with the flow rate of sample down to 1 µL/min at total flow rates of 20 µL/min from compensation from oil, maintaining the stable injection of jetting for high viscosity liquid samples while keeping the jet at speeds capable of replenishing sample at the high repetition rate at the European XFEL. Furthermore, the co-flow thickness also can be characterized and varied from 6 to 60 µm according to the flow rate. Future work includes confirmation of jet velocities at varying liquid flow rates while varying the helium gas pressures and application of the co-flow injector for serial crystallography at XFEL instruments including crystal suspensions with high viscosity such as photosystem II.

Diandra Doppler , Arizona State University

Electronically stimulated crystal droplets for reduced sample consumption during serial femtosecond crystallography

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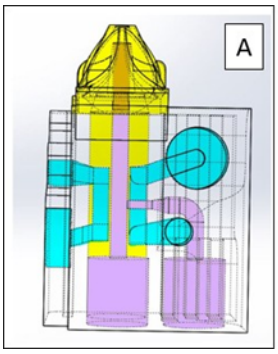
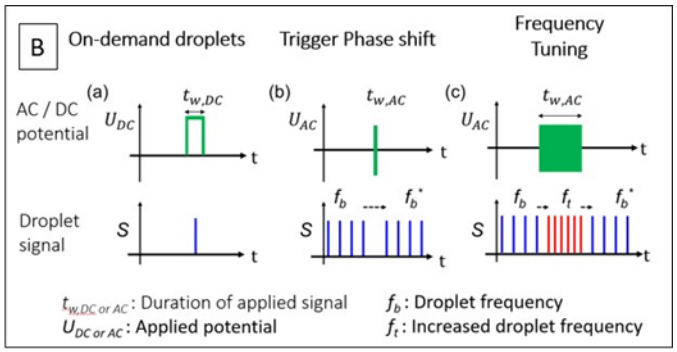
With recent advances of X-ray free electron lasers (XFEL), serial femtosecond crystallography (SFX) has enabled the structure determination of challenging proteins such as membrane protein complexes and their reaction dynamics. In SFX experiments with XFELs, the crystal experiences destruction after a single pulse of the XFEL, and therefore 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. Additionally, 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.





Our approach dynamically generates and triggers aqueous crystal droplets in an immiscible perfluorinated oil in various modes with electric pulses in 3D printed microfluidic devices. In the most recent triggering device, there are three primary components: a T-junction, the electrodes, and the nozzle. In figure A, the schematic is depicted where fluidic channels containing the T- junction is highlighted in purple, the electrodes are in cyan, and the nozzle (and gas line) are in yellow.

The droplets are generated at the T-junction, the electrodes are responsible for electrical triggering in mode 1-3, and the nozzle is designed as a gas dynamic virtual nozzle (GDVN) to inject sample into the path of the XFEL.

The three functional modes of the device, seen in figure B include i) on-demand droplet generation, ii) inducing a phase shift for a given droplet generation frequency to match that of the XFEL for synchronization, and iii) tuning the frequency of droplet generation in 3D printed microfluidics. In mode 1, droplets are generated at frequencies up to 1.6 Hz. Importantly, in mode 2, a phase shift in droplet generation frequency ranging from 2 ms to 80 ms can be achieved to synchronize droplets with the XFEL pulse. We demonstrate up to 80% droplet synchronization with consistent droplet volume (12.1 ± 0.2 nL) and frequency (10 ± 0.1 Hz) in an electronic feedback system with an external reference corresponding to 10Hz XFEL repetition rate. In mode 3, the droplet generation frequency can be tuned through the application of various AC potentials with up to 4-fold increase. A graphical representation of the three modes can be seen in Figure B. In addition, we combined the triggering device with a 3D printed nozzle to integrate the triggering approach in a single piece hybrid injector (Figure A).

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JAMES GEIGER, ARIZONA STATE UNIVERSITY

TBD

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Deepshika Gilbile, University of California-Davis

Functionalized polymer-graphene hybrid substrates for high-throughput, hydrated fixed target SFX

Gilbile, D.[1], Shelby, M.[2], Segelke, B.[2], Grant, T.D.[3], Hunter, M.S.[4], Meents, A.[5], Coleman, M.A.[1,2], Frank, M.[1,2], and Kuhl, T.[1]

[1]University of California at Davis, CA, USA. [2]Lawrence Livermore National Laboratory, Livermore, CA, USA. [3]Department of Structural Biology, Jacobs School of Medicine and Biomedical Sciences, Hauptman-Woodward Institute, SUNY University at Buffalo, Buffalo, New York, USA. [4]Linac Coherent Light Source, SLAC National Accelerator Laboratory, Menlo Park, California, USA. [5]Center for Free-Electron Laser Science, Hamburg, Germany.

Fixed target sample delivery is an exciting alternative to widely used liquid-jet based technologies for biological imaging using XFELs, offering advantages of low sample consumption, high hit rates, eliminating the danger of downtime due to clogging, and with emerging fast-scanning instrumentation, rapid data collection. Some of the key challenges involved in fixed target delivery include optimization of sample deposition and control of the sample environment during measurement, for example by maintaining sample hydration to obtain quality diffraction data, while minimizing added background from enclosing layers. While the use of single crystalline silicon or silicon nitride micropatterned chips with pipette-and- wick based deposition strategies have been demonstrated, the cost and fragility of the supports as well as sample loss from wicking remain challenging issues.

This poster will focus on new strategies to address these issues through the development of robust, cost- effective and rapidly fabricated COC or PMMA polymer-based substrates in conjugation with tunable graphene-polymer support layers that provide long term sample hydration and protection in vacuum. I will also present our latest developments in integrating Ni^{2+}-NTA functionalized polymer surfaces on chip to bind His-tagged proteins and grow protein crystals in desired locations to achieve optimal high hit- rates.

This work was performed, in part, under the auspices of the U.S. DOE by LLNL under Contract DE-AC52- 07NA27344. This work was supported by NIH grants R01GM117342 (NIGMS) and R21AI120925 (NIAID) and the National Science Foundation (NSF) BioXFEL Science and Technology Center Grant 1231306- 6230. Use of the LCLS, SLAC National Accelerator Lab, is supported by the U.S. DOE, Office of Science, under contract no. DE-AC02-76SF00515.

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Matt Goode, Arizona State University

Hybrid modeling theory shows *Francisella* lipoprotein 3 in motion

Matthew R. Goode[1], James D. Zook[1], Debra T. Hansen[1], Michael W. Moran[1], Emily K. Kaschner[1], Elizabeth P. Ramirez[1], Petra Fromme[1]

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Francisella tularensis is a highly virulent pathogenic bacterium that causes tularemia, a potentially fatal disease. *F. tularensis* is endemic to North America as well as Northern Europe and Asia. It is classified as a U.S. Tier 1 select agent in the fight against bioterrorism due to its high virulence and stability when aerosolized. A dose as low as 10 organisms can cause infection which is concerning as no current vaccine is available in the United States. *F. tularensis* lipoprotein 3, or Flpp3, is an outer membrane protein which has been shown to be vital to the virulence of the bacterium. There are two structures of the soluble domain of Flpp3, an NMR structure and an X-ray crystal structure, with a vital difference. The NMR structure shows an internal cavity that the X-ray structure lacks. To further investigate Flpp3 conformations, we combined small angle X-ray scattering with the NMR relaxation data to show a common native state ensemble of Flpp3. These results suggest that the pocket morphology is highly variable, and that Flpp3 can transition to different conformations at room temperature. With this information, the beginnings of ligand studies are being conducted on the protein to look for possible therapeutics against tularemia.

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IZUMI ISHIGAMI, ALBERT EINSTEIN COLLEGE OF MEDICINE

X-Ray radiation reduction of the Cytochrome C Oxidase

Izumi Ishigami[1], Syun-Ru Yeh[1] and Denis L. Rousseau[1]

[1]Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY, USA

Cytochrome *c* oxidase (C*c*O) is the terminal enzyme in the electron transfer chain. It reduces dioxygen to water and harnesses the chemical energy to drive proton translocation across the inner mitochondrial membrane. The enzyme is comprised of four redox centers: a copper center (Cu_A), a heme group (heme *a*), and a Cu_B - heme a_3 binuclear center where dioxygen is reduced to water. Many crystal structures of various forms of C*c*O have been reported. However, it is well-established that metal centers in proteins are susceptible to radiation damage such as photodissociation and photoreduction. As such, radiation damage may have contributed to the uncertainties in the structure of the fully oxidized form of bovine C*c*O (bC*c*O). The axial ligand has been assigned as a peroxide bridging Cu_B and the heme a_3 iron atom. However, its O-O distance is longer than expected for a peroxide ion and when 2 ligands are put in, one on the heme iron and one on Cu_B, they are too close. Here we have measured the *in situ* optical absorption spectra of bC*c*O after exposure to synchrotron radiation and observed that all of the metal centers have become reduced while the crystal structure showed that the protein matrix remained trapped in the oxidized form. After annealing the irradiated crystals, the subsequent crystal structure revealed that the protein matrix had relaxed into the fully reduced form. These data bring into question the ligand structure of the resting oxidized enzyme, which has been proposed to be a peroxide based on crystallographic measurements.

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Andrea Katz, Cornell University

Combining MISC injectors with spectroscopy to boost efficiency of XFEL beatmtimes

Katz, A.M., Calvey, G.D., Zielinkski, K.A., and Pollack, L.

School of Applied and Engineering Physics, Cornell University, Ithaca, New York, 14853

Mix-and-inject serial crystallography (MISC) is an emerging technique that provides atomically detailed snapshots of biomolecules as they carry out their functions. Early experiments have yielded exciting results, but these experiments are still challenging to perform. In particular, choosing the correct timepoints to capture a structural intermediate state is essential for a successful experiment. However, diffusion and chemical kinetics in crystals can differ significantly from solution, and there are currently limited options for measuring these properties *in crystallo*, making it very challenging to choose the best timepoints. Here, we detail new technology to interface MISC injectors with spectroscopic methods, allowing a measurement of kinetics in crystals. This method keeps the same mixers and flow conditions that would be used in an XFEL experiment. With only a fraction of the sample used for an XFEL run, the spectroscopic method can determine timepoints which have significant populations of structural intermediate states. Experimenters can then focus on these timepoints at their XFEL run, greatly boosting efficiency. A proof-of-principle experiment, where EPR spectroscopy is used to track the reaction of myoglobin and azide, is presented.

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ROSEMARIE MARTINEZ BORRERO, FLORIDA INTERNATIONAL U

MECHANISTIC STUDIES OF *MYCOBACTERIUM TUBERCULOSIS* TOPOISOMERASE I INHIBITION BY ENDOGENOUS TOXIN RV1495

Rosemarie Martinez Borrero [1], Pamela K. Garcia[1], Thirunavukkarasu Annamalai[1] and Yuk-Ching Tse-Dinh[1,2]

Department of Chemistry and Biochemistry, College of Arts, Sciences and Education, Florida International University, Miami, FL
Biomolecular Sciences Institute, Florida International University, Miami, FL, USA

Antimicrobial resistance is a worldwide public health concern as existing antibiotics are becoming ineffective. Tuberculosis is the leading infectious cause of death worldwide. According to the World Health Organization, in 2017, there were 558,000 cases of drug resistant tuberculosis (TB), thus there is an urgent need of new TB treatment against a novel target. Toxin-antitoxin (TA) systems participate as gene regulators within bacteria. Researchers believe that TA systems contribute to the long-term dormancy of *Mycobacterium tuberculosis (Mtb)* within the host-cell environment. A system of interest is *Mtb's* toxin Rv1495, a homolog of MazF that is part of the MazEF TA system, which has endoribonuclease activity. The elucidated crystal structure of the MazEF TA system displays a negatively charged pocket on the Rv1495 toxin when bound to its antitoxin. However, previous research showed that the *Mtb* toxin Rv1495 also functions as an inhibitor against *Mtb's* essential topoisomerase IA (TopA) by interacting with the 30 kDa C-terminal domain of TopA. We have developed a complementary assay using an *E. coli* strain with temperature-sensitive *topA* mutation to further locate the Rv1495 interaction site to the 30kDa C-terminal domain of TopA. Results from the complementation with C-terminal deletions allowed us to narrow down this protein-protein interaction somewhere within the 24 amino-acid positive C-terminal tail of TopA. Site-directed mutagenesis is utilized to identify the critical lysine residues within this C-terminal tail that are responsible for the protein-protein interaction. Complementation with the site directed mutagenesis mutants support the role of the lysine-rich C-terminal tail in interaction between TopA and toxin Rv1495. TopA C-terminal domain in mycobacteria are distinct from the C-terminal domain of most other bacteria. The biochemical and biophysical characterization of the mechanism of *Mtb* TopA inhibition will allow the advancement of therapeutic approaches against a new antibacterial target and are selective towards the essential topoisomerase IA enzyme within the pathogenic mycobacteria for treatment of both TB and diseases caused by the non-tuberculosis mycobacteria (NTM).

Acknowledgements: This work is supported by NIH grant R01 GM054226 and Research Supplement GM054226-S1.

Marc Messerschmidt, Arizona State University

TBD

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Diana Monteiro, Hauptman-Woodward Institute

The new photocaging lab at the Hauptman-Woodward Institute: light-triggering of non-naturally photoactivatable systems

Diana C.F. Monteiro[1] and Edward H. Snell[1]

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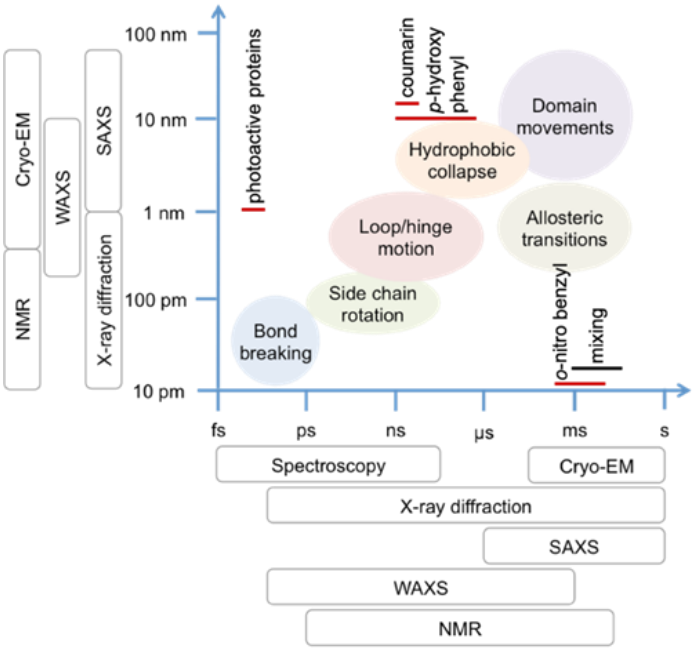
A new synthetic chemistry laboratory dedicated primarily to the synthesis of protein ligands bearing photocaging moieties has been integrated into the Hauptman-Woodward Medical Research Institute. The objective of this initiative is to develop a close relationship with members of the BioXFEL centre, as well as the wider time-resolved structural biology community, interested in pursuing light-triggered time-resolved experiments on systems that are not naturally photoactivatable.

Photocaging strategies involve the introduction of a light-cleavable protecting group onto a ligand/substrate of the protein target which, in the absence of light, remains inactive. A light pulse of suitable wavelength (usually 360 nm) and intensity (depending on the absorption cross-section of the compound) can be used to cleave the protecting group, allowing the free ligand to interact with its protein target and drive protein function and associated conformational changes.[1,2]

The design of such compounds is highly dependent on both the chemical structure of the ligand as well as the photo-decaging requirements. Typical photocages include *ortho*-nitrobenzyl, coumarinyl and *para*-hydroxyphenyl derivatives. The timescales of decaging can range from nanoseconds to 100s of milliseconds and absorption cross-sections are defined by the functional groups decorating the photocages. Quantum yields are highly dependent on the photocage structure as well as the molecule being released. The chemical structure of the final protected ligand has to be design with all these concerns in mind as well as the synthetic route leading to the final compound. A dedicated chemical synthesis has to be devised for each new project and in most cases optimized to yield sufficient high-quality caged compounds for both spectroscopic characterization and the final time-resolved experiments.



As first project, we are pursuing the photocaging of coelenterazine, the substrate of *R. reniformis* luciferase. We will investigate and answer long standing questions regarding the mechanism and dynamics of this reaction.[3] We will elucidate the binding pose of both the substrate and molecular oxygen within the active site as well as how the protein environment is conducive to the oxidation reaction in the absence of co-factors or metals.

1. Levantino, M., Yorke, B.A., Monteiro, D.C.F., Cammarata, M. & Pearson, A.R. (2015) *Curr. Opin. Struct. Biol.* **35** 41-48.

2. Josts, I.; Niebling, S.; Gao, Y.; Levantino, M.; Tidow, H.; Monteiro, D.C.F. *IUCrJ* **2018**, *5* (Pt 6), 667-672

3. Loening, A.; Fenn, T.; Gambhir, S. *J Mol Biol* **2007**, *374* (4), 1017 1028.

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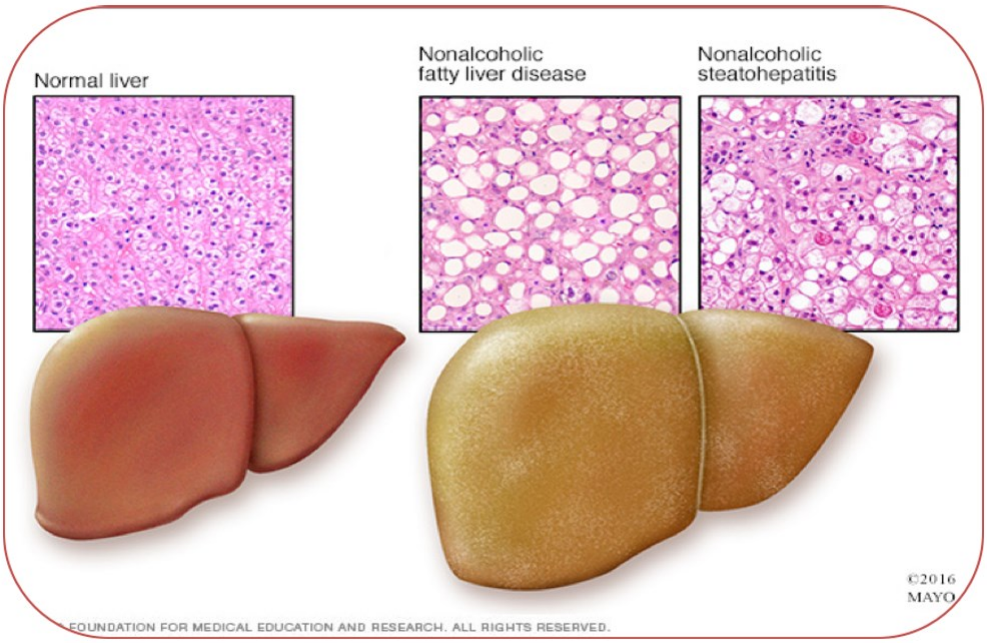
Michael Moran

NMR structure of G0S2 protein

Michael Moran[1,2], Matt Goode[1,2], Paige Ramirez[1,2], Vasiliki Laloudakis[1,2], Emily Kaschner[1,2], James Zook[2], Debra Hansen [1,2], Brian Cherry[1], Petra Fromme[1,2] Rebekka Wachter[1], Jun Liu[3], Xiadong Zhang[3], Wei Liu[1,2]

1. School of Molecular Sciences, Arizona State University, Tempe, AZ
2. Center for Applied Structural Discovery, Arizona State University, Tempe, AZ
3. Mayo Clinic, Scottsdale AZ

Abstract Non-alcoholic fatty liver disease occurs when triglycerides are stored in the liver, leading to irreversible scarring and damage of liver tissue. Inside the liver, adipose triglyceride lipase is responsible for the breaking down of triglycerides and is regulated by the inhibitor g0/g1 switch gene 2 or g0s2 . G0S2 is currently one of the targets against drug design for non-alcoholic fatty liver disease, and more information is needed on the structure of this protein to aid in drug discovery. Here we describe the expression of g0s2 in anE. coli system as well as purification and characterization of a functional, properly folded G0S2 in amounts viable for solution state NMR spectroscopy. Initial spectra of the isotopically labeled protein show well dispersed 15N resonance lines, clean 13C resonances, and dominant a-helices characteristics. These results show that a prepared g0s2 construct is suitable for solution NMR such that 20 amino acids are now assigned in the G0S2 portion of the protein, allowing for further NMR work with this protein for the purpose of structural discovery.



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Henrike Müller-Werkmeister, University of Potsdam

Watching an enzyme at work: Time-resolved serial crystallography with fixed targets and photocaged substrate from milliseconds to seconds

Müller-Werkmeister, H. M.1, Schulz, E. C.2, Mehrabi, P.2, Tellkamp F.2, Miller, R.J.D.2,3, Pai, E. F4

1. University of Potsdam, Physical Chemistry, Potsdam-Golm, Germany 2. Max-Planck-Institute for Structure and Dynamics of Matter, Hamburg, Germany, 3. Chemistry & Physics, University of Toronto, Toronto, ON, Canada, 4. Biochemistry, University of Toronto, Toronto, ON, Canada,

To fully capture the dynamics and structural changes during biochemical reactions over several orders of magnitude in time, is a major experimental challenge and became recently accessible by time- resolved serial femtosecond crystallography (TR-SFX), driven by the availability of XFEL sources.

The sample delivery tools originally developed for those studies at XFELs on the ultrafast time scale [I. Martiel, H. M. Müller-Werkmeister, A. E. Cohen, *Acta Cryst. D*, 2019, D75, 160–177] start to inspire similar experiments at synchrotrons [R. L. Owen, D. Axford, D. A. Sherrell, A. Kuo, O. P. Ernst, E. C. Schulz, R. J. D. Miller, H. M. Müller-Werkmeister, *Acta Cryst. D*, 2017, D73, 373-378.], thereby enabling time- resolved serial synchrotron crystallography (TR-SSX), which allows the study of protein dynamics on time- scales >us at ambient conditions.

We have recently demonstrated the *hit-and return* (HARE) approach [E. C. Schulz*, P. Mehrabi*, H. M. Müller-Werkmeister*, F. Tellkamp, A. Jha, W. Stuart, E. Persch, R. De Gasparo, F. Diederich, E. F. Pai, R. J. D. Miller, *Nature Methods*, 2018, 15 (11), 901-904.], which uses fixed targets for sample delivery of up to 25.000 protein crystals on an individual crystallography chip and allows the efficient data collection for several time points during a single synchrotron beamtime. Using the HARE approach, we were able to follow the full reaction cycle of an enzyme, fluoroacetate dehalogenase, and captured 18 time points from 30 milliseconds to 30 seconds during the non-reversible turnover.

The experimental details reveal four catalytic turnovers and show the entire reaction mechanism, including the formation of the covalent intermediate. [P. Mehrabi*, E. C. Schulz*, R. Dsouza, H. M. Müller- Werkmeister, F. Tellkamp, R. J. D. Miller, E. F. Pai, *Science* 365 (6458), 1167-1170, 2019.] The time-resolved structures further reveal the allosteric mechanism leading to the previously observed half-the-sites reactivity. While the secondary structure shows no conformational changes during the enzymatic reaction, the local water structure both on the protein surface and at the dimer interface shows a strong asymmetry between the subunits, revealing a “molecular phone wire” of water molecules, which seem to transmit the allosteric signal.

Crucial for this experiment was the efficient triggering of the enzymatic reaction using a photocaged substrate. TR-SX experiments mainly focus on protein dynamics that can be light-triggered, however the majority of proteins lack a native chromophore and time-resolved studies of these systems rely on external triggers, like photocages, for reaction initiation, thereby allowing to study light- insensitive, irreversible processes [J. J. Zaitsev-Doyle, A. Puchert, Y. Pfeifer, H. Yan, B. A. Yorke, H. M. Müller-Werkmeister, C. Uetrecht, J. Rehbein, N. Huse, A. R. Pearson, M. Sans, *RSC Adv.*, 2019, 9 (15), 8695– 8699.].

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Garrett Nelson , Arizona State University

Synchronized droplets for serial diffraction

K.Karpos[1 *], R. Nazari[1,2 *], S. Zaare[1], R. Alvarez[1], G. Nelson[1], U. Weierstall[1], and R. A. Kirian[1]

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*These authors have contributed equally

Time-resolved solution scattering (TR-SS) experiments at X-Ray Free Electron Lasers (XFELs) have utilized continuous liquid microjets in order to produce scattering profiles with low background noise. When conducting experiments at XFELs with pulse repetition rates on the order of 100 Hz, these continuous jets waste the vast majority of injected sample between shots, 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 under vacuum is a highly desired improvement for solution scattering experiments.

The use of microdrops rather than continuous jets can simultaneously increase the scattering signal while reducing the liquid flow rate and thereby improve the overall measurement efficiency by orders of magnitude. We have ongoing efforts to develop variants of 3D-printed gas dynamic virtual nozzles (GDVN) that produce periodic droplets that may be synchronized with XFEL pulses at ~100 Hz using a simple piezo-driven trigger.

JOSE OLMOS , RICE UNIVERSITY

PROTEIN CRYSTAL STRUCTURE OF FERREDOXIN FROM MYOVIRUS P-SSM2

Olmos, Jr., J.L.,[1] Campbell, I.J.,[1] Atkinson, J.T.,[1] Sparks, N.,[1] Xu, W.,[1] Miller, M.D.,[1] Silberg, J.J.,[1,2,3] & Phillips, Jr., G.N.[1,4]

Department of Biosciences, Rice University, 6100 Main St., MS-140, Houston, TX, 77005

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The cyanomyophage P-SSM2 encodes a viral ferredoxin that reroutes photosynthesis in the planet's most prevalent phototroph, *Prochlorococcus marinus*. Notably, it is unusual for viruses to encode a part of photosystems, and an incomplete understanding of how this phage ferredoxin reroutes electrons from photosynthesis to benefit the reproduction of the phage motivated our structure determination efforts. A better understanding of the phage ferredoxin could inform the role of cyanophage infection of

P. marinus, a key primary producer in oligotrophic ocean environments. Here, we report this new viral ferredoxin structure to a resolution of 1.6 Å. The ferredoxin contains a [2Fe-2S] cluster that is coordinated by cysteine residues. The asymmetric unit cell in the crystal structure contains two molecules. In molecule A, the electron density for the protein and iron-sulfur cluster is well defined; however, in molecule B, there is some ambiguity. We ruled out radiation damage as a cause of the disorder in molecule B. The precipitant solution contained high zinc concentrations. The literature suggests that zinc can disrupt iron-sulfur clusters. Analysis of the anomalous difference map allowed identification of multiple zinc-binding sites. In particular, at the iron-sulfur cluster binding site in molecule B, the map was consistent with a mixture of molecules that have a zinc ion bound and others with an iron-sulfur cluster. This is further supported by split occupancy refinements. This ferredoxin is relatively unstable (Tm = 29 °C) compared to other Fds (Tm = 60–70 °C). Here, we will discuss some of the structural and biophysical properties of the phage ferredoxin, including its structural similarity to cyanobacterial Fds, its midpoint potential, and its role in electron transfer, which we have confirmed with a cellular assay.

Protein Crystal Structure of PEX4 and PEX22 from *Arabidopsis thaliana*

Olmos, Jr., J.L.,[1] Bradford, S. E.,[1] Xu, W.,[1] Wright, Z.J.,[1] Miller, M.D.,[1] Bartel, B.,[1] & Phillips, Jr., G.N.[1,4]

Peroxisomes are essential organelles that sequester oxidative metabolic reactions in cells. Peroxins (PEX proteins) coordinate peroxisome biogenesis and function. Currently, no structures of peroxins from the reference plant *Arabidopsis thaliana* have been reported. Here we report the X-ray crystal structure of a peroxin complex from *Arabidopsis thaliana*: PEX4 and PEX22. PEX4 is an E2 ubiquitin-conjugating enzyme and PEX22 is an integral membrane protein that anchors PEX4 to the peroxisome membrane. PEX22 interaction is required for the ubiquitin-conjugating activity of PEX4. We co-expressed PEX4 in a translational fusion with the soluble PEX4-interacting domain of PEX22 in *E. coli* and compared the structure of the PEX4-PEX22 complex to the structures of yeast homologs in the Protein Data Bank. Although *Arabidopsis* PEX22 has low sequence identity to yeast PEX22, it maintains a similar architecture. Our structure may inform the altered peroxisomal function of *pex4* mutants isolated through forward-genetic screens. Guided by our structural data, we hypothesize that pex4-1 (P123L) may disrupt the activity of PEX4 or its interaction with the E1 conjugating enzyme, while pex4-3 (A144T) may disrupt the PEX4-PEX22 interaction. Studies are underway to test these hypotheses. Our findings will further inform the role of peroxins in peroxisome structure and function. This work is a part of the undergraduate thesis work of S.E.B., a 2018 BioXFEL summer intern at Rice.

This work was supported by the NSF STC BioXFEL Center Award No. 1231306.

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Valerie Panneels, Paul Scherrer Institute

Mammalian thodopsin dynamics using pump probe serial femtosecond crystallography

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Mammalian Rhodopsin, a prototype of the largest druggable G Protein-Coupled Receptors family (GPCRs), is our light receptor for night vision. Upon photon absorption, its chromophore 11-cis retinal undergoes one of the fastest events in biology, which happens in the femtosecond range, the isomerisation into all-trans. The whole rhodopsin photoactivation process lasts over about 10 orders of magnitude on a logarithmic time scale, until the coupling to the G protein transducin occurs. Our study aims at determining the structure of rhodopsin intermediates using time-resolved serial femtosecond crystallography (TR-SFX), which has been successfully used for the prokaryotic proton pump bacteriorhodopsin [1-3]. Rhodopsin microcrystals grown in the dark are successively injected in the light of a pump laser and directly probed after various time-delays from femtoseconds to milliseconds using an X-ray free electron laser. Several 'static' structures of dark and active states of rhodopsin have been characterized by X-ray crystallography in cryogenic conditions [4]. However, obtaining high-resolution structures of photoactivated intermediates in a time-resolved manner and at room temperature would provide important insights on the detailed mechanism of rhodopsin activation, e.g. retinal isomerization, changes in protonation states, rearrangement of amino acid side chains and water molecules. We have now prepared and characterized crystals of wild-type mammalian rhodopsin diffracting to a resolution of 2 Å. The crystals were obtained for the first time in a lipidic cubic phase, which offers various advantages, including an optimal constant speed of sample delivery. Pilot SFX tests at the SACLA (SPring-8 Angstrom Compact Free Electron Laser) and a TR-SFX test at the LCLS (Linac Coherent Light Source) showed a satisfactory hit rate. Data were collected at the SACLA and SwissFEL. A preliminary map shows, as a proof of principle, rhodopsin with the retinal in a batho conformation at the correctly earlier-predicted time-delay. Time-resolved serial femtosecond crystallography on rhodopsin will not only give details on the molecular activation of a class A GPCR, but will also give insights into the photophysical trigger of retinal excitation upon photon absorption.

[1] Nogly et al. Nat Commun. (2016) 7:12314.
[2] Nango et al. Science. (2016) 354(6319):1552-1557.
[3] Nogly et al. Science. (2018) 13;361(6398).
[4] Deupi et al. Biochem Soc Trans. (2012) 40(2):383-8.

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De-acylation of Escherichia coli lipopolysaccharides

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Water is considered one of the most indispensable starting material for different biotechnical applications such as cleaning, manufacturing, research and development, etc. The required water quality will depend on the intended purpose of the process. In biomedical applications, for example, it is of utmost importance that the quality of the water contains a minimum to none amount of toxins, particularly those from bacterial origin. The lipopolysaccharide (LPS) molecule is an important pathogen that comes from the gram-negative bacteria external cell wall and triggers a signal within the innate immune system.1 To occur the LPS agonist role in humans, it is necessary to stimulate the toll-like receptor 4 (TLR4) by using its six acyl chain from its lipid A moiety.2 The effect of this LPS triggered signal includes an initial strong inflammatory response by the immune system that subsequently is counteracted via the human enzyme acyloxyacyl hydrolase (AOAH). In nature, AOAH detoxifies LPS by removing fatty acids from lipid A component.3 The main purpose of this study is to mimic this biological response in-vitro by exploring additional enzymes in order to develop a sustainable water treatment technology. Removing LPS from water systems had been a challenge because of its high variability of their molecular weights. Furthermore, this molecule had shown relative stability to temperature and pH changes resulting in no structure change.4 In this study we aim to explore a new detoxification mechanism with a catalytic deacylation procedure using lipases. The product of the de-acylation reaction had been characterized via gas chromatography (GC), free fatty acid assays (FFA), dynamic light scattering (DLS), among others. Results have demonstrated viability of the reaction that will serve as the groundwork principle to develop further technology for water purification. The details for this investigation methods and analytical techniques will be presented. This work is supported by the NIH RISE program (Grant # 5R25GM061151-18).

1. Steimle, A.; Autenrieth, I. B.; Frick, J.-S. Structure and function: Lipid A modifications in commensals and pathogens. International Journal of Medical Microbiology 2016, 306 (5), 290-301.

2. Gorelik, A.; Illes, K.; Nagar, B. Crystal structure of the mammalian lipopolysaccharide detoxifier. Proceedings of the National Academy of Sciences 2018, 115 (5), E896-E905.

3. 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.

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.

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USING UNSUPERVISED ANOMALY DETECTION FOR DATA REDUCTION IN TEMPORAL AND SPATIAL DATA

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With the emergence of big data and big data analytics across a variety of application domains in science, engineering, and technology, comes a strong need for tools and methods of handling and sifting through the data. Data can be collected using various types of sensors, across various environments, and arrive in a number of different formats. The data collected can also exist in the temporal or spatial domain. Due to the ability to capture data in a streaming fashion with high temporal and/or spatial resolution, the size of the data collected can reach massive volumes fairly quickly. A typical goal of any data collection or analytic system is to identify which instances of data are relevant and which are irrelevant. The approach presented in this research uses the signature-measure-decision approach to anomaly detection in order to achieve this goal. It is generalizable, unsupervised, requires low communication overhead, and possesses online capability for use in streaming data systems. Experiments were conducted using smoke plume simulation data in the spatial and temporal domain, as well as physiological responses from real test subjects in the form of temporal data (multivariate time-series data). The results when using simulation data show a data reduction of over 80% with a recall of over 90%. In addition, the preliminary results when using real data show an average reduction of 43% with a classification accuracy of over 70%. The results are promising in that they can identify relevant portions of the data without the need of expert knowledge, and greatly help reduce the amount of data that needs to be saved and manually inspected and/or labeled. Furthermore, the approach and algorithms used are general enough that they can be applied across datasets with differing features, sampling frequencies, and application domains.

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Ishwor Poudyal, University of Wisconsin-Milwaukee

Pushing XFEL single-particle imaging towards near-atomic resolution

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X-ray free-electron lasers (XFELs) made it possible to obtain diffraction information from single biological macromolecules. This has been achieved by intense X-ray pulses so short that diffraction data can be collected before the sample is destroyed. By collecting a sufficient number of single-particle diffraction patterns, the three-dimensional electron density of a molecule can be reconstructed. The quality of the reconstruction depends largely on the number of diffraction patterns collected at the experiment. So far, XFEL-based single-particle imaging (SPI) of large biological entities, primarily viruses, has reached a resolution of ~10nm. Here we estimate by simulation how SPI could be applied to much smaller organic molecules and eventually reach near-atomic resolution. We choose thiol decorated gold nanoparticles with 102 gold atoms surrounded by thiol as a model system. We show that the strong scattering of the gold nanoparticles allows one to identify and orient the single-particle snapshots. By collecting 20,000 single-particle snapshots, the electron density of the organic thiol can be retrieved at near-atomic resolution. This approach of rigidly attaching a weakly scattering organic object to a strongly scattering nanoparticle offers a previously unexplored route to imaging biomolecular systems by XFEL single-particle techniques.

This work was supported by NSF-STC “BioXFEL” (STC-1231306).

Surya V. S. R. K Pulavarti, University at Buffalo

SAXS/NMR measurements suggest large conformational excursions of β-lactamase during substrate turnover

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Solution NMR experiments performed for β-lactamase (BlaC) during ceftriaxone (CEF) turnover suggest the presence of large conformational excursions from the 'ground state' associated with X-ray structures. To test this hypothesis, we envision mix-and-inject serial SAXS ('MISS-XFEL') to complement MISC-XFEL.[1] Towards this aim, we (i) expressed BlaC without His-tag, and (ii) collected SSRL SAXS data to confirm that high-quality SAXS samples were prepared. Subsequently, we collected SAXS data at different time points after mixing BlaC and CEF. While the SAXS profiles of BlaC are in excellent agreement with the MISC-XFEL crystal structures of both BlaC and BlaC-CEF complex, the profiles registered during CEF turnover deviate largely form those predicted from MISC-XFEL structures. These findings support the view that large conformational excursions, which are not detected in MISC-XFEL structural analyses, are associated with catalysis. The functional implications of these novel findings are unknown.

References cited on poster:

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

2. Tremblay, L. W. *et al.* Structures of the michaelis complex (1.2 Å) and the covalent acyl intermediate (2.0 Å) of cefamandole bound in the active sites of the *Mycobacterium tuberculosis* β-lactamase K73A and E166A mutants. *Biochemistry* **2010**, *49*, 9685-9687.

Lattman, E. E *et al.* Biological small angle scattering: Theory and practice. *Oxford University Press* **2018**.

Acknowledgements: This work was supported by the NSF STC award 1231306, NSF MCB-1615570 and SSRL proposal 11C9. We thank Marius Schmidt, Jose Olmos, and George Philipps for helpful discussions, Christopher Kupitz for supporting the BlaC sample preparation, and Thomas Weiss and Ivan Rajkovic for SAXS experiment setup at SSRL BL 4-2.

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Linda Quinones, Meharry Medical College

Trypanosoma brucei small TbTims show unique protein-protein interactions

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Trypanosoma brucei (*T. brucei*) is a parasitic protozoan that causes a deadly diseases affecting population in Sub-Saharan Africa known as African trypanosomiasis. The available treatments are highly inadequate and toxic therefore there is a need for development of better drugs to treat this disease. In eukaryotes, the small Tims are soluble intermembrane space proteins of the mitochondria those are involved in the import of hydrophobic inner membrane proteins. These small Tims form heterohexameric complex of 70 kDa where one monomer of a small Tim is alternatively link to another small Tim. For example, in yeast Tim9-10 complex is formed by three Tim9 and three Tim10 alternatively linked by salt bridges. *T. brucei* possesses six small TbTims: TbTim9, TbTim10, TbTim11, TbTim12, TbTim13 and TbTim8/13. Besides Tim9 and Tim10, other small TbTims are unique to trypanosomes. It has been shown by our lab and others that these small TbTims are associated with each other and with the membrane-bound translocase complex of the mitochondrial inner membrane (TbTIM) in *T. brucei*. On a native gel the small TbTims are found in the larger complex similar in size to the TbTIM as well as in a 70 kDa complex, suggesting that these small TbTims may form a similar hetero-hexameric complex. However, the arrangement of the small TbTims in this complex has not been understood. We are using 1) yeast-2-hybrid analysis, co-expression and co-immunoprecipitation analysis of the recombinant small TbTims in different combination, and 3) in-situ interaction studies in *T. brucei*, e.g., proximity-dependent ligation assays (PLA) to elucidate the interaction pattern of these small TbTims. We are also applying site-directed mutagenesis analysis to determine the important amino acid residues critical for their interactions. Identification of the unique structural domain(s) for protein-protein interaction will be helpful to define the target for novel therapeutic intervention against this deadly disease.

This work is supported by the following NIH grants: 5RO1AI125666, 2SC1GM081146,

and R25 GM05994

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SAMINATHAN RAMAKRISHNAN, NATIONAL CANCER INSTITUTE

VISUALIZING LIGAND TRIGGERED CONFORMATION CHANGE AND PHASE TRANSITIONS IN RIBOSWITCH CRYSTALS USING ATOMIC FORCE MICROSCOPY

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Martensitic phase transition in crystals is an important phenomenon in material science and biology. Particularly, solid to solid (SS) phase transition in crystals have critical biological functions, for instance docking of T4 bacteriophage on the bacterial host and motion control in bacteria. However, the martensitic transition has been studied extensively only in organic, inorganic materials and polymers. Here we present a new approach to record time-lapsed ligand induced phase transition in adenine riboswitch crystals using Atomic Force Microscopy (AFM). Under optimized ligand concentration and temperature, the crystal lattice transition from initial apo state to trans state and final bound state was recorded along with multiple intermediate structures at the rate of 23 seconds per frame and 100nm scan size. Moreover, our time-resolved serial femtosecond crystallography (SFX) data obtained using an XFEL, which also illustrate the phase transition in adenine riboswitch crystals, are consistent with our AFM observations. AFM data could provide a preliminary understanding of the phase transition, and observed lattice parameters throughout the transition could be useful in the SFX data analysis.

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Josué Rodríguez Cordero, University of Puerto Rico

High-throughput crystallographic screening method for Membrane proteins at membrane potentials

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Membrane proteins comprise approximately 26% of the human proteome. These include receptors involved in cell-cell signaling, pores, channels, and pumps. Over 40% of the current targets of drugs are membrane proteins, but to date only around 180 *unique* human membrane protein structures have been determined. There are thousands of potential drug targets to be discovered. This is an extraordinary incentive to obtain high-resolution 3D structural data of membrane proteins. It is well known that membrane proteins must be solubilized prior to crystallization, a procedure that comes with the potential for destabilization. Furthermore, standard crystallization methods include performing large screenings over many different parameters, including sample concentration, pH, precipitants, and temperature, oftentimes yielding negative results. There is a *critical need* for a reliable method of crystallizing membrane proteins. Reproducibility is of particular importance in crystallography methods. The ability to consistently produce high-quality membrane protein crystals opens up the possibility of utilizing different ligands during the crystallization process in order to probe their effects on the conformation of the proteins. All membrane proteins are embedded in a membrane that includes polar lipids and differential ionic concentrations on either side, which results in an electric field across them. Thus, it is fair to hypothesize that the structural conformations of localized domains in membrane proteins are voltage dependent. This principle is utilized in the high-throughput crystallographic screening device for membrane proteins that we are designing, prototyping, and testing. The device applies a physiological resting membrane potential to a protein-detergent complex in a lipid matrix (RMP@LMx device, US Patents No. 10,155,221 and 10,358,475). It is also capable of applying voltage ramps and other waveforms. This device is being used to conduct high-throughput screening of membrane protein crystals. The overall objective of this project is the design, fabrication, and testing of an instrument that performs high-throughput membrane protein crystallization trials. Bacteriorhodopsin is currently being used as a model protein in crystallization trials with the RMP@LMx device, to optimize its operational parameters and assess its effects in crystallization.

This work is supported by NIH grants 1R01GM098343, 5R25GM061151-18 and NSF award 1231306.

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Silivia Russi, SLAC - SSRL

Diffraction Quality Optimization and Data Collection at Ambient Temperatures under Humidity Controlled Conditions

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The Structural Molecular Biology (SMB) group of the Stanford Synchrotron Radiation Lightsource (SSRL) has developed a system for automated sample mounting and serial diffraction experiments at controlled humdity at the Macromolecular Femtosecond X-ray Crystallography Instrument at LCLS for general users. At SSRL, this instrumentation will be applied for a new remote access program for crystallography data collection and crystal screening at near physiological temperatures and controlled humidity. The utility of controlled hydration to change the properties of protein crystals is well documented, beginning with the pioneering dehydrated hemoglobin and myoglobin studies of Perutz, Kendrew and Huxley (1940's-1950's). Crystal dehydration increases the protein/solvent ratio and may trigger changes in crystal packing, unit cell dimensions and space group which can change the internal order of the crystal lattice (mosaicity, diffraction power) and crystal/cryo-solution dynamics. Integrating controlled hydration within the pipeline of room temperature data collection will enable optimization per protein crystal sample.

This capability also opens the door to new investigations on protein dynamics. To this end, a novel plate has been developed for crystallization, sample transport and automated sample mounting for diffraction data collection at ambient temperatures.

Future automation at SSRL-SMB will support a rapid transition for diffraction data collection and crystal screening at ambient temperatures to 100K using a rapid nozzle switcher.

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Sarthak Saha, University of Massachusetts-Amherst

X-ray Compatible Microfluidics for Advanced Protein Crystallography

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Serial femtosecond crystallography requires the use of upwards of a million crystals for a single data set. Microfluidic and microfabricated devices are a powerful strategy to facilitate the efficient mounting of a large number of crystals. In particular, our efforts are focused on the design and fabrication of polymer-based fixed target devices for serial crystallography. The use of polymeric materials provides low background while circumventing challenges associated with stray diffraction signals from crystalline materials. This approach also has the potential to take advantage of low-cost continuous manufacturing strategies and further facilitate translation of these strategies from XFELs to synchrotrons. We have developed photoresist based microfluidic platform designed for high throughput serial crystallography experiments in room temperature and cryogenic conditions, as well as under vacuum. The architecture of the device allows photoresist film to act as an ultra-thin X-ray diffraction window while sealing the device to prevent significant water loss for weeks. The use of cleanroom photolithography enables custom tailoring of these devices based on the requirements, allowing easy integration with various X-ray sources and beamline automation. Our fabrication scheme also allows for the straightforward incorporation of graphene into our devices, opening up a myriad of applications. In particular, we have demonstrated the ability to use integrated graphene sheets as X-ray transparent electrodes to enable electric field based studies on various protein samples. We have also demonstrated the ability to use graphene layers as a gas diffusion barrier to enable the room temperature diffraction analysis of oxygen sensitive targets.

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AMIT SAMANTA, DESY

CREATING AND CONTROLLING CRYOGENICALLY-COOLED BEAMS OF SHOCK-FROZEN, ISOLATED, BIOLOGICAL AND ARTIFICIAL NANOPARTICLES

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Single-particle diffractive imaging (SPI) is emerging as a new technique for 3D imaging of aerosolized nanoparticles at x-ray free-electron lasers (XFELs). However, one of the primary bottlenecks in realizing SPI is the efficient delivery of isolated, reproducible target particles into the x-ray focus [1]. Here, we present novel approaches for the production of cold and high-density beams [2] of a broad variety of biological nanoparticles, ranging from single-domain proteins, including membrane proteins, to multi- subunit protein complexes and molecular machines, designed for use in XFEL experiments. This will also enable us to gain a better understanding of the ultrafast dynamics across extended biological systems. Fast freezing from ambient temperature to 10 K in less than 10 µs will help freezing room-temperature equilibrium state distribution and even trapping reaction intermediates.

Furthermore, we have developed a numerical simulation infrastructure that allows quantitative simulation of isolated particle trajectories throughout the setup [3]. This allowed us to improve injection geometries and build aerosol injection systems optimized for specific particle sizes in order to produce the highest-density particle beams [2, 4]. We propose an optimised setup with cooling rates for few- nanometers particles on nanoseconds timescales. The produced beams of shockfrozen, isolated nanoparticles provide a breakthrough in sample delivery, e.g., for diffractive imaging and microscopy or low-temperature nanoscience. The produced cryogenically-cooled particle beams can subsequently be further manipulated and controlled using electric [5] or optical fields, such as hollow-core vortex laser beams [6].

[1] M. M. Seibert, et al, *Nature* **470**, 78 (2011)

[2] A. K. Samanta, et al, *Submitted* (2019)

[3] N.Roth, et al, *J. Aerosol Sci.* **124**, 17 (2018)

[4] V. Singh, et al, *Phys. Rev. A* **97**, 032704 (2018)

[5] Y.P. Chang, et al, *Int. Rev. Phys. Chem.* **34**, 557 (2015)

[6] N. Eckerskorn, et al, *Phys. Rev. Appl*. **4**, 064001 (2015)

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OXYHEMOGLOBIN PROFILE IN THE PRESENCE OF HYDROGEN SULFIDE

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Hydrogen Sulfide (H_2S) had been conventionally considered as a toxic molecule until it was suggested for physiological function. H_2S is produced endogenously at low concentration by enzymatic pathways in humans, via cystathionine β-synthase, cystathionine ɣ-lyase, and 3-mercaptopyruvate sulfur transferase. H_2S is highly lipophilic, it can penetrate a cell by simple diffusion and interacts with some hemoproteins. The interaction of H_2S with hemoglobin (tetrameric protein) and myoglobin (monomeric protein), in the presence of O_2 or H_2O_2 can generate the species sulfhemoglobin and sulfmyoglobin with a characteristic optical band around 620 nm and 618 nm, respectively. This as a result of a covalent heme modification in which one of the pyrrole rings is modified by the incorporation of the sulfur atom across the β-β double bond of the pyrrole. As a result, the oxygen binding capacity of sulfhemoglobin and sulfmyoglobin is reduced by a factor of ~135 and 2,500 in comparison of native hemoglobin and myoglobin, respectively. In 1986, Peisach et. al. studies using isoelectric focusing and optical spectrometry shown that both partially and fully sulfurated tetramers can be found in sulfhemoglobin samples. The A_{625nm}/A_{576nm} absorbance ratio was used as indicator of degree of sulfuration, in which values close to 1 represents fully sulfurated tetramers. This research is focused in the relation between the hemoglobin sulfurated tetramers as a function of physiological range of hydrogen sulfide concentration. The study was carried out evaluating the formation reaction of sulfhemoglobin at physiological conditions using different hydrogen sulfide concentration. The sulfhemoglobin complex sample was prepared by mixing oxyhemoglobin, with H_2S in UV-Vis cuvette. The UV-Vis spectroscopy was used to monitor the sulfhemoglobin complex as well A_{625nm}/A_{576nm} absorbance ratio. The results showed a positive correlation between the sulfhemoglobin absorbance as a function of H_2S concentration and suggest a partially sulfurated tetramers in the sulfhemoglobin samples.

References:
(1) Whiteman, M.; Le Trionnaire, S.; Chopra, M.; Fox, B.; Whatmore, J. *Clin. Sci. (Lond).* **2011**, *121*, 459–488.
(2) Li, Q.; Lancaster, J. R. *Nitric Oxide* **2013**, *35*, 21–34.
(3) Ríos-González, B. B.; Román-Morales, E. M.; Pietri, R.; López-Garriga, J. *J. Inorg. Biochem.* **2014**, *133*, 78–86.
(4) Kolluru, G. K.; Shen, X.; Bir, S. C.; Kevil, C. G. *Nitric Oxide* **2013**, *35*, 5–20.
(5) Román-Morales, E.; Pietri, R.; Ramos-Santana, B.; Vinogradov, S. N.; Lewis-Ballester, A.; López-Garriga, J. *Biochem. Biophys. Res. Commun.* **2010**, *400*, 489–492.
(6) Park C, Nagel R, Blumberg W, Peisach J, Magliozzo R. *J. Biol. Chem*.,261, **1986**, 19, 8805-8810.

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Robin Schubert

Biological sample preparation and characterization at the European XFEL

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

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

The XBI User Consortium has funded and the European XFEL now operates sample preparation and characterization facilities for biological samples. This laboratory offers a wide range of biochemical and biophysical characterization techniques including TEM, ESEM, AFM, SONICC, nativeMS, DLS and AUC. The Sample Environment and Characterization and XBI staff can support users in all steps from gene expression through protein purification, biophysical characterization, and crystallization up to sample delivery of both crystalline and non-crystalline samples of a wide range of biological systems at the instruments of the European XFEL.

The poster will present an overview about available equipment in the labs. In addition, possible workflows for sample preparation and characterization using the proteins actin and formin are shown. Formin was recombinantly produced using *E. coli* and purified using affinity chromatography, while actin was purified from pig acetone powder. Results from the characterization of the Formin-Actin complex will be presented that can be further used for crystallization as well as single particle imaging of larger complexes as well as fibers. It serves as a roadmap to optimize samples step by step in order to ensure the best sample quality for experiments at European XFEL.

ROBIN SCHUBERT

NATIVE MASS SPECTROMETRY FOR SINGLE PARTICLE IMAGING WITH AN XFEL

Lorenzen, K.[2], Kádek, A.[1,2], Lu, Y.[1,2], Bandelow, S.[3], Schweikhard, L.[3], Commandeur, J.[4], Papanastasiou, D.[5], Uetrecht, C.[1,2]

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The European XFEL has entered user operation. With its unparalleled peak brilliance and repetition rate, it has the potential to drive science in the field of SPI at XFELs, which is thus far limited to large and medium-sized viral particles. SPI will allow imaging protein complexes without the need for crystallization. This eventually renders transient conformational states accessible for high-resolution structural studies yielding molecular movies of biomolecular machines. A major bottleneck is the wealth of data required to reconstruct a single structure leading to long processing times. Main reasons for this are intrinsic sample heterogeneity, low signal/high noise and unknown relative particle orientation.

MS SPIDOC will overcome this data challenge by developing a native MS system for sample delivery, named X-MS-I. It will provide mass and conformation selected biomolecules, which are at least partially oriented along their dipole axis upon imaging. This will enable structural reconstruction from much smaller datasets speeding up the analysis time tremendously. Moreover, the system features low sample consumption and a controlled low background easing pattern identification.

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CHENGHUA SHAO, PROTEIN DATA BANK, RUTGERS UNIVERSITY

XFEL/SSX DEPOSITION AND DATA CONTENT SUPPORT AT THE PROTEIN DATA BANK

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

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The Protein Data Bank (PDB) is the single global repository for experimentally determined three-dimensional structures of biological macromolecules and their complexes with ligands. The Worldwide Protein Data Bank (wwPDB) is the international collaboration that manages the PDB archive according to the FAIR Principles: Findability, Accessibility, Interoperability, and Reusability. PDB archive now holds and freely disseminates ~160,000 experimentally -determined structures of biological macromolecules. Consistent growth in the number of structures coming from X-ray Free-Electron Lasers (XFEL) has been observed in the past few years, and such growth is seen across the globe. Of the ~100 XFEL structures deposited into PDB in 2019, 1/3 were from US, 1/3 from Europe, and 1/3 from Asia.

We have worked with wwPDB PDBx/mmCIF Working Group and experts from the XFEL community to develop new PDBx/mmCIF metadata extensions that support the XFEL metadata collection at the wwPDB OneDep system, so that PDB users can review the experimental data pertaining to each XFEL structure. Such metadata are also collected on Serial Synchrotron X-Ray Crystallography (SSX) structures, for which we have seen a significant growth as a result of the demand and technical advancement of implementing time-resolved structural study at the conventional synchrotron light sources.

This presentation briefly describes the XFEL/SSX structures statistics and geographical distribution in the PDB archive. We also demonstrate XFEL/SSX deposition and annotation at wwPDB OneDep system (https://deposit.wwpdb.org/), focusing on collecting metadata specific to XFEL/SSX experiments such as light source, sample delivery, and reflection data processing. The wwPDB is committed to working closely with federal funders and XFEL/SSX users to ensure faithful preservation and representation of their data in the PDB core archive.

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Megan Shelby, Lawrence Livermore National Lab

A fixed target platform for serial femtosecond crystallography in a hydrated environment

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X-ray Free Electron Lasers (XFEL) are enjoying an increasing impact as techniques utilizing their ultrafast, intense, and highly coherent pulses for structural biology are developed. These include serial femtosecond crystallography (SFX), or coherent diffractive X-ray imaging (CDXI) which entail collection of nearly damage-free diffraction patterns for crystalline and non-crystalline samples. However, delivery of the sample into the X-ray beam path 1) while maintaining low X-ray scatter background, 2) with minimal sample consumption, 3) at a rate that matches the pulse repetition rate of the XFEL (120 Hz in the case of LCLS), and 4) in such a way that sample hydration is maintained to prevent degradation remains a technical challenge for some experiments, especially where this methodology is applied to relatively low-ordered samples or those difficult to purify and crystallize in large quantities.

Our strategy towards background minimization for weakly diffracting samples is to explore the use of large area few-layer graphene (FLG) in conjunction with polymer thin films as encapsulating materials for fixed target delivery of hydrated samples in vacuum conditions as a generally applicable method for high-throughput and high-resolution biological imaging at room temperature.[1] A thin (tens of nm) polymer backing imparts mechanical robustness, flexibility, and allows for easy handling while graphene's excellent water barrier properties allow for the minimization of the polymer thickness. We used these hybrid films as enclosing layers to maintain sample hydration for room temperature in-vacuum studies at the Coherent X-ray Imaging (CXI) end station with micropatterned fabricated Si substrates compatible with a rapid scanning fixed target approach utilizing Roadrunner towards a long-term goal of low-background measurements on weakly diffracting samples. As a proof of principle, we used microcrystals of the 24 kDa rapid encystment protein (REP24) to provide a benchmark for polymer/graphene sandwich performance. Comparative SFX experiments were performed in the humidified environment at the Macromolecular Femtosecond Crystallography (MFX) end station without encapsulation.

The REP24 microcrystal unit cell obtained from our sandwiched in-vacuum sample was consistent with previously established unit-cell parameters and with those measured by us without encapsulation in humidified helium, indicating that the platform is robust against evaporative losses. While significant scattering from water was observed because of the sample- deposition method, the polymer/graphene sandwich itself was shown to contribute minimally to background scattering.

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

1. Shelby, M. L.; Gilbile, D.; Grant, T. D.; Seuring, C.; Segelke, B. W.; He, W.; C., E. A.; Pakendorf, T.; Fischer, P.; Hunter, M. S.; Batyuk, A.; M., B.; Meents, A.; Coleman, M. A.; Kuhl, T.; Frank, M., A Fixed Target Platform for Serial Femtosecond Crystallography in a Hydrated Environment. *IUCrJ.* **2019,** (Accepted).

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Andrew Shevchuk, Arizona State University

Present and future prospects for incoherent diffractive imaging

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

We continue our approach to studying IDI through theory and simulation with a model of inner-shell fluorescence generated by semiclassical dipole radiators (i.e., excited high-Z atoms). We also report on recent experimental IDI-related efforts, particularly an upcoming IDI experiment at EuXFEL led by the Chapman group and the advantages of sub-femtosecond X-ray pulses that the compact XFEL being built at DESY will bring to this technique.

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

This work is supported by BioXFEL award 1231306.

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Petr Skopintsev, Paul Scherrer Institute

Femtosecond to millisecond structural changes in a light-driven sodium pump

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Light-driven sodium pumps actively transport positively charged ions across cellular membranes with applications as optogenetic tools in neuroscience. While resting state structures of the prototypical sodium pump *Krokinobacter eikastus* rhodopsin 2 (KR2) have been solved, it is unclear how structural alterations over time allow sodium translocation against a concentration gradient. Using the new Swiss Free Electron Laser, we have collected serial crystallographic data at ten pump-probe delays from femtoseconds to milliseconds. High-resolution structural snapshots throughout the KR2 photocycle show how retinal isomerization is completed within a few hundred femtoseconds and changes its binding pocket in the early nanoseconds. Subsequent rearrangements and deprotonation of the retinal Schiff base allow sodium to path an electrostatic gate in microseconds. In the millisecond range, structural and spectroscopic data in combination with computer simulations indicate transient binding of sodium ions close to the retinal and later at an extracellular exit site. These results provide direct molecular insights into the dynamics of active cation transport across biological membranes.

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Mukul Sonker , Arizona State University

Microfluidic Devices for Rapid Screening of Protein Crystallization Conditions and Fixed-Target Applications

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X-ray crystallography is an excellent tool for determining structures of biomolecules leading to a better understanding of their functions and roles. However, the need for growing high quality crystals to obtain suitable diffraction data poses a major limitation towards the progress of the field. Protein crystallization is a laborious process that requires skilled personnel and multiple sample preparation steps, and may take numerous trials to optimize ideal crystallization conditions for a given protein. Membrane proteins pose even more challenges for crystallization and require specific crystallization conditions similar to their native environments. Another limitation is the high sample consumption during continuous injection for crystallographic experiments that can be minimized and ~100% crystal hit rate can be achieved using a fixed-target for holding crystals. Thus, an automated crystallization screening tool for membrane proteins capable of being directly used as a fixed-target for diffraction experiments will prove beneficial. Previously, we reported a multilayer polydimethylsiloxane (PDMS) nanowell microfluidic device that can rapidly produce 170 unique crystallization conditions via a concentration gradient generator design, requiring ~5 µL of protein solution.[1] Here, we report further development to this device to enable integrated on-chip protein crystallization and fixed-target applications. The improved nanowell device employs a hybrid multilayer design embedding a thin PDMS membrane sandwiched between patterned cyclic olefin polymers (COP) sheets to create valves that can be actuated on the application of vacuum. In the absence of vacuum, the valves remain closed to seal the individual protein well to preserve a particular crystallization condition established during the filling process. This allows vacuum compatibility in crystallography experiments. In addition, COP offers an opportunity for directly exposing sample proteins to X-rays on-chip based on high X-ray transmission properties. Furthermore, we are also developing methods to conduct on-chip sponge-phase crystallization using this COC-PDMS hybrid device for the screening of crystallization conditions for membrane proteins such as Adenosine A_{2A} receptor. Such a device will offer a rapid screening of in-LCP membrane protein crystallization conditions and can be used directly as a fixed target for X-ray diffraction experiments in the future.

B. G. Abdallah et al. Crystal Growth and Design, 2016, 16 (4), 2074–2082 .

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TIMOTHY STACHOWSKI, HWI

Structural insights into conformational switching in latency-associated peptide between TGFβ-1 Bound and Unbound States

Structural insights into conformational switching in latency-associated peptide between TGFβ-1 bound and unbound states

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Transforming growth factor beta-1 (TGFβ-1) is a secreted signaling protein that directs many processes including tissue organization, proliferation, and immunity [1]. The primary endogenous activity regulatory mechanism of TGFβ-1 is sequestration by its pro-peptide, latency-associated peptide (LAP). LAP non- covalently binds TGFβ-1 to sterically prohibit it from interacting with cell-surface receptors and triggering signaling cascades. For this reason, recombinant LAP and antibodies targeting LAP are promising therapeutic strategies for modulating TGFβ-1, particularly as cancer treatments to promote anti-tumor immunity [2]. Although it is known that LAP undergoes a large conformational change during TGFβ-1 binding/unbinding [3,4,5], the mechanism is not completely understood.

Using a combination of crystallography, X-ray solution scattering, and cellular assays, we developed a mechanism of how TGFβ-1 is sequestered by LAP that reveals several potential regulatory features for therapeutic exploitation [6]. Comparison of the crystal structure of apo LAP (solved here) to the previously solved LAP-TGFβ-1 complex structure [4] suggests that LAP is in a more open and extended conformation when unbound to TGFβ-1. Our analysis suggests a mechanism of binding TGFβ-1 through a large-scale conformational change in LAP that includes a contraction of the inter-monomer interface and caging by the 'straight-jacket' domain that may be coordinated through a loop-to-helix transition in the core fold. Cellular experiments support the importance of this helix, as mutants exhibited a reduced ability to form the LAP-TGFβ-1 complex compared to wild-type LAP. X-ray scattering based modelling also supports the notion that LAP is in a more open conformation when unbound to TGFβ-1 and reveals possible orientations and ensembles in solution. Lastly, this analysis indicates that the conformational change in LAP during TGFβ-1 binding does not include a repositioning of the integrin-binding motif as previously suggested [7]. Together, the results from these experiments have provided novel insights into the required conformational changes in LAP for TGFβ-1 binding that can potentially aid the development of therapeutics targeting or using LAP to modulate TGFβ-1 activity.

References:

Annes *et al.*, *J Cell Sci*, 2003

Gabriely *et al.*, *Sci Immunol*, 2017

McMahon *et al.*, *Biochem J,* 1996

Shi *et al.*, *Nature*, 2011

Stachowski *et al.*, *J Synch Rad*, 2019

Stachowski *et al.*, *IUCrJ*, In Review

Munger *et al.*, *Mol Biol Cell*, 1998

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Miklos Tegze, Wigner Research Centre for Physics

Comparison of EMC and CM methods for orienting diffraction images in single particle imaging experiments

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The short and intense pulses of X-ray Free Electron Lasers (XFELs) make diffraction experiments on single particles possible [1]. Identical particles are injected into the X-ray beam with random orientations and diffraction patterns can be recorded in a 2D detector before the particle is destroyed by radiation damage. Individual diffraction patterns of small particles or molecules are noisy and contain insufficient information to solve the structure of the particle. Therefore, to assemble a single set of consistent diffraction data thousands or millions of diffraction patterns must be recorded. The crucial step is to find the relative orientations of the individual patterns.

Here we compare the efficiency and reliability of the EMC [2] and the CM [3-6] orientation algorithms. The EMC algorithm is slightly modified by introducing polar coordinates and using FFT and the correlation theorem. This modification speeds up the calculation and improves its time scaling with the complexity of the particle (R^5 instead of R^6). Both algorithms were optimized for parallel computing on GPUs.

Our test molecules for the comparison were lysozyme and RNA polymerase II. Many thousands (20,000 for lysozyme and 100,000 for RNA polymerase II) of diffraction patterns of the molecules in random orientations were calculated. Poisson noise was generated for several values of XFEL pulse intensity in the range of 10^{27}-10^{28} ph/m^2 for lysozyme and $2*10^{25}$- $5*10^{26}$ ph/m^2 for RNA polymerase II. Then attempts were made by both methods to find the relative orientations of the patterns and to construct a 3D intensity distribution. The reliability of the results was tested by calculating the correlation with the ideal 3D intensity distribution constructed from the patterns using their true orientations.

For the relatively small lysozyme, the results for the two methods do not differ much, although the CM method is somewhat faster. For the more realistic case of RNA polymerase II, we have found that the CM algorithm is not only faster, but more reliable than the EMC method. There is a region of XFEL intensities, where the EMC method seems to converge, but gives inaccurate results. In the same region, the CM method converges to a solution with very low angular errors and gives a reliable 3D intensity distribution. At even lower XFEL intensities, both algorithms fail.

In conclusion, at higher intensities and for less complex objects, both the EMC and CM algorithms give reliable result. For the more demanding case of low intensities and a more complex molecule the CM algorithm is more reliable and gives better results. While in this simulation it was easy to verify the results, we would like to stress the importance of using reliable figure of merits (e.g. the C-factor [5]) and correlation maps [5,6] to validate the results in the case of real measurements.

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

References:

1. G. Huldt, A. Szőke & J. Hajdu, J. Struct. Biol. 144, 219 (2003).
2. N. D. Loh, and V. Elser, Phys. Rev. E 80, 026705 (2009).
3. M. Tegze, and G. Bortel, J. Struct. Biol. 179, 41 (2012).
4. M. Tegze, and G. Bortel, J. Struct. Biol. 183, 389 (2013).
5. M. Tegze, and G. Bortel, Acta Cryst. A 72, 459 (2016).
6. M. Tegze, and G. Bortel, Acta Cryst. A 74, 512 (2018).

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Jennifere Vargas Santiago, University of Puerto Rico - Mayaguez

Immobilized Hemeprotein in Collagen Matrix

Immobilized Hemeprotein in Collagen Matrix

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Hydrogen sulfide (H_2S) has been transformed from a toxic gas, with a characteristic smell of rotten eggs, to a crucial signaling molecule with important physiological function, such as regulation of blood pressure, muscle relaxation, anti-inflammatory, anti-oxidative, tumor suppressor, among others. H_2S dissolution is accompanied by the formation of HS^- (pK_a=7.04) and S^{2-} (pK_a=11.96). At pH 7.4, the total sulfide exists at approximately 18.5% H_2S and 81.5% HS^-. Currently, it is unknown which of the two species has the biological effect; the H_2S or the derived species. For this reason, it is a challenge to develop a robust sensor and/or biomarkers that quantify free or bound H_2S, making it difficult to determine physiological concentrations causing inconsistencies in the literature. Hemoglobin I (HbI), found in the mollusk *Lucina pectinata,* is known for the extraordinary affinity with H_2S. The goal of this project is to create a transparent matrix where HbI can be immobilized into and over a biocompatible environment to scavenge and quantify H_2S. Therefore, this work proposes to investigate the transfer of hydrogen sulfide in a biological system with HbI, and to extend the results obtained so far, to further understand its reaction with other proteins. These issues will be addressed through the following specific aims: (1) To encapsulate HbI-H_2O (metHbI) inside collagen scaffold by electrochemical method, (2) To study the reaction between H_2S and metHbI encapsulated in collagen, (3) To immobilize HbI on collagen layer, and (4) To study the reaction between H_2S and metHbI encapsulated onto collagen.

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STEPHANIE WANKOWICZ, UNIVERSITY OF CALIFORNIA SAN FRANCISCO

TBD

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JENNIFER WIERMAN, SLAC, STANFORD UNIVERSITY

Serial Crystallography Structural Biology Research at SSRL and LCLS

Serial Crystallography Structural Biology Research at SSRL and LCLS

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The standard sample environment available at the new Macromolecular Femtosecond crystallography instrument (MFX) at LCLS is a highly automated goniometer setup for fixed-target serial femtosecond diffraction experiments, supported by the Structural Molecular Biology (SMB) group at the Stanford Synchrotron Radiation Lightsource (SSRL). The experimental front-end is based on developments at SSRL and LCLS XPP to provide an efficient framework to carry out goniometer-based experiments using automated strategies tailored to handle a variety of sample requirements, crystal sizes and experimental goals. These developments coupled with improvements in data processing algorithms make it possible to derive high resolution crystal structures using only 100 to 1000 still diffraction images using just a handful of sample mounts.

Similarities in instrumentation and software environments will form the foundation of a synergistic relationship between the new MFX and a new, next-generation undulator microfocus beam line at SSRL, BL12-1. Through a Gateway approach with MFX, BL12-1 provides a preeminent capability for MC research in the US. BL12-1 is outfitted with a broad bandpass capability which provides exceptional brightness, small microbeams and a high number of reflections when rastering on the fly or using crystal injectors. It is equipped with a high frame rate EIGER detector and a high speed goniometer, enabling new approaches for data collection, phasing and studies of structural dynamics.

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Sharah Yasharahla, Howard University

Study of the energy capacity of lithium ion phosphate batteries with reduced graphene oxide

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Research has shown that adding reduced graphene oxide (RGO) to Lithium Iron Phosphate (LFP) batteries can be used to improve conductivity and performance. This improvement has been demonstrated with LFP/RGO composites delivering an initial discharge capacity of 158mAhg-1 at 0.1C, which is comparable to pristine LFP capacity of 170 mAhg -1. Our research is focused on exploring the addition of high weight percentages (20 - 60wt%) of reduced graphene oxide to LFP cathodes. Initial results for discharge capacity show an increase of the specific capacity in batteries where 60wt% of reduced graphene oxide is added to LFP cathode material. In contrast, batteries with 20wt% of reduced graphene oxide added to the LFP cathode material had similar specific capacities in comparison with batteries made with pristine cathode material. Initial conductivity values are shown to decrease when higher weight percentages of reduced graphene oxide are added to the cathode composition.

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Brenda Hogue, Arizona State University

Virus preparation optimization for single particle structural studies

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Bacteriophage PR772, a member of the *Tectiviridae* virus family, is a double-stranded, linear DNA virus with an approximately 70 nm diameter icosahedral capsid and internal lipid bilayer. Previous studies with a related virus revealed that the internal membrane is released from one of the capsid vertices to form a lipidic nanotube that facilitates delivery of the viral genomic DNA to the host cell during infection[1]. Bacteriophage PR772 was used as a model virus for single-particle imaging (SPI) experiments as part of the SPI Initiative at LCLS. Experiments focused on optimization of sample preparation, experimental conditions and data analysis. Initial SPI experiments produced diffraction snapshots and low-resolution ~9-10 nm 3D structures of PR772 were determined by multiple groups[2-6]. All of the structures exhibited deviations from true icosahedral symmetry. In some cases, off-center positioning of the genome toward one of the vertices was noted and without imposing icosahedral symmetry, projection of a tubular structure from a fivefold vertex was observed. Because of the apparent heterogeneity, we placed a major focus on further optimization to produce more homogeneous, high concentration preps of PR772 for further SPI experiments which aimed at obtaining a higher resolution 3D structure than was previously achieved. In addition to optimizing PR772 samples for SPI experiments, parallel cryo-EM imaging was done, with the goal to directly compare with XFEL results and obtain a high-resolution structure. An optimized protocol for virus preparation was established. Diffraction images were collected during LCLS X341 beamtime. Preliminary analysis indicated that some diffraction patterns extended to 7 nm resolution, but too few single hit snapshots were collected to generate a 3D structure at the higher resolution. Parallel cryo-EM imaging allowed for direct visualization of PR772 particles. Homogeneous, high concentration particles were observed by TEM. However, when particles were plunge frozen and imaged a large number of particles were damaged, with some having extended nanotube-like structures and some appearing to have lost the viral genome. Nonetheless, intact single particle images were manually selected and classified based on variations in structural features. Images were averaged to create projection views which clearly show particle differences. Preliminary volume analysis indicates that some particles exhibit true icosahedral symmetry, but there is clear particle heterogeneity. Overall, the results indicate that the optimized growth and purification protocol yields high quality PR772 preps, but the virus is apparently sensitive to manipulations such as plunge freezing, at least under the conditions that were used. Sample delivery conditions used for XFEL studies may also trigger structural changes in PR772, which could account for some of the results. It remains clear from the last beamtime that improvements must be made to increase the number of single-hit snapshots to achieve high-resolution structures. Other less complex, conformationally sensitive, viruses should also be considered for future XFEL single-particle experiments as the technology is continuing to be developed.

1. Peralta B, et al. *PLoS biology* 2013; **11**(9): e1001667.

2. Reddy HKN, et al.. *Scientific data* 2017; **4**: 170079.

3. Hosseinizadeh A, et al. *Nature methods* 2017; **14**(9): 877-81.

4. Kurta RP, Donatelli JJ, Yoon CH, et al. *Physical review letters* 2017; **119**(15): 158102.

5. Rose M, et al. *IUCrJ* 2018; **5**(Pt 6): 727-36.

6. Shi Y, et al. *IUCrJ* 2019; **6**(Pt 2): 331-40.

Acknowledgements: Funded by the National Science Foundation STC award number 1231306.

XFEL experiments were done at the LCLS at SLAC. LCLS is supported by DOE contract DE-AC02-76SF00515.

JAMES GORDON, SWISSCI

VERSATILE MICROPOROUS POLYMER-BASED SUPPORTS FOR SERIAL PROTEIN CRYSTALLOGRAPHY

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Serial data collection has emerged as a major tool for data collection at state-of-the-art light sources, including microfocus beamlines at synchrotrons, free electron lasers and diffraction limited storage rings. Challenging targets, characterized by small crystal sizes, weak diffraction and stringent dose limits, benefit most from these methods. We report here a polymer-based thin membrane support developed for performing serial data collection or screening on a wide range of protein crystals suspended in liquids, especially more challenging cases. The sample deposition method is simple and robust, but flexible and rationally adaptable to a variety of cases. It results in an optimally thin specimen providing both low background and an environment as close as possible to the growth conditions of the crystals. The large free-standing area enables deposition of drop-scale amounts of material. Imaging and visualization are straightforward on the highly transparent membrane. We demonstrate its use at the SwissFEL and at the Swiss Light Source on a variety of cases including membrane protein samples.

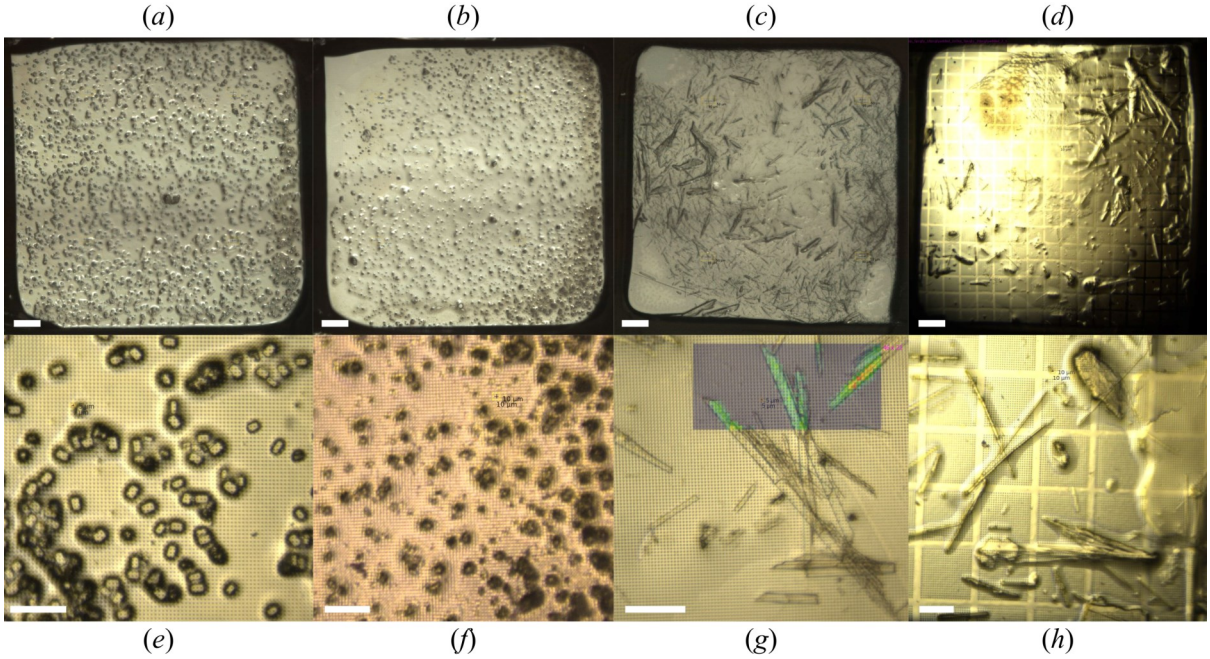

Figure: Online views of chips prepared with some of the tested protein crystals: (a, e) hen-egg white lysozyme microcrystals, (b, f) thaumatin microcrystals, (c, g) TD1 crystals, (d, h) miniG_0-rhodopsin crystals. Scale bars are 200 µm in (a-d) and 50 µm in (e-h).

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Till Stensitzki, FU-Berlin

Comparison of the initial photoreaction of Bacteriorhodopsin in microcrystals

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Thee light-activated proton-pump bacteriorhodopsin (BR) has one of the best studied photo-reactions. The initial photoreaction of BR consists of the isomerization of the 13-cis retinal to the all-trans form on a sub-picosecond scale and was studied by almost every kind of available spectroscopic technique: BR is readily available and easily handled, so it is often used as a benchmark system for novel techniques.

Recently technological advances made time-resolved x-ray crystallography in the femtosecond regime possible, therefore several time-resolved x-ray studies on BR were published and it is now possible to compare spectroscopic with structural changes on a femtosecond timescale. This comparison is necessary, since it is not known if initial photo-reaction is affected by crystallization of the protein. Furthermore, recent x-ray experiments used critically high excitation powers. How and if this affects the reaction is unclear.

Here we use polarization resolved femtosecond pump-probe spectroscopy, which is sensitive to the structure, to show that the initial photo-reaction in BR in identical in LCP-microcrystals and in the purple membrane. To simulate the conditions used in the XFEL experiments, we repeated the experiments with pump powers approaching XFEL conditions. Under these conditions, we observed drastic signal changes.

A distinct feature of the XFEL results are the low-frequency oscillations, which were not yet observed by optical spectroscopy. Using Vis-pump IR-probe spectroscopy we present evidence linking these atomic oscillations to spectroscopic features.

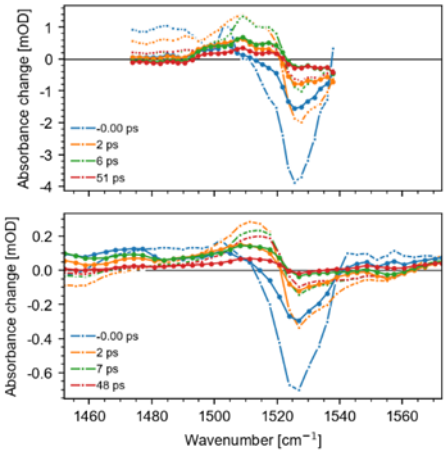

Figure 1: Polarization resolved transient IR spectra in the region of the retinal C=C stretching vibration. Light lines show the difference spectra for parallel pump-probe polarizations, normal lines for perpendicular polarizations. The ratio these two signals depends the structure. *Top:* transient spectra measured on a BR micro-crystal slurry. *Bottom:* transient spectra of BR in the purple membrane. Both the dichroic ratio and the dynamics are identical.

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Elsie Pares-Mato, University of Puerto Rico

Molecular modelling and crystallographic studies of the cytoplasmic domain of Wsc1p

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Saccharomyces cerevisiae, the budding yeast, must remodel initial cell shape and cell wall integrity during vegetative growth and pheromone-induced morphogenesis. The cell wall remodeling is monitored and regulated by the cell wall integrity (CWI) signaling pathway. Wsc1p, together with Wsc2p and Wsc3p, belongs to a family of highly O-glycosylated cell surface proteins that function as stress sensors of the cell wall in S. cerevisiae. Wsc1p possesses a cytoplasmic domain where two conserved regions of the sequence have been assessed to be important for Rom2p interaction. Molecular structures of the cytoplasmic domain of these Wsc-family members were generated using the standard and fully-automated ORCHESTAR procedures provided by the Sybyl-X 2.1.1 program. Their protein models were validated with Procheck-PDBsum and ProSA-web tools, and subsequently used in docking-based modeling of proteinprotein and protein-compound interfaces for extensive structural and functional characterization of their interaction. The results obtained from these computer-based analyses have aimed us to foster the optimal conditions necessary for the crystallization of the cytoplasmic tail of Wsc1p.

Reference: Vélez-Segarra V, Carrasquillo-Carrión K, Santini-González JJ, Ramos-Valerio YA, Vázquez-Quiñones LE, Roche -Lima A, Rodríguez-Medina JR, Parés-Matos EI. Comput. Biol. Chem. (2019) 78:338-352. doi: 10.1016/ j.compbiolchem.2019.01.001. PMID: 30654316

Grant Acknowledgement: This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20 GM103475-14. Also, it was supported by RCMI grant U54 MD007600 (National Institute on Minority Health and Health Disparities) from the National Institutes of Health and by BioXFEL (Biology with X-ray Free Electron Lasers) from the National Science Foundation (2019-2020).

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Eduardo Cruz-Cho, University of Puerto Rico

High-throughput single particle identification of XFEL images using Nystrom Extension

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High-repetition-rate XFELs produce large datasets of 2D diffraction patterns of biomolecules, containing up to millions of snapshots. In principle, such datasets should allow researchers to determine biomolecular structures and conformations, which can be used to compile molecular movies and energy landscapes. However, these datasets not only contain diffraction patterns of single particles; but also snapshots of multiple particles and molecular aggregates. An automated sorting procedure is needed to extract single-particle snapshots from very large XFEL datasets.

Here, we present a Nyström Extension Protocol [1] as an efficient method to identify single-particle snapshots. The protocol relies on manifold embedding, which maps the high-dimensional space of diffraction patterns into a representative lower-dimensional space. Previously, it has been shown that manifold embedding can identify single-particle hits [2], but it required multiple iterations and visual inspection of the different manifold regions. This is overcome by using a training dataset that contains simulated diffraction patterns of single particles only. By using the technique of "Diffusion Map embedding", the training dataset defines a manifold on which the single particle datapoints are clustered. The test dataset, which contains single- and multiple- particles, is mapped into the training manifold, resulting in closely spaced points for the single-particles images, whereas multiple particle images fall into the far manifold-distance regime.

As proof of principle, we tested this method with a dataset from PR772 virus. First, we evaluated a test dataset of simulated diffraction patterns for single and multiple PR772 particles, obtaining above 90% identification of single particles. Second, we employed an experimental XFEL dataset collected at the SLAC National Accelerator Laboratory [3], to demonstrate near-perfect identification of single- and multiple- particles. Overall, our results indicate Nyström Extension Protocol is a promising high-throughput data analytical technique for single-particle identification in large XFEL datasets.

[1]. Lafon, S. et al., "Data fusion and multicue data matching by diffusion maps". *IEEE Transactions on pattern analysis and machine intelligence*, **28**, 1784-1797 (2006).

[2]. Hosseinizadeh, A. et al., "Conformational landscape of a virus by single-particle X-ray scattering". *Nature Methods* **14,** 877–881 (2017).

[3]. Reddy, H.K.N. et al., "Coherent soft X-ray diffraction imaging of coliphage PR772 at the Linac coherent light source", *Scientific Data*, **4**, 170079 (2017).

This work was supported by NSF-STC "BioXFEL" (STC-1231306).

Guillermo Calero (University of Pittsburgh)

Transcription with a laser: Towards a molecular movie of Nucleotide addition

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DNA-directed RNA Polymerase II (Pol II) is a highly-conserved protein among eukaryotic organisms and plays a fundamental role in cellular life, specifically the transcription of genes into messenger RNA (mRNA). Structural studies of Pol II, traditionally X-ray but more recently single particle cryo-electron microscopy have provided snapshots of initiation, elongation, and interactions with factors, DNA damage, and chromatin. Due to the nature of previous experiments (structural snapshots) and limited resolution, mechanisms of Pol II catalysis and the role of the essential "trigger loop" (TL, Rpb1 residues 1077-1096) have been limited. Specifically, by the inability to capture the TL in its closed or "on" conformational state, which to date has been achieved in essentially two datasets published in 2006. In this seminal work, the TL is stabilized in the closed conformation by the presence of a template-specified matched nucleotide. This structure represented a clear breakthrough in our knowledge of the mechanisms of transcription. However, this study was limited by the low resolution of the data (4.0 Å). Furthermore, electron density for the two catalytic Mg^{2+} ions (A- and B-site) was not clearly discernable, meaning the catalytic conformation has not yet been directly observed. Using a newly discovered crystallization condition, and an automated protocol for fixed goniometer free electron laser data collection, we have obtained a radiation-damage free dataset of wild-type Pol II (WT) at 3.0 Å, which is the highest resolution of WT elongation complex yet recorded. Our experiments show *the first static picture* of a pre-inserted nucleotide in the catalytic pocket; the TL in the "on" conformation; strong electron density for sites A and B metals; and a putative third Mg^{2+} (C-site) never before observed. Given that our crystallization condition allowed consistent visualization of all the structural elements involved in the process of transcription by Pol II, we performed time resolved X-ray crystallographic experiments *to elucidate the time evolution* of the molecular events during Pol II nucleotide addition cycle. Our data shows the first molecular movie of Pol II in the act of transcription.

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SANKAR RAJU NARAYANASAMY (SLAC/UB)

NOVEL GAS, LIQUID, PLASMA IMAGING SETUP AT LINAC COHERENT LIGHT SOURCE

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A major problem for XFEL experiments with aerosols and gaseous samples is the wasted XFEL beamtime and inefficient sample use during the long and tedious alignment of the laser with the sample stream. Here, traditional off-axis optical microscopy imaging setups struggle to visualize the sample stream forcing the users to perform time-consuming hit rate scans over the area of interest. Here, we present a novel, robust, live imaging technique that allows to visualize typically "invisible" fluids, such as gases and aerosols, in a single frame and with better contrast than existing optical fluid imaging techniques. This setup combines both coherent Schlieren- and phase contrast imaging principles and has been tested for GDVNs, liquid sheet jets and glass capillaries. Being a second derivative imaging method [1,2], it can furthermore be utilized to visualize liquids, gases, plasmas and laser-liquid interactions. Helium and nitrogen gas streams were successfully visualized experimentally in both atmospheric and partial vacuum environments with sub-micron pixel resolution. Additionally, with the inclusion of surrogate modelling the regime of unknown fluid parameters can be explored.

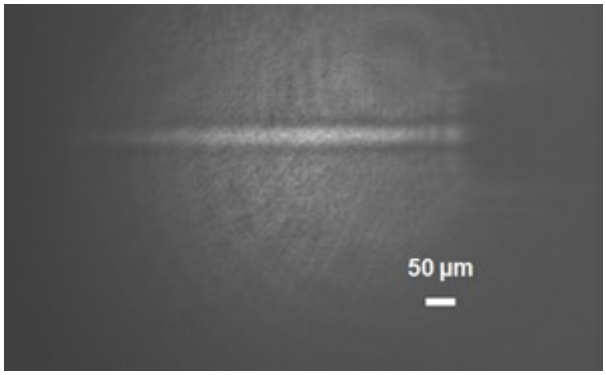

Fig.1. Helium gas from GDVN at 150 psi in atmospheric conditions

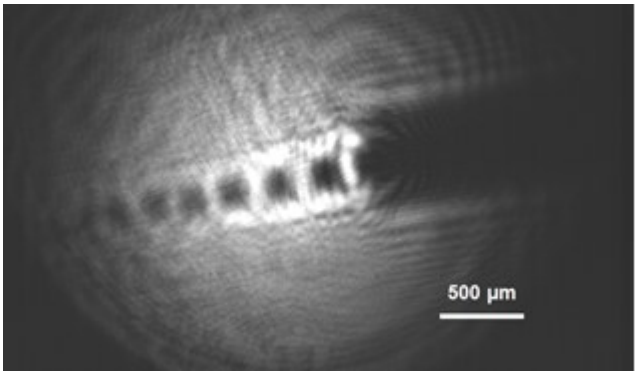

Fig.2. Nitrogen gas from capillary tubing at 400 psi forming Diamond Shock wave patterns in atmospheric conditions

References:

[1] P. Davis, T. Doppner, S.H. Glenzer, et al. X-ray scattering measurements of dissociation-induced metallization of dynamically compressed deuterium, *Nature Communications* **7**, 11189 (2016)

[2] L.B. Fletcher, U. Zastrau, S. H. Glenzer, et al. High resolution x-ray Thomson scattering measurements from cryogenic hydrogen jets using the Linac Coherent Light Source, *Review of Scientific Instruments* 87, 11E524 (2016)P. Davis, T. Doppner, S.H. Glenzer et al. *Nature Communications* **7**, 11189 (2016)

Lars Paulson (University at Buffalo)

Leveraging additive manufacturing techniques to create CFD-inspired microfluidic devices

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Computational fluid dynamics (CFD) is a powerful numerical analysis tool for designing and characterizing microfluidic devices. However, the complex three-dimensional structures suggested by CFD simulations are often difficult to build at micron and submicron scales. By leveraging recent advances in additive manufacturing technologies and developing new manufacturing techniques, we realize these structures and create microfluidic devices with higher performance and new applications

We use a variety of techniques to optimize and fabricate the finished microfluidic devices:

- True-3D manufacturing. Additive manufacturing techniques such as two-photon polymerization allow the manufacture of high -resolution 3D structures not realizable with layer-based techniques.
- Surface treatment. Techniques such as chemical coatings or plasma etching allow control of the chemical and physical properties of the devices, reducing crystal adhesion and promoting laminar flow
- Imaging and characterization. After a device has been fabricated, evaluating the geometry and operation with optical and electron microscopy ensures a defined manufacturing process, accurate translation of the CAD geometry and provides inspiration for new designs.

By combining these diverse methods, we are able to fully take advantage of CFD modeling, and translate the computational solutions into physical devices, ready for use in experiments. Recently, flat liquid sheet jet geometries were fabricated using two-photon polymerization.

This material is based upon work supported by the National Science Foundation BioXFEL grant no. DBI-1231306

Celestino Padeste (Paul Scherrer Institut)

Polymer supports for serial protein crystallography at the SwissMX endstation At SwissFEL

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We are presenting the fabrication of polymer-based supports for serial crystallography, which were designed for application at the SwissMX endstation at SwissFEL, Switzerland's X-ray free-electron laser. Supports for serial crystallography usually consist of fine grid structures or microporous membranes, onto which the protein crystal suspension is deposited and separated from the mother liquid through their sieve-function. We are focusing on polymer-based devices, thus taking advantage of the low X-ray absorption and scattering background of polymer materials, absence of X-ray diffraction if using amorphous polymers, the high design flexibility and the potential mass-fabrication at low cost. Here, we will discuss our developments for measurements at cryo-conditions, where efficient blotting of the crystallization buffer is of highest importance, as well as for studies at room temperature, where specially designed enclosures protect the crystals from drying out and denaturation.

For cryo-applications, perforated polymer films as thin as 2–3 µm are produced using nano-imprint lithography. A stamp with arrays of micro-pillars is embossed into a spin-coated double-layer, which consists of 2–3 µm of COC on top of a water-soluble interlayer (1, 2). After a short plasma etch to remove the residual layer in the embossed holes (3), a supporting frame is applied from the top (4), and the supports are released from the underlying silicon wafer by immersion into water (5), which dissolves the water-soluble polymer. Perforations of 2–3 µm in diameter at 4–6 µm period were found to provide high blotting efficiency, while the film maintained sufficient stability for deposition of the crystal suspension and flash-cooling. X-ray diffraction with very low scattering background signal has been demonstrated in experiments at both, SLS and SwissFEL. For measurements at room temperature, we developed an entirely polymeric enclosure system consisting of 3D-printed top and bottom frames with a suspended continuous polymer film of a few micrometers in thickness, which can be slided onto a frame holding a central support membrane. In this case, the central membrane was 25-50 µm thick with imprinted pyramidal shaped holes. It was designed for capturing individual protein crystals at predefined positions, in order to optimize the hit-rate of the probing X-ray beam. The enclosure system maintained enough humidity to keep protein crystals stable during XFELexperiments of up to one hour.

Mitch Miller (Rice University)

Cryo-trapping crystal studies of photoreceptor PixJ yield insights into its photoconversion mechanism

Miller, M.D.,[1] Clinger, J.A.,[1] Burgie, E.S.,[2] Cohen, A.,[3] Kern, J.,[4] Orville, A.M.,[5,6] Vierstra, R.D.,[2] Phillips, Jr., G.N.[1,7]

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Proteins are dynamic, moving molecules that inhabit an ensemble of states and can change conformation to drive signaling cascades and catalyze reactions. To understand how these signaling cascades work at the atomic level, we are performing cryo-trapping experiments with crystals of the photosensing GAF domain from the blue/green-light photoreversible cyanobacterial photoreceptor PixJ from *Thermosynechococcus elongatus*. The crystal form we are using maintains reversible photo-conversion in the crystalline state. Temperature-scan cryo-crystallography and pump-quenching are two cryo-trapping techniques that we are applying to observe intermediate structures along the photoconversion pathway. The temperature-scan data shows small changes permitted at very low temperatures via $F_{O\,(illuminated)}$-$F_{O(dark)}$ difference electron density maps, which correspond to early intermediates during the photoconversion reaction, as PixJ transitions from its dark-adapted Pb state to its activated Pg state. These smaller changes include the chromophore isomerization and reorientation in the binding pocket. Pump quench experiments allow us to observe larger changes and later intermediates that occur along the photoconversion pathway with time resolution, including binding pocket rearrangements. These cryotrapping techniques enable a more complete understanding of the photoconversion pathway as compared to studying the static Pb and Pg end point structures alone. The PixJ crystals are suitable for serial femtosecond crystallography (SFX) and have yielded a 1.55 Å room temperature structure. Further SFX experiments would complement the cryotraping studies and should allow the capture earlier time points in the photoconversion pathway.

Supported by NSF Science and Technology Center, BioXFEL, NIH, Houston Area Molecular Biophysics Training Grant, Diamond Light Source, and the Welcome Trust. Data were collected at the Stanford Synchrotron Radiation Laboratory beamlines 12-2 and 9-2, at the LS-CAT beamline 21-ID-D and GM/CA-CAT beamline 23-ID-B at the Advanced Photon Source and the MFX instrument at the Linac Coherent Light Source.

Jose Julian Del Toro (University of Puerto Rico)

Cryo-trapping crystal studies of photoreceptor PixJ yield insights into its photoconversion mechanism

The nicotinic receptor α4β2 naturally varies in the number of subunits resulting in two possible stoichiometries: $\alpha4_{(2)}\beta2_{(3)}$and $\alpha4_{(3)}\beta2_{(2)}$. Moreover, studies suggest that especially this complex is critical for cognitive functioning and has an active role in nicotine addiction. High resolution structures have not been resolved, but x-ray and cryoEM data is available. The problem lies in the natural occurring structural heterogeneity that hampers acquisition of high-resolution data. To tackle this, we propose a novel approach in which a histidine tag is cloned in the β2 subunit in order to separate both conformations into pure, or nearly pure solutions. Primers were designed to linearize the plasmid and purify the insert (gene + tag). Then, the latter were added to the Gibson's assembly mix for subcloning. Subsequently, the mix is used for transformation in Dh5α competent cells and for colony polymerase chain reaction (PCR) for validation. Results show that the cloning was successful, nonetheless sequencing is necessary to fully know is it was completed successfully.

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Rebecca Jernigan (Arizona State University)

New structural insights into the function of the active full length human Taspase 1: a novel anticancer therapeutic targert

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Taspase1 (threonine aspartase 1) is an endopeptidase overexpressed in primary human cancers that has been identified as a novel potentially potent anticancer drug target. Loss of Taspase1 activity has been demonstrated to disrupt proliferation of human cancer cells in vitro and in mouse tumor xenograft models of glioblastoma. By functioning as a non-oncogene addiction protease, Taspase1 coordinates cancer cell proliferation, invasion and metastasis. Taspase1 encodes a highly conserved 50 kDa inactive proenzyme that undergoes auto-proteolytic cleavage becoming an active heterodimer that displays an overall αββα structure. The crystallographic structures of the proenzyme and a truncated version of activated Taspase1 are known. In this study, the crystallographic structure of the full length active human Taspase1 is shown to 3.1Å. For the first time, a key structure element has been identified: a long helix of about 50 residues that was missing in previous reported structures of the activated enzyme. Previously his helix was predicted to have a helix-turn-helix conformation lying right on top the catalytic site of Taspase1; however the crystallographic structure of the full-length taspase1 shows a straight helix conformation. This opens new insights in the enzymatic mechanisms for possible substrate recruitment of Taspsae1 and suggest the long fragment as a novel target for the design of medical drugs that inhibit the function of Taspase1 enzyme.

Acknowledgement:

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

Juan Lopez-Garriga (University of Puerto Rico)

Lactoperoxidase catalytically oxidize hydrogren sulfide via sulfheme turnover to sulfer species

Lactoperoxidase Catalytically Oxidize Hydrogen Sulfide via Sulfheme Turnover to Sulfur Species

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Hydrogen sulfide (H_2S) is produced endogenously in organs and mammalian tissues by four enzymes: cystathionine b-synthase (CBS), cystathionine g-lyase, 3-mercaptopyruvate sulfurtransferase, and D-amino acid oxidase. H_2S is implied in cancer, diabetes, chronic kidney disease, cardiovascular, and other diseases, as well as in potential therapeutic effects, such as neuromodulation, neuroprotection, vasodilation, inflammation, and cytoprotection. It is highly lipophilic and hence could penetrate cells by simple diffusion. Because the possible nature of H_2S as an antioxidant agent, the chemistry and biology of hydrogen sulfide have as targets relevant hemeproteins, such as myoglobin (Mb), hemoglobin (Hb), lactoperoxidase (LPO), myeloperoxidase and other heme proteins. The results show that sulfheme, a product of Mb and Hb, in the presence of H_2S and H_2O_2 or O_2 are energetically more favorable than its native analog by 130 Kcal/mol and 60 Kcal/mol, respectively. The theoretical and experimental electron paramagnetic results show that H_2S inhibits the formation of ferryl Compound I, (FeIV=O Por•+) radical species via sulfheme formation, and no ROS amino acid species are present at the end of the reaction.

Our results show that under strictly anaerobic conditions the addition of H2S to native LPO does not form the heme-H_2S complex. Furthermore, data indicate that the presence of aerobic conditions (O_2 or H_2O_2) in the presence of H_2S leads to the characteristic spectrum of sulfheme LPO derivatives with the electronic transitions at 638 nm and 727 nm, assigned to the ferrous and ferric states, respectively. Interestingly, in the presence of H2S, throughout the reactions of LPO with H_2O_2, a continuous turnover of the formation of ferrous and ferric sulfLPO followed by a recovery of native LPO was observed, indicating LPO catalyzed oxidation of hydrogen sulfide by the peroxide. This catalytic oxidation of H_2S is not consistent with sulfheme decomposition to regenerate hydrogen sulfide; in other words, H_2S transport via sulfheme is not possible in biological systems, in agreement with the theoretical results. Instead, product analysis suggests that the turnover process generates oxidized sulfur species, most likely sulfate (SO_4^{-2}) and polysulfides (HSx-x = 2-9) as products.

The transformation of sulfLPO to these inorganic sulfur derivatives allows suggesting the process as a mechanism to dispose of H_2S from related environment. Also can be speculated that if sulfate accumulates in the *in vivo* system it can lead possible tissue inflammation process. Besides, electron paramagnetic resonance data suggest that during sulfheme turnover, H_2S can act as a scavenger of H_2O_2 in the presence of LPO without detectable formation of any carbon-centered protein radical species, suggesting that H_2S protects the enzyme from radical-mediated damage and ROS environments.