BioXFEL

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9th International Conference

February 8-10, 2022

POSTER COMPETITION ABSTRACTS

POSTER SESSION

TUESDAY 5:30-7:00 PM - EVEN NUMBERS PRESENTED
WEDNESDAY 5:30-7:00 PM - ODD NUMBERS PRESENTED

#	FIRST NAME	LAST NAME	POSTER TITLE
1	Nadia	Ali	Mitigating Oxidative Stress via p47 Interaction with Plasma Membrane
3	Adil	Ansari	Generating a co-axial aerosol beam with a novel focuser to improve particle hit rate as part of single particle imaging
4	Michael	Brown	Femtosecond X-Ray Scattering Reveals Functional Dynamics of G-Protein-Coupled Receptor
5	Sebastian	Cardoch	Temporal shaping of X-ray fluorescence emission using high-intensity X-ray pulses
6	Melissa	Carrillo	Fixed-Targets for Protein Dynamic Studies at SwissFEL
8	Joe	Chen	Towards phasing from crystal stacking fault
9	Eduardo	Cruz Chu	Retrieving Biological Function from Single-Particle Images of Time-Resolved Experiments
10	Gabriela	Diaz-Figueroa	From Alignment Laser to 3D Structures: Determining Structures of GPCRs in a Compact X-ray Light Source
11	Diandra	Doppler	Electronically Stimulated Droplet Microfluidics for Reduced Sample Consumption During SFX
12	Raison	Dsouza	Extraction of continuous conformational change from single-particle data: A benchmarking study
13	Mariola M.	Flores Rivera	Synthesis, Crystal Structure and Biomedical Application of Ferrocene-Hormone Complexes
14	Roshanak	Etemadpour	Energy Landscape of the SARS-CoV-2 spike protein in complex with ACE2 receptor
15	Petra	Fromme	Molecular Movies of Biomolecules “in Action” with X-ray Free Electron Lasers
16	Martin	Fuchs	Advanced data collection at FMX – the Frontier Microfocusing Macromolecular Crystallography Beamline at National Synchrotron Light Source II
17	Marina	Galchenkova	Strategy for data compression in protein crystallography experiments

POSTER SESSION, continued

#	FIRST NAME	LAST NAME	POSTER TITLE
18	Leland	Gee	Developments of X-ray Spectroscopy at the LCLS Towards Applications to Biomedical Research
19	Deepu	George	Impact of protein crystal contact forces on intramolecular picosecond dynamics
20	Elijah	Gonzalez	Ab initio Phasing of High Solvent Content Crystals Using a Hybrid Input-Output Algorithm
21	Debbie	Hansen	Advances in the structural biology of the outer surface proteins from the Lyme disease pathogen support the structure-based design of needed therapeutics
23	Rick	Hewitt	Towards FOSS Processing of Pink-Beam Rotation Series A case study on E. coli Dihydrofolate Reductase
24	Konstantinos	Karpos	Time-Resolved Drop on Demand at the LCLS Macromolecular Femtosecond Crystallography (MFX) Endstation
25	Thomas	Kierspel	MS SPIDOC: Mass Spectrometry meets Single Particle Imaging
26	Hyun Kyung	Lee	Photo-activated solid-to-solid-phase transition in adenine riboswitch aptamer RNA crystals.
27	Zhongrui	Liu	Polymer-based fixed target chips for low-background, hydrated fixed target SFX
28	Tek	Malla	Time Resolved Study on Beta Lactamase Reacting with an Inhibitor
29	Abhik	Manna	Microfluidic Devices for Membrane Protein Sample Delivery to X-ray Sources
30	Andrea	Markelz	Simplifying Protein Collective Vibrational Assignment Through Symmetry
31	Jeffrey	McKinney	Photoprotective Structural Change Led by Intramolecular vibrational switching
32	Benjamin	Mobley	A Metropolis Monte Carlo Algorithm for Merging Single-Particle Diffraction Intensities
34	Jessika	Pazol	Understanding the in-vitro de-acylation response of bacterial lipopoly-saccharides (Ra LPS) via enzymatically activated hydrolysis in aqueous systems
35	Anthony Rafael	Rivera-Barreto	The DNA binding diversity of the SIX family of transcription factors in Drosophila melanogaster and Heliconius erato.
36	Jessica	Rodríguez-Ríos	Investigating the DNA-binding specificity of cardiac transcription factors complexes
37	Sarthak	Saha	A Microfluidic Device for Room Temperature Crystallography

POSTER SESSION, continued

#	FIRST NAME	LAST NAME	POSTER TITLE
38	Ana Sofía	Santiago-Russe	Structural Characterization and Structure-Guided Engineering of MfnG SAM-dependent MTase
39	Manoj	Saxena	A structural view of chromatin remodelling by BAF at human Lin28b nucleosome locus
40	Gebhard F.X.	Schertler	Retinal isomerization in vertebrate visual pigments measured at SwissFEL
41	Madan Kumar	Shankar	The pyrrole water amplifies the chromophore action - The preparative state prior to isomerization of the D-ring
42	Chenghua	Shao	Enhanced Deposition of XFEL/SSX Structures at the Protein Data Bank
43	Alexandra	Tolstikova	Recent Developments in Cheetah
45	Stephanie	Wankowicz	Ligand binding remodels protein side chain conformational heterogeneity
46	August	Wollter	Background impact on EMC reconstruction and phasing in single particle imaging of biological samples
48	Iris	Young	Curiosity: A Tool for Exploring Maps
50	Joshua	Rodriguez	Measuring Temperature in Macromolecular Crystallography Experiments using Water Scattering and Molecular Dynamics
51	Ravindra	Singh Rawat	Taxifolin inhibits Adenosine deaminase from Mycobacterium tuberculosis (MtbADA): Structural and Kinetic studies
52	Juan	Valentin-Goyco	Reconstitution and Biochemical Characterization of CYP11B Enzymes in Nanodiscs
53	Victoria	Mazlova	xxx
55	Alfredo	Bellisario	Enhancing orientation recovery in biological X-ray SPI with neural networks
56	Reza	Nazari	3D Printed Gas Dynamic Virtual Nozzles To Synchronize Droplets With The XFEL (X-Ray Free Electron) Pulses
57	Sabine	Botha	Serial Macromolecular Crystallography at ALBA Synchrotron Light Source
58	Maximillian	Wranik	Molecular snapshots of drug release over eleven orders of magnitude in time

POSTER #1

Nadia Ali

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RETURN TO INDEX

Mitigating Oxidative Stress via p47 Interaction with Plasma Membrane

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The overall focus of this project is the structure of the PX domain of p47 and its binding to potential inhibitors. p47 is one protein within the NADPH oxidase 2 (NOX2) enzyme complex. This peripheral membrane protein produces reactive oxygen species, compounds that can trigger apoptosis. Oxidative stress induced diseases include cancer and cardiovascular disease. Determining molecules that block p47 activation would be the first auspicious step in the development of an anti-cancer drug.

Structural studies of the PX domain of p47 were conducted using both X-ray crystallography and NMR spectroscopy techniques. Using this data, fragment screenings for ligand binding were performed. The principles of fragment-based drug design were implemented in this study.

Several fragments show strong binding to p47 (Kd < 1 mM). These ligands will be studies and developed further to yield more energetically favorable and efficient binding. Once their interactions with p47 are fully characterized, novel ligands will be synthesized and tested for inhibition ability.

POSTER #3

Adil Ansari

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RETURN TO INDEX

Generating a co-axial aerosol beam with a novel focuser to improve particle hit rate as part of single particle imaging

Adil Ansari[1,2], Richard A. Kirian[1], Reza Nazari[1,2], Roberto Alvarez[1,3], Konstantinos Karpos[1], Judicael Tombo[1], Sahba Zaare[1], John C.h. Spence[1]

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Aerodynamic Lens Stacks (ALSs) have been developed over the past 15+ years to focus aerosol beams for the purpose of Single-Particle Imaging (SPI) development at XFEL facilities. The ALS systems used in SPI experiments are now well-optimized and produce roughly 10 micrometer diameter particle beams.[1] However, the fraction of XFEL pulses that intercept a particle remain low when directing a ~100 nm x-ray beam at individual protein molecules (i.e. targets that are not large protein complexes such as viruses).[2] Here we consider a novel aerodynamic focuser that exploits inertial focusing, but which uses a special geometry that allows the x-ray beam to run parallel to the aerodynamically focused aerosol beam.[3] We expect that such a geometry would increase the hit fraction substantially as the entire ~200 micron Rayleigh length of the x-ray beam focus could be utilized. We will present our preliminary simulation studies to investigate the behavior of the focuser with particle-fluid models.[4]

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Acknowledgments

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POSTER #4

Michael Brown

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RETURN TO INDEX

Femtosecond X-Ray Scattering Reveals Functional Dynamics of G-Protein-Coupled Receptor

Michael F. Brown[1,2], Thomas D. Grant[3], Suchithranga M.D.C. Perera[1], Leslie A. Salas-Estrada[4], Andrey V. Struts[1], Konstantinos Karpos[5], Udeep Chawla[1], Steven D.E. Fried[1], C. Swathi K. Menon[1], Nipuna Weerasinghe[1], Domingo Meza[6], Derek Mendez[5], Alan Grossfield[4], Petra Fromme[6], Richard A. Kirian[6]

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Development of ultrafast X-ray free-electron lasers (XFELs) gives a new avenue to experimentally investigate protein dynamics in the picosecond and femtosecond time regimes. For G-protein-coupled receptors (GPCRs) like rhodopsin, an important question is how the rapid local dynamics of the ligand initiate the functional protein transitions. However, structural evidence is lacking because until now the extreme reaction speed has precluded direct X-ray diffraction approaches. Here we show how solution X-ray scattering combined with molecular simulations informs the functional dynamics of membrane proteins in ways that could previously only be imagined. To follow the protein structural changes due to light absorption by retinal, we conducted time-resolved pump-probe small-angle X-ray scattering (TR-SAXS) studies of detergent-solubilized rhodopsin. Femtosecond solution X-ray scattering using an XFEL detected the protein structural change in the absence of a crystal lattice and allowed the first direct detection of *cis–trans* isomerization of retinal bound to visual rhodopsin as it occurred in real time. We discovered a significant difference scattering signal within the first few time points immediately following the pump laser triggering event. Our analysis of the difference scattering profiles showed two dominant states within first 10 picoseconds due to the photorhodopsin and bathorhodopsin intermediates. Molecular dynamics (MD) simulations furthermore allowed interpretation of the structural motions corresponding to the *cis–trans* isomerization of retinal within the protein binding pocket. Upon light excitation the localized retinal isomerization initiates ultrafast global movements of the transmembrane helices that propagate at the speed of sound throughout the entire protein. The overall volumetric expansion is driven by the global structural changes over longer time scales that trigger the visual signaling cascade. Our experiments directly reveal the initial structural steps of rhodopsin leading to the metarhodopsin equilibrium, G-protein activation, and subsequent visual signaling. This research was carried out at LCLS during beam times LE71, LM59, LN60, LW75, LP99, and LU18.

Acknowledgments

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POSTER #5

Sebastian Cardoch

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RETURN TO INDEX

Temporal shaping of X-ray fluorescence emission using high-intensity X-ray pulses

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Incoherent diffractive imaging has been suggested as a novel technique to determine structure employing high-intensity X-rays. Emission from independent atoms within a temporal coherence of a few femtoseconds can generate an interference pattern that encodes relative emitter positions. Detection of this signal depends on the duration of the incident pulse, where a good signal-to-noise ratio mandates a photon-dense pulse that is as short as possible. Pulse lengths of 10 fs currently produced at X-ray free-electron lasers might not be sufficient to achieve the required signal. In this work, we computationally studied the temporal shaping of a Gaussian X-ray pulse at 9 keV using copper. The sample becomes plasma within femtoseconds, and we modeled the photon-matter interaction via collisional radiative plasma simulations. We studied dynamical changes of the transmitted and emitted pulse near copper's K-alpha fluorescence and found a temporal reduction in both cases of around a few femtoseconds. Due to the number of photons deposited, the sample becomes partially transparent and achieves a non-equilibrium transient state. We investigated the mechanisms responsible for these changes and explored the possibility to engineer shorter pulses.

Acknowledgments

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POSTER #6

Melissa Carrillo

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RETURN TO INDEX

Fixed-Targets for Protein Dynamic Studies at SwissFEL

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Various design aspects contribute to the advancement of serial femtosecond crystallography (SFX). Developments such as x-ray light sources, x-ray detectors and sample delivery methods play a strong role for the success of experiments. Ideal sample delivery systems allow for fast data collection, low consumption of crystalline sample, low background noise and short data collection time[1]. Meeting these requirements makes time-resolved SFX experiments possible, which require large amounts of data[2].

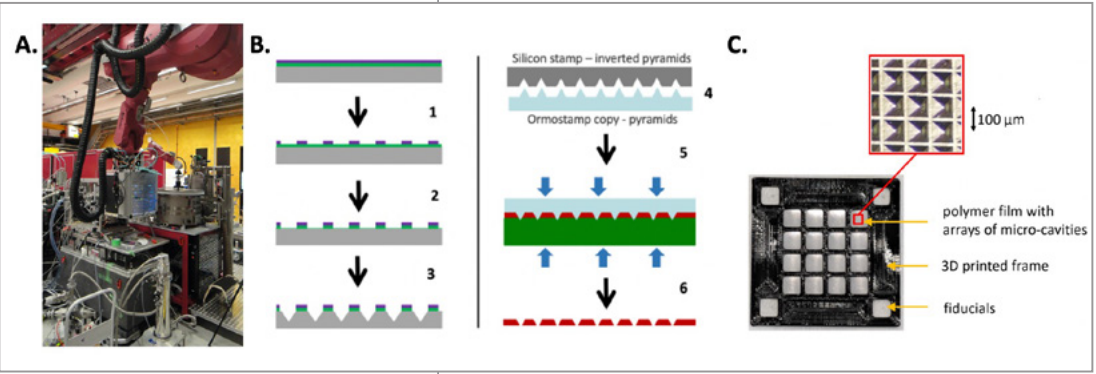

Figure 1. A. Fixed target endstation at SwissFEL B. Fabrication of a silicon stamp through reactive ion etching (1-2) and KOH etching (3). An ormostamp copy is created from the silicon stamp through UV imprinting (4), which is hot embossed through a polymer film (5); final product after plasma etching (6) C. First-generation SwissMX fixed target

When using fixed targets, the crystalline samples are deposited on a chip prior to data collection. Fixed-target SFX sample delivery methods have mostly focused on chips microfabricated from silicon wafers that offer an inert support for the immobilized crystals and a precise aperture array for rapid alignment strategies[3,4]. These chips are reusable but brittle. They can give strong Bragg reflections along the Si(111) axis when misaligned, potentially damaging x-ray detectors.

Here we present preliminary data on the fabrication of polymer-based fixed-targets being developed for TR-SFX at SwissMX, the new end station dedicated to fixed-target SFX at SwissFEL, Switzerland's X-ray free-electron laser. The polymer-based film provides low x-ray absorption and scattering background, high design flexibility and the potential mass-fabrication at low cost. We have designed inverted pyramidal shaped wells in membranes ranging from 25-50 µm in thickness. This design enables single crystals to funnel into predefined positions, optimizing the hit-rate of the probing X-ray beam.

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Acknowledgement:

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POSTER #8

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RETURN TO INDEX

Towards phasing from crystal stacking fault

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Arrangements of molecules deviating from perfect, infinite lattices give diffracted intensities between Bragg peaks. These inter-Bragg intensities can yield an increased information content that allows for model-free structure determination[1-7].

We have previously developed an occupancy formalism that is able to describe the diffraction from finite, arbitrarily truncated crystals, and an associated phase retrieval algorithm that successfully phases such diffraction[8]. Here we show that the same approach can be applied to phasing the inter-Bragg intensities arising from crystals with stacking faults.

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POSTER #9

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RETURN TO INDEX

Retrieving Biological Function from Single-Particle Images of Time-Resolved Experiments

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Recent progress in data analysis of single-particle cryogenic Electron Microscopy (cryoEM); such as Geometric Machine Learning algorithms, allows one to retrieve the conformational spectrum of heterogeneous molecular ensembles. Until recently, most efforts have been attempted under *equilibrium* conditions[1,2], where the conformational spectrum is time-independent, and thus directly yields the free-energy landscape. However, biological systems operate far from equilibrium to sustain the processes that constitute life.

Out of equilibrium, the functional pathways are time-dependent, and the evolution of the conformational spectrum can be described by a Fokker-Planck equation, however with an unknown operator. State-of-the-art developments in Machine Learning, the so-called Physics-Informed Neural Networks (PINN)[3], allows one to retrieve the underlying Fokker-Planck operator from sparse observations alone[4], providing a complete physics-based description of the out of equilibrium process. Moreover, time-resolved cryoEM experiments have recently become practical[5]. Together, this enables us to combine the advantages of time-resolved serial crystallography ('out-of-equilibrium processes') with the advantages of single-particle methods ('avoid averaging over unlike particles').

Based on these opportunities, we establish a conceptional and algorithmic framework to extract functional pathways *out of equilibrium* from a collection of time-resolved single-particle images. This constitutes an unexplored route in studying biological function and structural dynamics *out of equilibrium*.

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POSTER #10

Gabriela Diaz-Figueroa

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RETURN TO INDEX

From Alignment Laser to 3D Structures: Determining Structures of GPCRs in a Compact X-ray Light Source

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The Compact X-ray Light Source (CXLS) at Arizona State University produces diffraction patterns with intense X-ray by Inverse Compton Scattering (ICS). In ICS, a photon acquires energy from an electron to produce an X-ray. With this system, we can study G-Protein Coupled Receptors (GPCRs), which are peripheral membrane proteins targeted by 60% of FDA approved drugs. When GPCRs are activated, a series of reaction can occur. For example, opening and closing of ion channels, activation or deactivation of enzymes, and protein hydrolysis. The structural characterization of GPCRs can lead to optimization of drug development, by assessing a better targeting mechanism that will lead to fewer side-effects and better drug biding. We conducted a laser alignment in the CXLS using a YAG screen, which is a fluorescent screen that helps determine the size and intensity of the beam. Furthermore, we provide the crystallization process of GPCRs using Lipidic Cubic Phase (LCP) and describe its potential for structure determination using the CXLS and CXFEL.

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POSTER #11

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RETURN TO INDEX

Electronically Stimulated Droplet Microfluidics for Reduced Sample Consumption During SFX

Diandra Doppler[1,2], Mukul Sonker[1,2], Ana Egatz-Gomez[1,2], Garrett Nelson[2,3], Mohammad Towshif Rabbani[1,2], Abhik Manna[1,2], Cole Errico[1,2], Jorvani Cruz Villarreal[1,2], Jose Manuel Martin Garcia[4], Rebecca Jernigan[1,2], Sahba Zaare[2,3], Konstantinos Karpos[2,3], Roberto Alvarez[2,3], Sabine Botha[2,3], Gihan Ketwala[1,2], Thomas Grant[5], Angel L. Pey[6], Alice Grieco[7], Miguel Angel Ruiz-Fresneda[7], Alexandra Tolstikova[8], Reza Nazari[2,3], Uwe Weierstall[2,3], Valerio Mariani[9], Petra Fromme[1,2], Richard Kirian[2,3], and Alexandra Ros[1,2]

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

Previously, we had developed a 3D-printed device capable of generating and electronically stimulating aqueous crystal droplets segmented by an immiscible oil phase.[1,2] The device creates crystal-laden droplets and allows injection with 3D-printed gas dynamic virtual nozzles (GDVNs). The phase and frequency of the droplets is optically detected and then shifted, through continuous electronic stimulation, to match the phase of the XFEL.

Most recently, A feedback mechanism for adjusting the droplet generation frequency and phase compared to the XFEL was developed with custom Python scripts to control the delay generator and signal amplifier through a Raspberry Pi with additional monitoring through LabChart software. The modular 3D-printed devices for sample delivery along with its intricate Raspberry Pi data processing was implemented at the Macromolecular Femtosecond Crystallography (MFX) instrument at the Linear Coherent Light Source (LCLS) in 2020 and 2021 to deliver NAD(P)H: quinone oxidoreductase 1 (NQO1) and phycocyanin crystals. The feedback mechanism successfully recorded droplets of both proteins and electrically stimulated them to synchronize with the 120Hz XFEL while feeding the droplet signal into the MFX EPICS data stream to directly compare the droplet phase with the droplet and crystal hit rate in the OnDA monitor (OM)[3]. Additionally, Python scripts were used to automate the scan of droplet position to and after a 4-run compilation in OM, the optimal droplet position corresponded to a 7x increase in crystal hit rate. Furthermore, the droplet injection reduced required flow rates for sample to 4 µL/min, amounting in 75% sample conservation (compared to a continuous injection) while still collecting 4Å resolution diffraction patterns of both proteins, NQO1 and phycocyanin, for structure determination. Future work will include studying the nature of the droplets within the jet after it leaves the nozzle and modifications to the geometry allowing for more stable flow which will ideally increase the droplet hit rate to above 90% while still conserving more than 90% of sample.

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POSTER #12

Raison Dsouza

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RETURN TO INDEX

Extraction of continuous conformational change from single-particle data: A benchmarking study

Raison Dsouza, Ghoncheh Mashayehki, Peter Schwander, Abbas Ourmazd

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Biomolecular machines have evolved to perform specific tasks through a concerted sequence of conformational motions. Because of the complex structure of biomolecules, each machine can be thought of to exist essentially in many conformational substates (CS). The CS of a given machine has the same overall structure and performs the same biological function but differs in local structural details and possibly in the rate at which the function is performed. In other words, the conformational free energy space exhibits valleys separated by high energy barriers[1]. There is growing recognition that such motions involve continuous conformational motions, rather than jumps between a small number of CSs[2]. Apart from disordered proteins, each state of the conformational continuum is associated with a different energy. The probability of a conformational state being occupied is determined by the Boltzmann factor, which drops exponentially with increasing energy.

For near-equilibrium processes, function proceeds along minimum-energy pathways on the energy landscape, because higher-energy conformational substates are weakly occupied. A growing number of data-analytical tools for determining continuous conformational motions are emerging. Only a small subset of these tools can determine functionally relevant motions. Here, we report the first comparative benchmarking study of the performance of four leading data-analytical tools for determining the conformational energy landscapes, functionally relevant conformational paths on these landscapes. Such benchmarking is essential for systematic progress in compiling atomic-level movies of biomolecular function.

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Acknowledgement:

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POSTER #13

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RETURN TO INDEX

Synthesis, Crystal Structure and Biomedical Application of Ferrocene-Hormone Complexes.

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Approximately 52% of breast cancer cases are related to over-expression of the estrogen receptor (ERα). Conventional metal-based therapeutic drugs, such as cisplatin and its derivatives, are still used to inhibit this abnormal cellular proliferation rate. However, cisplatin-based drugs are highly cytotoxic, triggering a series of side effects that become detrimental to the body due to their lack of selectivity between healthy and cancerous tissue. In 1984, Köpf-Maier, and his co-workers first reported the anticancer properties of ferrocene. This organometallic compound leads to the formation of radical oxygen species that cause oxidative damage to DNA, inducing cell apoptosis. Our research group has recently successfully incorporated ferrocene with estrone and estradiol at estrogen's rings A and D showing cytotoxic activity on hormone dependent and hormone independent breast cancer cell lines. The ferrocene-hormone complex substituted at estrogen's ring A proved to have cytotoxic activity similar to conventional therapeutic agents such as cisplatin and tamoxifen, and dockings studies showed effective interaction. This work seeks to deliver a new approach to enhance the selectivity to effectively target hormone dependent cancers, specifically, ER+ breast cancer. We present a series of novel ferrocene-hormone complexes with its characterization by X-Ray Diffraction (XRD). Computational studies of the interaction of the ferrocene-hormone complexes with ERα protein were performed which demonstrated the possibilities of docking interaction of these drugs in the ligand binding pocket of the ERα.

POSTER #14

Roshanak Etemadpour

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RETURN TO INDEX

Energy Landscape of the SARS-CoV-2 spike protein in complex with ACE2 receptor

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The SARS-CoV-2 spike protein and its interactions with the Angiotensin-Converting Enzyme 2 (ACE2) play a key role in the mechanism by which COVID infection takes place. We use a data-driven machine-learning approach to map the energy landscape and functional conformational motions of the spike protein in the presence of ACE2.

POSTER #15

Petra Fromme

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RETURN TO INDEX

Molecular Movies of Biomolecules “in Action” with X-ray Free Electron Lasers

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X-ray Free Electron Lasers (XFELs) have opened a new avenue for structural discovery of the function and dynamics of biomolecules. Processes in biology are highly dynamic and the study of their dynamics is one of the grand challenges of Structural Biology as most structures determined so far only provide a static picture of the molecule. Serial Femtosecond Crystallography (SFX) provides a novel concept for structure determination, where X-ray diffraction “snapshots” are collected from a fully hydrated stream of nanocrystals, using femtosecond pulses from high energy X-ray free-electron lasers (XFELs)[1-4]. The XFEL pulses are so strong that they destroy any solid material, but a femtosecond is so short (1 fs =10 $^{-15}$*s*) that X-ray damage is diminished and diffraction from the crystals is observed before destruction takes effect[3]. Structural Biology with X-ray Free electron lasers allows data collection at near physiological conditions at room temperature[5-13] thereby opening new avenues for the study of light-driven systems in pump probe experiments[7-12] as well as the study of medical important proteins that could enhance structure-based drug design with SFX studies[5,13]. The poster will show an overview of XFEL studies on medical important proteins, including the first XFEL studies on an important enzyme from SARS-CoV2 as well as reporting on our most recent time-resolved studies on Photosystem I and II.

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Acknowledgement:

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POSTER #16

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RETURN TO INDEX

Advanced data collection at FMX – the Frontier Microfocusing Macromolecular Crystallography Beamline at National Synchrotron Light Source II

Fuchs, M.R., Shi, W., Andi, B., Gao, Y., Kreitler, D., Jakoncic, J., Bernstein, H.J., Lazo, E.O., Soares, A., Myers, S.F., Aishima, J., Schaffer, R., Qian, K., Shea-McCarthy, G., Langdon, T., Sweet, R.M., Berman, L.E., Schneider, D.K., McSweeney, S.

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TThe FMX beamline in sector 17-ID-2 at NSLS-II covers a photon energy range from 5 - 30 keV, and delivers a flux of 4×1012 ph/s at 1 Å into a 1×1.5 µm2 to 10×10 µm2 (V×H) variable focus. The achievable flux densities, and consequently dose rates, surpass most current MX beamlines by up to two orders of magnitude (Schneider et al., 2021), ideal for high-speed (Gao et al., 2018), and high time resolution data collection. The microbeam enables fast rastering localization of microcrystals down to a micron in size, and local characterization of large irregular crystals. Serial- and multi crystal crystallography, fully automated data collection of crystals of sizes 40 µm and up, and humidity-controlled room temperature and multi temperature crystallography are further special measurement modes. For long wavelength native phasing, we added a He flight path to minimize scattering and air absorption. The beamline's high throughput proved crucial for fragment binding studies on the SARS-CoV-2 Nsp3 macrodomain (Schuller et al., 2021), and opportunities provided by multi temperature crystallography were demonstrated in a temperature-dependent study of the SARS-CoV-2 main protease (Ebrahim et al., 2021). In a new development project, we are establishing time-resolved serial crystallography at the beamline.

The FMX beamline is operated by the Center for Biomolecular Research (CBMS) (https://www.bnl.gov/nsls2/lifesciences/) through a NIH, NIGMS Center Core P30 Grant (P30GM133893), and by the DOE Office of Biological and Environmental Research (KP1607011).

Research proposals for beamtime can be submitted through the NSLS-II website: https://www.bnl.gov/nsls2/userguide/

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POSTER #17

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RETURN TO INDEX

Strategy for data compression in protein crystallography experiments

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Elucidating the atomic details of protein structure is crucial for understanding the processes of life at the molecular and atomic levels. Serial crystallography (SX) is one of the possible experimental methods, which main benefit is to carry time-resolved studies at room temperature. But to get 3D structural information of the studied protein a lot of diffraction snapshots are needed. Moreover, combined with new X-ray detectors and performing experiments at the modern synchrotrons and Free Electron Lasers (FELs) SX invariably leads to high storage consumption. Some numbers: the detector Eiger2 XE 16M can produce up to 13 GB/s in 16-bit mode of uncompressed data (1-4 GB/s with compression), which results in ~1 PB/day (or 100-400 Tb with compression). Detectors like JungFrau (JF), AGIPD or ePix10k can generate even more data. For example, one 1 Mpix detector at eXFEL normally generates 1 PB/week, so 3 experiments running in parallel may result in 3 PB/week. Considering the price for 1 PB storage system as ~150k Euro this data rate results in 0.5 M Euro/week just for storing data! One of the possible ways to overcome this problem is to delete the raw data after a short period of time and keep only the averaged intensity of Bragg peaks. Such an approach can be justified only in normal protein crystallography (MX) because of the well optimized pipeline. Unfortunately, for FELs and for more complicated synchrotron SX experiments it is not possible yet – reprocessing of raw data can improve the result a lot due to the improvements in the processing pipeline and better detector calibration. For example, recently we have shown that reprocessing of the data measured 10 years ago at LCLS led to resolution improvement by up to 1 A. Thereby, to save more storage we are keen on reducing data by using lossless or lossy compression approaches applied to raw data. Lossless compression is always preferable because it doesn't influence the data quality, but lossy compression can give us a higher compression rate.

The evaluation of the existing lossless compressors was performed using packages such as h5py, PyTable and h5plugin, compatible with the Python 3.6. Several datasets from different facilities (eXFEL and Petra III) and different detectors (AGIPD and Eiger 16M) were chosen for the tests. For the integrating detectors as AGIPD raw datasets have a natural floating point type of the stored data and the values are usually quite high. Counting detectors like Eiger 16M, used at synchrotrons, have an integer type of data and usually quite low values. Thus, these two examples cover a wide range of possible SX cases. The results of testing suggest that floating data types are not compressible and bzip2 with optimized parameters will always give the best compression rate.

Applying lossy compressors on the data requires careful check of the final data quality. Data loss should not result in the inability to reconstruct the protein structure and should not give much worse characteristics of datasets. We have tested several approaches for lossy compression of SX data: binning the data, saving only hits, quantization, etc. Data from different experiments at synchrotrons and FELs with different detectors and different samples were used. By checking the resulting statistics of compressed data (like CC*/Rsplit, Rfree/Rwork) we have demonstrated that the volume of the measured data can be greatly reduced (10-100 times!) while the quality of the resulting data was kept almost constant. Some compression strategies, tested on SX and MX datasets, can be used for other types of experiments, even with different sources (for example electron diffraction). Moreover, we tested compression techniques on the SAD dataset (thaumatin collected at 4.57keV measured at the SwissFEL with JungFrau 16M detector) that is much more sensitive to data quality. It allowed us to determine the limits of application for most of the considered compression algorithms.

POSTER #18

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RETURN TO INDEX

Developments of X-ray Spectroscopy at the LCLS Towards Applications to Biomedical Research

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Over the last decade X-ray spectroscopic methods at the LCLS XFEL have found substantial success studying increasingly complex systems at atomic lengths and fundamental timescales. With the advancement of the LCLS to LCLS-II – and the future LCLS-II-HE upgrade – we are concomitantly developing our X-ray spectroscopy instruments to keep pace with, and utilize, the new XFEL capabilities. To further these developments, we recently received an NIH P41 Biomedical Technology Research Resource grant that supports methodological developments to enable a collection of "Driving Biomedical Projects" (DBPs) in collaboration with our user community. To these ends, we are currently developing three new X-ray spectrometers: an X-ray polycapillary spectrometer system that will enable lower sample concentrations and low-signal techniques (e.g. EXAFS); a multi-line Von Hamos geometry X-ray emission spectrometer that can be configured to collect two X-ray emission lines simultaneously; and a high-throughput tender X-ray spectrometer. Further, we are developing near-/real-time feedbacks to monitor/optimize sample delivery conditions including integration of a UV-Vis spectrometer into the Macromolecular Femtosecond Crystallography (MFX) end station. Collectively, these innovations will ensure the concerted growth in spectroscopic capabilities with the LCLS-II upgrades to produce new and impactful research especially in the biomedical sciences.

POSTER #19

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RETURN TO INDEX

Impact of protein crystal contact forces on intramolecular picosecond dynamics

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While many protein dynamics studies depend on crystal phase measurements, it is not known how the intermolecular interactions effect these dynamics. We examine the dynamical impact of intermolecular interactions by measuring the collective structural vibrations using anisotropic terahertz microspectroscopy and tuning interactions through controlled dehydration of the crystal water content. Anisotropic terahertz microspectroscopy (ATM) isolates protein collective vibrations from a highly congested spectrum based on the direction of the vibrational transition dipole[1, 2]. For tetragonal and triclinic crystals no frequency shifts are observed. Instead, the bands initially narrow then broaden with dehydration, consistent with increased and then decreased ordering as found in X-ray crystallography[3]. For monoclinic crystals on the other hand, we find strong anisotropic bands at 14.8 and 21.6 cm-1, which blue shift by 5.0 and 3.1 cm-1 respectively. This blue shift in the vibrations suggests a stiffening of the potential due to either a reduction in the solvent, an increase in the interaction with the neighboring molecules or both. Monoclinic lysozyme crystals are known to reversibly transform to another monoclinic form due to dehydration[4], thus it is possible that the spectral shifts are associated with this new ordering and not changes in the interactions. We explore these different possible mechanisms by comparison with Normal Mode Ensemble Analysis calculations[5].

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Acknowledgment

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POSTER #20

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RETURN TO INDEX

Ab initio Phasing of High Solvent Content Crystals Using a Hybrid Input-Output Algorithm

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The elucidation of a protein's three-dimensional structure provides crucial information regarding its biological function. Concerning structure determination, X-ray crystallography is among the principal techniques used to resolve electron density maps of proteins at an atomic resolution through a Fourier transform of crystallographic diffraction patterns. This Fourier transformation requires a measured amplitude and phase for each diffracted reflection spot. However, due to physical restraints, phase information is systematically lost during diffraction measurements, and the so-called phase problem must be solved. While further crystal experimentation can provide additional data that may supplement these missing phases, these experiments are not always feasible for biological systems. Desirably, computational methods can also retrieve these phases, however existing algorithms typically require prior known structural information. Here, we demonstrate that a hybrid input-output (HIO) algorithm can solve the phase problem directly from the intensities measured during diffraction–that is, ab initio–for high solvent content crystals. In particular, we show that this HIO phase retrieval method can reproduce electron density maps of several previously resolved protein structures belonging to multiple space groups within the protein database (PDB). Additionally, our work investigates the sensitivity of how a crystal's solvent fraction is defined when running an HIO job by interpreting the associated effects on algorithm convergence. Ultimately, we aim to use this HIO method to resolve the structures of novel proteins on a broader scale of interest.

Acknowledgment

This material is based upon work supported in part by the STC Program of the National Science Foundation (NSF) through BioXFEL under Agreement No. 1231306, the Big-Data Private-Cloud Research Cyberinfrastructure MRI-award funded by NSF under grant CNS-1338099 and by Rice University's Center for Research Computing (CRC).

POSTER #21

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RETURN TO INDEX

Advances in the structural biology of the outer surface proteins from the Lyme disease pathogen support the structure-based design of needed therapeutics

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Advances in structural biology technologies support the structure-based design of drugs against infectious diseases. To support novel drug development against Lyme disease, here we review structural biology efforts for the tick-borne pathogen and spirochete bacterium *Borrelia burgdorferi*. Annually, Lyme disease infects over 400,000 Americans, of whom 90% are unaware that they have been infected due to problematic diagnoses and to symptoms that are variable and non-specific. Some 10-20% of those infected suffer from lifelong, untreatable, and debilitating symptoms of Lyme arthritis, heart inflammation (Lyme carditis), and neurological complications (Lyme neuroborreliosis). *B. burgdorferi* accomplishes pathogenesis and invasion of various human tissues by the action of over a hundred outer membrane lipoproteins that are unique to the genus *Borrelia* and that are upregulated only upon mammalian infection. To date, there are no published reports of the application of X-ray free electron lasers or cryo-electron microscopy to any spirochetal pathogen. Existing reports of atomic-resolution structures of the outer membrane proteins of *B. burgdorferi* were accomplished using synchrotron X-ray crystallography and, importantly, relied on recombinantly expressed protein that was not directed to the bacterial cell membranes. Our group recently pioneered the membrane-translocated recombinant expression of outer membrane proteins from B. burgdorferi, in which we identified the novel result that the arthritogenic BBA57 outer membrane lipoprotein forms an oligomeric (homo-multimeric), alpha-helical pore that crosses the outer surface of the Lyme disease pathogen. This pore is analogous to those previously identified by X-ray crystallography in the outer surface of pathogenic proteobacteria and that function in the formation of biofilms and toxins, adhesion to tissues, evasion of the immune system, secretion of biomolecules, and active drug export. Our efforts are expected to reveal the first atomic-resolution structure of a membrane-translocated outer membrane protein from *B. burgdorferi*. These structures will enable the structure-based design of anti-arthritic drugs and anti-infectives that will prevent Lyme disease and aid patients who suffer from persistent symptoms.

POSTER #23

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RETURN TO INDEX

Towards FOSS Processing of Pink-Beam Rotation Series; A case study on E. coli Dihydrofolate Reductase

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X-ray crystallography allows for the structural analysis of biological molecules in atomic detail. Many X-ray sources, including both synchotrons and XFELs, have the ability to produce greater intensity light if a broad wavelength spectrum is used. This capability is particularly promising for single-shot “diffract-before-destroy” and time-resolved X-ray experiments in which monochromatic observations are necessarily partial. The primary obstacle to widespread adotopn of pink-beam experiments has been the difficulty of data analysis. Here, we present progress towards a free and open-source software solution to pink-beam data analysis based on the modular DIALS framework and cctbx. We illustrate initial results using diffraction data obtained for E. coli dihydrofolate reductase, and show state-of-the-art performance.

Acknowledgment

This work is funded by a research agreement with the BioCARS national user facility run by the University of Chicago.

POSTER #24

Konstantinos Karpos

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RETURN TO INDEX

Time-Resolved Drop on Demand at the LCLS Macromolecular Femtosecond Crystallography (MFX) Endstation

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Sample consumption is a common problem in time-resolved small- and wide-angle x-ray scattering experiments (TR-SWAXS). The problem is particularly severe for irreversible systems that cannot be recycled, such as the ultrafast light-activated visual rhodopsin GPCRs that motivate our own TR-SWAXS studies. In an effort to reduce sample consumption and simultaneously increase the signal-to-noise ratio, an off-the-shelf drop-on-demand system was studied and characterized to determine the feasibility of using triggered droplets at x-ray Free Electron Laser (XFEL) facilities. In a first-of-its-kind experiment, a piezo-driven triggered droplet source was installed and tested at the LCLS Macromolecular Femtosecond Crystallography (MFX) endstation with water, CHAPS buffer, and bovine visual rhodopsin in a light-activated TR-SWAXS experiment. We will present our findings along with detailed simulations that helped determine the optimal experimental design.

POSTER #25

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RETURN TO INDEX

MS SPIDOC: Mass Spectrometry meets Single Particle Imaging

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Native mass spectrometry (MS) enables the ionization and transfer of structurally intact non-covalent protein complexes into the gas-phase. As such, it is a perfect tool to study proteins and their assembly intermediates in a mass and conformation specific manner. However, structure follows function, and the structural information that can be gained with techniques like top-down MS or ion mobility is limited. Accordingly, other experimental approaches such as X-ray diffractive imaging are necessary to get a full understanding of the proteins and their assemblies.

MS SPIDOC (Mass Spectrometry for Single-Particle Imaging of Dipole Oriented Protein Complexes) is a Horizon 2020 funded research and innovation program1 aiming at the combination of both experimental techniques. In particular, well established methods from MS like m/z selection, ion trapping or ion mobility are adapted as part of the sample delivery system for X-ray diffraction. In contrast to conventional diffractive imaging of crystallized proteins, the proteins here are delivered as single particle without the need for crystallization. This increases naturally the requirement to the X-ray source. Thus, single-particle X-ray diffractive imaging (SPI) is only conducted at X-ray free electron lasers2, the worlds brightest X-ray sources in the world.

This contribution will highlight the ongoing efforts of the MS SPIDOC consortium to develop this sample delivery system for the use at beamlines of the European XFEL3. The current state of the designing and manufacturing of the instrument prototype will be presented as well as the results of the first testing of individual component modules. Furthermore, theoretical simulations as well as experimental device design for particle dipole orientation in strong electric fields will be previewed.

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POSTER #26

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RETURN TO INDEX

Photo-activated solid-to-solid-phase transition in adenine riboswitch aptamer RNA crystals.

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Solid-to-solid phase transitions have been commonly observed in both organic and inorganic crystals. However, in macromolecular crystals, transitions resulting from large conformational changes are difficult to investigate, as they often disrupt lattice packing and other stabilizing interactions. We are interested in phase changes observed in crystals of adenine riboswitch aptamer RNA (riboA) that are induced by ligand binding. Previously, we reported microscopy and diffraction data that characterized the stages of phase transition upon mixing with ligand. However, with large ligand-induced changes, the kinetics and cooperativity of the phase transition are likely influenced by diffusion rates and non-uniform saturation of ligand binding sites upon mixing. To mitigate this effect, we are exploring the use of photo-caged adenine (pcADE) to trigger the phase change. The riboA crystals are first saturated with pcADE solution, and the binding and subsequent conformational changes are then activated by a single UV LED pulse. The solid-to-solid phase transition is monitored using polarized video microscopy, and characterized by changes in crystal birefringence intensity. The efficiency of adenine uncaging is investigated using high-performance liquid chromatography. With this study, we hope to provide insight to performing pump-probe experiments using an XFEL, where the use of smaller crystals and a high-intensity UV laser may enable cooperative transition throughout the entire crystal, an essential factor for characterizing conformational changes through time-resolved diffraction.

POSTER #27

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Polymer-based Fixed Target Chips For Low-background, Hydrated Fixed Target SFX

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A National Science Foundation Science and Technology Center

Abstract

- Improved fixed target microfluidic chips for efficient data collection of protein crystals at XFEELs and synchrotrons
- Maintain crystal hydration during storage and data collection, robust enough to load and transport, low background
- Fabricate rapidly at low cost, highly flexible
- Potentials for aided crystallization: hydrogel, functionalizable polymer brushes

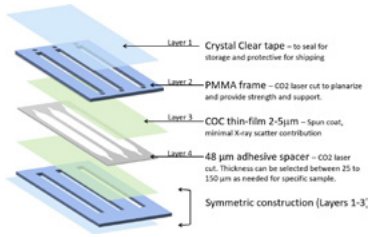


Fig.1: Cross section of layers in improved microfluidic fixed target chips

Improvements

- Previous generation cyclo olefin copolymer (COC) thin-film based microfluidic chips (Lab on a Chip 2021, DOI: 10.1039/d1lc00810b).
- Improved design has 240% more working area and loads smoothly without further treatments.
- New design does not require cleanroom equipment to fabricate. Production is much more rapid and flexible.

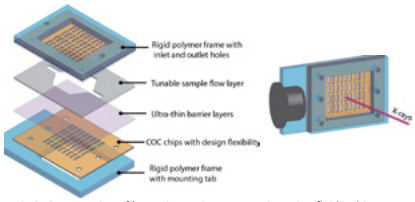
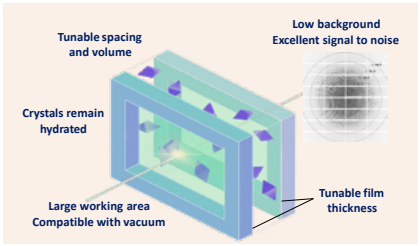


Fig.2: Cross section of layers in previous generation microfluidic chips

Features

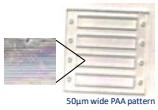
- 2 – 3 μm COC thin-film encapsulating layers at X-ray imaging area
- Sample thickness can be varied from 25, 48, 81 to 142 μm .
- Crystals in the chip can be stored for weeks and transported easily within protective frames, unused chips good for months
- Polymer brushes can be grafted in patterns on the inner surfaces of the chip by UV initiated photografting



Easy load with pipette:
- **Crystallization solution**
- **Dissolved hydrogel**
- **Crystal slurry**

Protected for shipping and storage

Flexible design:
- **Tailored to different beamline setup**
- **Tunable polymer brush**
- **Modify dimensions and fabricate within 2 days**



50μm wide PAA pattern

Maintain hydration for days; hydrogel aided crystallization compatible

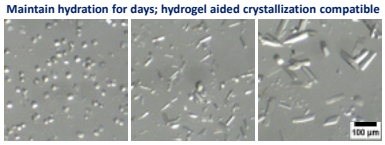


Fig.2: From left to right: Lysozyme crystallized in chip (1)after 10+ days (2) with 0.3%agarose (3) 0.4% agarose

Results at Synchrotron

NendoU protein from R. Jernigan, Fromme group at ASU

Diffraction signal
- Excellent signal to noise ratio
- Full scan of model proteins: lysozyme and thaumatin
- Resolved diffraction patterns of NendoU H234A protein for the first time, directly crystallized within the chip, or loaded crystal slurry

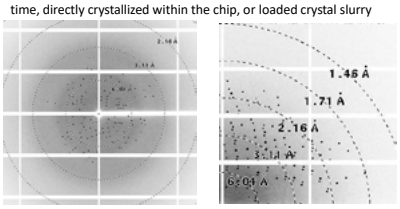


Fig.2: Left: Single diffraction frame of NendoU H234A protein crystallized within the chip. Right : Diffraction from lysozyme crystal

Background Contribution
- Low background scattering from thin film and buffer
- major scattering from air can be eliminated by operating in vacuum
- Film thickness and spacing for buffer (volume) can be tuned to match specific needs

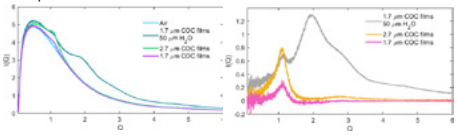


Fig.2: Contributions to background scatter: Left: overall background of empty and loaded chips. Right: Air scattering subtracted

Future Work

- Already demonstrated high resolution protein diffraction at LCLS MFX
- Open "half-sandwich" (layers 1-3)demonstrated at LCLS CXI (2/1/22)
- Compatible with samples not limited to protein (metal nanoparticles)
- Electric field aided crystallization on chip
- Patterned functionalization of thin film tailored to different proteins
- Compatibility with liquid crystal phase studies and spectroscopy

Acknowledgments

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.

RETURN TO INDEX

POSTER #28

Tek Malla

University of Wisconsin-Milwaukee

RETURN TO INDEX

Time Resolved Study on Beta Lactamase Reacting with an Inhibitor

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In mix-and-inject serial crystallography (MISC) small protein crystals are mixed with substrates at ambient temperature. The mixing triggers the reaction and the resultant change in structure is probed by X-rays. Using this technique, we studied the reaction of M. tuberculosis beta-lactamase (BlaC) microcrystals with sulbactam inhibitor. Seven different timepoints from 3ms to 700ms after reaction initiation were investigated. These results allowed us to visualize the enzyme-catalysis in action on millisecond time scale at atomic resolution. We also managed to capture an interesting intermediate on the catalytic pathway, whose detection would have been difficult without the MISC experiment.

Now we are working to apply singular value decomposition (SVD) to this data. SVD is a mathematical tool used in analysis of spectroscopic data and molecular dynamics simulation for deconvolution and noise reduction. SVD is applied to the time dependent difference Fourier maps. SVD helps reduce noise present in the difference electron density maps. And with a suitable kinetic mechanism time-independent structures of the intermediates can be recovered. In addition, the relaxation times of the kinetics can also be determined.

POSTER #29

Abhik Manna

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RETURN TO INDEX

Microfluidic Devices for Membrane Protein Sample Delivery to X-ray Sources

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GPCRs are an important class of membrane proteins present in the cell membrane which regulate transport in cell. Moreover almost 60% of the drug molecules target these membrane proteins. It is of utmost importance to know the structure of GPCRs in atomic resolution to specifically design drug molecules. X-ray crystallography has been established as a reliable method for structure determination with atomic resolution which requires delivery of crystals into the X-ray beam. To obtain crystals, extensive screening of crystallization conditions is required. Additionally, the instability of membrane proteins outside the cell membrane is the major bottleneck for obtaining sufficient sample for extensive screening. The membrane proteins can be stabilized and crystallized outside the cell membrane using sponge phase, a disordered lipidic cubic phase (LCP).

A previously developed three-layer PDMS microfluidic device can be used for screening of several crystallization conditions using minimal amount of precious protein sample.[1] This device comprised of 204 nanowells separated from one another by valves which can be actuated by applying vacuum. Here, we have modified and developed the same three-layer microfluidic device to be used as a screening fixed-target device. This device was fabricated with X-ray compatible thermoplastic material so that the same can be used to deliver crystals to state of art X-ray sources for screening of crystallization conditions and on chip crystal quality determination. Two layers of the device were imprinted on ~500 µm thin cyclic olefin copolymer (COC) sheets by hot embossing. A ~15 µm thin polydimethylsiloxane (PDMS) membrane was integrated as the middle layer, by chemical modification of the COC surface, to achieve valve functionality. Valve actuation was demonstrated by applying a vacuum pressure of 500 mbar and Lysozyme was crystallized on chip. Another two-layer basic fixed-target COC device was developed for delivering thousands of crystals to X-ray beam to collect complete diffraction data for structure determination. This ~ 1mm thick two-layer device consisted of an imprinted layer and one featureless COC slab. The imprinting was performed by hot embossing and two layers were bonded thermally. Both screening fixed-target device and basic fixed-target device were tested by filling sponge phase to confirm the compatibility of the material with the lipidic bilayer which was confirmed by the stability of the designs after 7 days of incubation. To lower the background scattering from the thermoplastic material, the thickness of the device was further reduced by replacing the ~500 µm thick COC cover slide with a ~5 µm thin COC film. The thin film was obtained by spin coating dissolved COC on a glass slide and was subsequently bonded to the imprinted layer activated through chemical treatment. This device was successfully filled in by capillary action and no leakage was observed. The basic fixed-target device has been further developed for crystallization of membrane proteins in zero gravity.

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POSTER #30

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RETURN TO INDEX

Simplifying Protein Collective Vibrational Assignment Through Symmetry

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Anisotropic terahertz microspectroscopy (ATM) isolates protein collective vibrations from a highly congested spectrum based on the direction of the vibrational transition dipole[1, 2]. ATM changes with inhibitor binding and photo excitation reveal that the vibrational bath evolves with functional state. By comparing the measurements to calculations, specific structural motions can be assigned to spectral bands. The complexity of the anisotropic spectral structure is dependent on the level of alignment of the molecules. This alignment is achieved using protein crystals, which are routinely used for structure determination and typically have 30-70% water by volume. One expects that the lowest symmetry crystals will have the most spectral structure, whereas higher symmetry crystals will have decreasing structure due to the dipole cancellation. Here we examine crystal symmetry effects for hen egg white lysozyme crystals (HEWL). ATM measurements of triclinic, monoclinic and tetragonal HEWL crystals find that indeed the spectral structure decreases with increasing symmetry. We compare the measurements to normal mode ensemble analysis calculations and find good agreement with the resulting symmetry in the anisotropic spectra. By comparing the spectrum from different crystal symmetry groups of the same protein CEWL, we identity the conserved vs unique spectral features across molecular arrangements in lattices. Peak near 40 cm^{-1} and 55 cm^{-1} were common among three lattice systems while a peak near 20 cm^{-1} was observed only in triclinic CEWL. This serves as a guide in determining the direction of the modes with respect to the molecule and identifying whether crystal contact forces influence the spectrum. The experimental spectrum is compared and contrasted to an ensemble averaged calculated anisotropic spectrum.

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2 Niessen, K. A.; Xu, M. Y.; George, D. K.; Chen, M. C.; Ferre-D'Amare, A. R.; Snell, E. H.; Cody, V.; Pace, J.; Schmidt, M.; Markelz, A. G., Protein and RNA dynamical fingerprinting. *Nat. Comm.* 2019, 10.

Acknowledgment

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POSTER #31

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RETURN TO INDEX

Photoprotective Structural Change Led by Intramolecular vibrational switching

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Picosecond protein structural dynamics enable efficient conformational transitions during biological function. We examine the switching of these long range collective structural vibrations of the photoprotective protein orange carotenoid protein (OCP) using anisotropic terahertz microspectroscopy, which provides dynamical fingerprinting for biomacromolecules by anisotropic absorption of protein crystals[1,2]. OCP controls efficiency of the phycobilisome (PBS), the light harvesting antenna in cyanobacteria, to prevent oxidative damage. In low light, carotenoid bound OCP appears orange (OCPo). Absorption of blue-green light results in a 12Å translocation of the carotenoid into the N-terminal domain (NTD) and conversion to an active red state (OCPR) which interacts with the light harvesting antenna, the phycobilisome (PBS), to induce fluorescence quenching[3]. We examine the changes in the collective vibrations with photoexcitation under two conditions. Under excitation similar to the solution phase switching we detect reversible photoswitching of the THz vibrational bands through double difference analysis. However, switching of the OCP crystal optical absorbance is not observed. Using conditions to achieve optical absorbance switching in the crystal, we confirm that the intramolecular vibrational changes arise from the transition to the photoprotective state. We compare the measured spectral changes to those calculated using normal mode ensemble analysis (NMEA) for apo and holo OCP. NMEA accounts for population sampling of the rugged energy landscape[4]. The calculated spectral difference in the 38-55 cm-1 range is strikingly similar to the observed changes. To assign the spectral features to structural motions, we examine the correlations in backbone motions for the 38-41 cm-1 spectral peak. Incredibly, long-range correlations persist with the averaging over the energy landscape. The protein's change in internal dynamics with the initial photoexcitation may provide the dynamical bias towards the reorganization of the NTD for the translocation of the carotenoid and the separation of the NTD from the CTD necessary for the interaction with the phycobilisome.

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Acknowledgment

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POSTER #32

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RETURN TO INDEX

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

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Single-particle imaging with X-ray free-electron lasers depends crucially on algorithms that merge large numbers of weak diffraction patterns despite missing measurements of parameters such as particle orientations. The Expand-Maximize-Compress (EMC) algorithm is highly effective at merging single-particle diffraction patterns with missing orientation values, but most implementations exhaustively sample the space of missing parameters and may become computationally prohibitive as the number of degrees of freedom extends beyond orientation angles. Here we describe how the EMC algorithm can be modified to employ Metropolis Monte Carlo sampling rather than grid sampling, which may be favorable for cases with more than three missing parameters. Using simulated data, this variant is compared to the standard EMC algorithm. Higher dimensional cases of mixed target species and variable x-ray fluence are also explored.

POSTER #34

Jessika Pazol

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RETURN TO INDEX

Understanding the in-vitro de-acylation response of bacterial lipopolysaccharides (Ra LPS) via enzymatically activated hydrolysis in aqueous systems

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Water is considered one of the most indispensable starting materials that is used in myriad of biotechnical and biomedical applications. More specifically, the water quality for biomedical applications must contain a minimum to no contaminants, particularly those from bacterial origin sources. In this context, lipopolysaccharides (LPS) are one of the strongest pathogens groups that can stimulate the human immune system.[1] LPS also known as endotoxin comes from the outer membrane of Gram-negative bacteria cell wall that can be found in water sources. Gram-negative bacteria shed-out LPS in the form of blebs[2] from its outer membrane particularly after biofilms successfully mature in events where stagnated waters occur. Despite its pathophysiological toxicity mechanism are well-established, exploring additional conditions to deactivate the molecule via enzymatic hydrolysis is still of interest. Furthermore, understanding the physical and chemical characteristics of LPS in water media will help to elucidate its initial physical and chemical condition to design specific inhibiting treatments. LPS are amphipathic molecules that can self-aggregates into different sizes and structures.[3] Inhibiting LPS in water systems had been a challenge because of its high variability in size and structure.[4] In this work we studied and characterized the release of free fatty acids from LPS molecules by using a lipase in aqueous media to induce a change in its molecular constituents. The product of the de-acylation reaction was characterized via dynamic light scattering, surface potential, infrared spectroscopy, small angle x-ray scattering, among others. The details for this investigation methods and analytical techniques will be presented. This work was supported by the NIH RISE program (Grant # 5R25GM061151-19).

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POSTER #35

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RETURN TO INDEX

The DNA binding diversity of the SIX family of transcription factors in *Drosophila melanogaster* and *Heliconius erato.*

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Transcription factor (TF) DNA-bindning specificity is determined by how their DNA-binding domain interacts with DNA. DBDs are highly conserved and used to classify TF into families. Members of a family usually recognized the same binding motif, but small changes in the DBD can lead to diversification in binding specificity. TFs members of the sine oculis homeobox (SIX) family are found from sponges to humans and are consider atypical members of the homeodomain family. They regulate numerous phenotypic features spanning from eye development in flies to red color pattering in the Heliconius butterflies wings. How evolutionary related TFs DNA-binding specificity diversifies is not fully understood. Using full length SIX proteins (Sine Oculis, SIX4 and Optix) from *Drosophila melanogaster* and *Heliconius erato,* we have performed *in vitro* Systematic Evolution of Ligands by Exponential Enrichment (SELEX-seq) to identify the DNA binding specificity, between the SIX TFs orthologs. Our preliminary data has shown that they bind to their canonical binding motif (TGATAC), however the way they bind can be different. Our data shows that optix from *Heliconius,* can bind both as a monomer and a homodimer with preferred 3-bp spacing between binding sites. In contrast to Heliconius optix, the ortholog from *Drosophila* prefers a GA flank 5' to the core motif. Currently we are working with our data to provide new insights in the binding diversity of the SIX TFs, allowing us to predict the genomic targets of the SIX genes in both *Drosophila melanogaster* and *Heliconius erato*.

POSTER #36

Jessica Rodriguez-Rios

University of Puerto Rico

RETURN TO INDEX

Investigating the DNA-binding specificity of cardiac transcription factors complexes

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Transcription factors (TFs) are sequence-specific DNA-binding proteins responsible for cellular differentiation and development. Human transcription factors are known for binding DNA as multimeric complexes to regulate gene expression. Transcription factors GATA4, NKX2-5, and TBX5, are fundamental elements of the gene regulatory network that controls mammalian heart development. More importantly, GATA4, NKX2-5, and TBX5 synergize to activate target genes. However, little is known about the impact of cooperative DNA-binding on specificity and its role in transcriptional regulation. Our objective is to identify the intrinsic DNA-binding preferences of the heterodimers complexes formed by NKX2-5:TBX5 and GATA4:TBX5, including binding site orientation and spacing patterns. In this work, I determined the in vitro DNA-binding specificity of the NKX2-5:TBX5 and GATA4:TBX5 complexes using Systematic Evolution of Ligands by Exponential Enrichment (SELEX-seq). Our SELEX-seq results show that both heterodimers bind DNA with specific binding site orientation and spacing. Specifically, NKX2-5:TBX5 prefers a spacing preference of -1bp, 3bp, and 1bp, in contrast to GATA4:TBX5 that prefers -1bp, 2bp, and 3bp spacing between binding sites. Identified binding sites were validated by Electrophoretic Mobility Shift Assays (EMSA). Published data from ChIP-exo confirmed that these binding sites occur in bound genomic regions. These findings will help understand the spatial and temporal gene regulation during heart development.

POSTER #37

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RETURN TO INDEX

A Microfluidic Device for Room Temperature Crystallography

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X-ray crystallography is the primary technique for determining the 3D structure of proteins and facilitates understanding the effects of protein structure on function. One of the key bottlenecks of X-ray crystallography remains the need to loop out protein crystals from the crystallization buffer and cryo-cool them for X-ray data collection. The process of crystal manipulation is very sophisticated and often ends up damaging the protein crystals. Although data collection at cryogenic temperature stabilizes the crystal, it also freezes out functional motions. Collecting room temperature data allows for the observation of conformational ensembles which can help in understanding protein function. We have developed a microfluidic device that allows on chip crystallization and room temperature data collection. We have employed UV curable polymer to fabricate the device with a high level of X-ray transparency. This enables data collection without the need to harvest the crystals. We have demonstrated the room temperature data collection of the hub domain of human calcium/calmodulin-dependent protein kinase II (CaMKII). The crystals were grown inside the device and RT data collection yielded 2.5Å resolution data. We also observed serval extra residues not visible in previous cryogenic data. This device not only eliminates the need for manual manipulation of crystals for room temperature data collection, but it also facilitates serial crystallography at the synchrotron.

POSTER #38

Anna Sofia Santiago-Russe

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RETURN TO INDEX

Structural Characterization and Structure-Guided Engineering of MfnG SAM-dependent MTase

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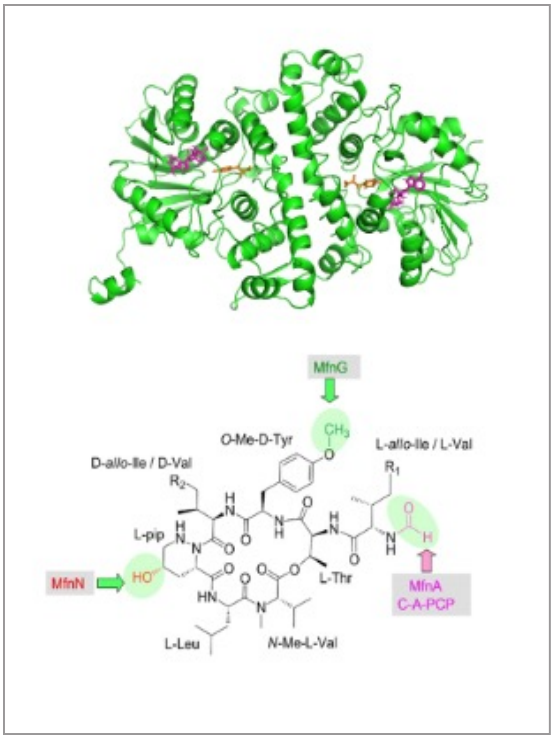
Methylation has drawn interest for its role in various bioprocesses, its effect on the chemical properties of compounds, and its use in the development of new pharmaceuticals. S-Adenosyl-L-Methionine (SAM)-dependent methyltransferases (MTases) catalyze the transfer of methyl groups. MfnG is a SAM-dependent MTase that methylates oxygen in tyrosine's side chain. Many MTases have accepted SAM analogues and synthesized new products, but MfnG has been shown unable to do this. Characterization of MfnG with bound tyrosine and SAH, the degraded product of SAM, has been done; however, the crystal structure of MfnG with SAM in the catalytic site has not been determined. In order to characterize MfnG's methylation mechanism, time-resolved crystallography experiments will be done to generate a motion picture for the methylation of MfnG's tyrosine. We also performed structural analysis to make MfnG more promiscuous by expanding the SAM binding pocket through the mutation of key residues. The development of these mutants proves our ability to perform structure-guided protein engineering on other MTases that could create new natural product-based compounds that may have therapeutic relevance.



POSTER #39

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RETURN TO INDEX

A structural view of chromatin remodelling by BAF at human Lin28b nucleosome locus

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Human chromatin is a highly dynamic identity consisting of the nucleosome core particle (NCP) as its basic unit. During various cellular events like DNA repair, transcription, and replication, the accessibility of the DNA wrapped around these NCPs to the participating proteins is tightly regulated. The ATP dependent chromatin modellers are an important class of protein complexes that helps to regulate the accessibility of the otherwise inaccessible NCP DNA by wrapping and unwrapping of the NCP DNA. BAF is an important chromatin remodelling complex in humans and is found mutated in nearly 20% of all human cancers making it an important target for drug discovery.

In this work, we have solved the structure of human BAF complex bound to an endogenous DNA NCP using cryo-EM.

Our results show important details of early nucleosome remodelling events at Lin28b nucleosome found in the human genome. A comparison of the previously solved structure of the Lin28b nucleosome alone and the BAF bound to Lin28b nucleosome helped us to understand the initial changes in the NCP during the remodelling event. As all the previously reported structures of BAF NCP are based on artificial DNA sequences, our structure of the first endogenous human BAF bound to a genomic nucleosome could potentially help us better understand the role of BAF in humans.

POSTER #40

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RETURN TO INDEX

Retinal isomerization in vertebrate visual pigments measured at SwissFEL

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We have developed a new crystal form of rhodopsin which is suitable for room-temperature serial crystallography. At the Japanese free electron laser SACLA and at SwissFEL we were able to collect data and we determined a better then 2 angstrom rhodopsin structure from the rhodopsin ground state. Following from this, we were able to collect a number of time-resolved FEL data sets. We have been able to observe the development of the retinal isomerization and we have a good 1 picosecond time point. The retinal isomerization can be characterized in detail and we can compare bacteriorhodopsin with rhodopsin and we can already present the isomerized distorted retinal conformation that exists 1 picosecond after light activation. The results can be compared to spectroscopic studies and to computational models that were created for the retinal isomerization. This work lays the foundation for more extensive time-resolved studies with free-electron lasers. In rhodopsin, light converts the antagonist 11-cis retinal into the agonist all trans retinal and time-resolved studies are shedding light on the initiation of the conformational changes that are conditional for G protein activation.

POSTER #41

Madan Kumar Shankar

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RETURN TO INDEX

The pyrrole water amplifies the chromophore action - The preparative state prior to isomerization of the D-ring

Madan Kumar S.[1,2], Weixiao Y. W.[1], Claesson E.[1], Amke N.[1], Piotr M.[1,2], Andrea C.[1], Janne I.[3], Tek N. M.[4], Suraj P.[4], Marius S.[4], Emina S.[5], Luo F.[6], Rie T.[6], Sebastian Westenhoff[1,2]

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Phytochromes are red-light photoreceptor proteins, which control many light-dependent functions in plants, fungi and bacteria. Light is absorbed by a bilin chromophore, which switches the structure and function of the entire phytochrome. The chromophore binding pocket is highly conserved in the entire phytochrome superfamily, with the so-called pyrrole water centered in the four-ringed bilin as an outstanding feature. The primary photoreaction of the bilin chromophore have not been characterized structurally. Claesson et al., reported the primary evolution of the structural changes in the biliverdin binding pocket at 1 ps after photoexcitation. Here we determine several crystallographic snapshots of the CBD from *D. Radiodurans* within the 1ps timescales using time-resolved serial femtosecond crystallography. We find that small structural changes in the B- and C-ring of the biliverdin causing 1 Å movement of the conserved pyrrole water within 800 fs. This causes a break of a crucial hydrogen bond of the pyrrole water to His260, which in turn releases the C-ring propionate and associated water molecules, which surround the D-ring. The result of this primary photoreaction is that the environment around the D-ring is loosened up, preparing it to isomerize at later time points. The pyrrole water thus amplifies small structural changes of the chromophore via a hydrogen bond release mechanism. The mechanism clarifies the primary photoreaction in phytochrome photoreceptor proteins.

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POSTER #42

Chenghua Shao

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RETURN TO INDEX

Enhanced Deposition of XFEL/SSX Structures at the Protein Data Bank

LChenghua Shao, Jasmine Y. Young, Ezra Peisach, Zukang Feng, Yuhe Liang, Irina Persikova, and Stephen K. Burley

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Protein Data Bank (PDB) is the single global repository for experimentally determined three-dimensional structures of biological macromolecules and their complexes with ligands, etc. 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. The PDB archive currently holds and freely disseminates more than 185,000 experimentally-determined structures of biological macromolecules. Consistent year-on-year growth in the number of structures coming into the PDB from X-ray Free-Electron Laser (XFEL) facilities has been observed. Current PDB holdings include 600+ XFEL structures. About half of the XFEL structures were deposited in the past two years, together with 200+ Serial Synchrotron Crystallography (SSX) structures (determined using diffraction data collected at the conventional light sources).

Together with wwPDB PDBx/mmCIF Working Group and experts from the XFEL user community, wwPDB has developed definitions for new PDBx/mmCIF Dictionary data items that support metadata collection for XFEL structures deposited via wwPDB OneDep structure deposition, validation, and biocuration system. Similar metadata are also collected during deposition of Serial Synchrotron X-Ray Crystallography (SSX) structures. We are also working on extending PDBx/mmCIF Dictionary data definitions for inclusion of unmerged XFEL/SSX diffraction data and the parameters describing reflection data collection and process.

Our presentation will briefly review PDB archive growth from a broad perspective of molecular contents and features. We will focus on XFEL/SSX structures statistics, features, and classification within the PDB archive and go on to demonstrate XFEL/SSX structure deposition, validation and biocuration using wwPDB OneDep system (https://deposit.wwpdb.org/). We will also discuss metadata specific to XFEL/SSX experiments such as light source, sample delivery, and reflection data processing. wwPDB is committed to working closely with federal funders and XFEL/SSX users to ensure faithful preservation and representation of their structures, experimental data, and metadata in the PDB archive.

POSTER #43

Alexandra Tolstikova

University of California San Francisco

RETURN TO INDEX

Recent Developments in Cheetah

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an increasing proportion of near-atomic-resolution macromolecular structures, this makes comparisons across maps quite difficult.

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

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

References

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

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

Acknowledgment

This work is supported by NIH/NIGMS fellowship F32GM133129.

POSTER #45

Stephanie Wankowicz

University of California San Francisco

RETURN TO INDEX

Ligand binding remodels protein side chain conformational heterogeneity

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

While protein conformational heterogeneity plays an important role in many aspects of biological function, including ligand binding, its impact has been difficult to quantify. Macromolecular X-ray diffraction is commonly interpreted with a static structure, but it can provide information on both the anharmonic and harmonic contributions to conformational heterogeneity. Here, through multiconformer modeling of time- and space-averaged electron density, we measure conformational heterogeneity of 743 stringently matched pairs of crystallographic datasets that reflect unbound/apo and ligand-bound/holo states. When comparing the conformational heterogeneity of side chains, we observe that when binding site residues become more rigid upon ligand binding, distant residues tend to become more flexible, especially in non-solvent exposed regions. Among ligand properties, we observe increased protein flexibility as the number of hydrogen bonds decrease and relative hydrophobicity increases. Across a series of 13 inhibitor bound structures of CDK2, we find that conformational heterogeneity is correlated with inhibitor features and identify how conformational changes propagate differences in conformational heterogeneity away from the binding site. Collectively, our findings agree with models emerging from NMR studies suggesting that residual side chain entropy can modulate affinity and point to the need to integrate both static conformational changes and conformational heterogeneity in models of ligand binding.

POSTER #46

August Wollter

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RETURN TO INDEX

Background impact on EMC reconstruction and phasing in single particle imaging of biological samples

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Single particle diffraction of biological molecules was proposed by Neutze et al. in 2000[1], using very intense x-rays with ultrafast pulses, and although the sample would be totally disintegrated most of the diffraction would occur before the destruction. By imaging identical samples multiple times one can use the Expand Maximize and Compress (EMC)[2] algorithm to assemble these images into a 3D diffraction intensity. This would allow you to attempt to phase the sample in 3D, and recover the full electron density, which is the goal.

Experimental diffraction images are not perfect however. The signal photons are few, so you need many images to compensate. There are many background photons measured from injector gas and from other sources. These circumstances cause EMC to fail sometimes, and knowing the limits of EMC is useful when designing experiments.

In my study I use background images from the Eu-XFEL from an injector gas only run, which I scale to various levels. I combine these with simulated diffraction patterns of different intensities and perform the EMC algorithm, with the goal of quantitatively finding a signal to background limit for the EMC algorithm. I also attempt to phase the recovered 3D diffraction intensities, to assess the feasibility of the experiment.

Although the analysis is not complete it is clear that more background photons and less signal photons is challenging for the EMC algorithm, and even more challenging for the phasing algorithms. A quantitative signal to background ratio would be very useful for experiments, and that is my goal.

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POSTER #48

Iris Young

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RETURN TO INDEX

Curiosity: A Tool for Exploring Maps

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

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

References

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POSTER #50

Joshua Rodriguez

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RETURN TO INDEX

Measuring Temperature in Macromolecular Crystallography Experiments using Water Scattering and Molecular Dynamics

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Temperature-jump crystallography is a method that allows for the determination of non-equilibrium protein conformations. Pulsed infrared lasers are used to transiently excite the water surrounding a protein crystal, rapidly heating the protein and moving it away from conformational equilibrium. Then, ultrafast x-ray pulses are used for time-resolved protein structure determination. To obtain the most detailed information about protein conformational changes, and their relationship to function, it is helpful to know the absolute temperature of the sample before and after temperature jump. In theory, temperature can be measured using the background x-ray scattering from water that is captured in each diffraction image. However, the absolute temperature of the sample remains difficult to determine. In this study, molecular dynamics simulations of water at various temperatures were used to differentiate between temperature-jumped and room-temperature samples. Future work will aim to relate x-ray scattering of water to the temperature of the water, guided by molecular dynamics. Our ultimate goal is to create a general tool to measure the temperature of a protein sample in real-time during a macromolecular crystallography experiment. We expect this tool will be useful for a variety of experiments that use temperature as a direct perturbation of protein structure, as well as for other experiments involving other perturbations, such as light and electric fields, that can lead to unwanted heating.

POSTER #51

Ravindra Singh Rawat

Gautam Buddha University, India

RETURN TO INDEX

Taxifolin inhibits Adenosine deaminase from Mycobacterium tuberculosis (MtbADA): Structural and Kinetic studies

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Mycobacterium tuberculosis causes a chronic pulmonary disease called tuberculosis. Tuberculosis is one of the leading causes of death across the world. According to World Health Organization, 1.5 million people of whom around 14% were HIV positive died from TB. TB is only second to Covid-19 infections with respect to the mortality by infectious agents. Moreover, the clinical management of the disease has been exacerbated by the emergence of multi-drug resistant (MDR) strains of *M.tuberculosis*. Therefore, exploring new therapeutic targets and molecules against the targets appears as a never ending task. In our in-silico and biochemical studies we have successfully demonstrated that taxifolin inhibits MtbADA. Taxifolin is a flavonol, a type of polyphenol produced by plants. Due to its natural occurrence and low cytotoxicity, it can be used as an adenosine deaminase inhibitor to inhibit *M.tuberculosis* growth, which may result in the development of a therapeutic molecule. Although, various inhibitors of this enzyme have been reported, the most potent inhibitor of ADA is transition state inhibitors such as deoxycoformycin. However, due to its cytotoxicity, the use of deoxycoformycin as a therapeutic molecule is limited. In our work we have used deoxycoformycin as a control molecule to validate our experimental procedures.

POSTER #52

Juan Valentin-Goyco

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RETURN TO INDEX

Reconstitution and Biochemical Characterization of CYP11B Enzymes in Nanodiscs

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The finely tuned aldosterone production controls blood pressure by regulating water and sodium retention. The overproduction of aldosterone, however, leads to primary aldosteronism, the major form of secondary hypertension. This mineralocorticoid hormone is produced by the mitochondrial cytochrome P450 11B2 (CYP11B2). While lowering aldosterone levels through inhibition of CYP11B2 has been established as a potential therapeutic approach, a few challenges are associated with this tactic. For example, CYP11B2 shares a 93% sequence identity with the cortisol-producing P450 11B1 (CYP11B1). Despite the similarities between CYP11B1 and CYP11B2, key functional and structural differences exist that could be exploited. To characterize these distinctive traits, CYP11B enzymes have been successfully expressed and purified in *E. coli*. However, our current reconstitution systems, albeit useful, have failed to recapitulate their activity in *in-vitro* cellular assays fully. As such, this research aims to optimize the reconstitution of these isoenzymes. Here we report the successful incorporation and purification of CYP11B-incorporated nanodiscs. As reported in the literature, nanodiscs have only been employed to study microsomal P450 enzymes, which often results in poor activity compared to liposomes. Our studies show that both CYP11B isoenzymes are active in nanodiscs composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS). Furthermore, CYP11B-incorporated nanodiscs exhibit an increased affinity (Kd) for their endogenous ligands and similar or higher activity than liposome and detergent micelle systems. Altogether, our results indicate that nanodiscs provide significant advantages over traditional reconstitution systems, such as improved stability, solubility, and controlled monomerization. This will enable more in-depth structural and functional studies, such as hydrogen-deuterium exchange mass spectrometry (HDX MS), to understand the subtle differences between CYP11B1 and CYP11B2 and aid drug development.

Acknowledgement

This work was supported by grant I01BX005084-01A2 from the U.S. Department of Veterans Affairs (to R.J.A.) and grant T32 GM008353-2 (to J.V.G.) from the National Institutes of Health.

POSTER #53

Victoria Mazlova

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RETURN TO INDEX

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affect online and offline data analysis. Our work examined deep learning as a tool to denoise and demask diffraction patterns. The goal is to map Poisson sampled and masked 2D diffraction intensities into continuous Fourier amplitudes. We trained a convolutional neural network on 9900 diffraction patterns, each simulated from an independent PDB file, and studied the model's performance under different signal strengths and mask sizes. The model proved its robustness to new unseen simulated data and outperformed a low-pass filtering algorithm. Finally, we showed that EMC's orientation recovery on denoised and demasked data succeeds with significantly fewer patterns. The algorithm also seems to be more stable and less prone to rotational error fluctuations when using data denoised and demasked with our pipeline. By introducing a new data analysis step into the current SPI data processing, this work can improve experimental diagnostics and enhance the performance of offline algorithms such as EMC. Restoring diffraction intensities might pave the way for heterogeneity classification and studying rare conformational states for which data is scarce.

References

1 Sobolev, Egor, et al. "Megahertz single-particle imaging at the European XFEL." Communications Physics 3.1 (2020): 1-11.

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POSTER #55

Alfredo Bellisario

Uppsala University, Uppsala, Sweden

RETURN TO INDEX

Enhancing orientation recovery in biological X-ray SPI with neural networks

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Developments in X-ray single-particle imaging enabled the study of increasingly smaller biological macromolecules and now aim to provide a new tool for protein structure determination[1]. These experiments use high-speed X-ray detectors[2] to collect photons scattered from the sample at an unknown orientation. Algorithms, such as EMC[3] or manifold embedding[4], have been developed to overcome the complex challenge of finding the relative orientation of the collected 2D diffraction patterns into a 3D volume. However, it is yet to be determined the robustness of these algorithms to noise, missing data regions, and background signal. Moreover, it is a challenging theoretical problem to calculate the lowest orientation error that these algorithms can provide[5]. In addition, Poisson noise and missing data regions affect online and offline data analysis. Our work examined deep learning as a tool to denoise and demask diffraction patterns. The goal is to map Poisson sampled and masked 2D diffraction intensities into continuous Fourier amplitudes. We trained a convolutional neural network on 9900 diffraction patterns, each simulated from an independent PDB file, and studied the model's performance under different signal strengths and mask sizes. The model proved its robustness to new unseen simulated data and outperformed a low-pass filtering algorithm. Finally, we showed that EMC's orientation recovery on denoised and demasked data succeeds with significantly fewer patterns. The algorithm also seems to be more stable and less prone to rotational error fluctuations when using data denoised and demasked with our pipeline. By introducing a new data analysis step into the current SPI data processing, this work can improve experimental diagnostics and enhance the performance of offline algorithms such as EMC. Restoring diffraction intensities might pave the way for heterogeneity classification and studying rare conformational states for which data is scarce.

References

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POSTER #56

Reza Nazari

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RETURN TO INDEX

3D Printed Gas Dynamic Virtual Nozzles To Synchronize Droplets With The XFEL (X-Ray Free Electron) Pulses

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

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Continuous liquid microjets have been widely used in time-resolved solution scattering (TR-SS) experiments at X-Ray Free Electron Lasers (XFELs) to deliver samples to the intense focus of the x-ray beam. When conducting experiments at XFELs with pulse repetition rates on the order of 100 Hz, these continuous jets waste the vast majority of injected sample between shots, which is a major problem particularly in cases of irreversible reactions that do not allow for sample recycling. While there are several commercially available options to trigger droplets in an atmospheric pressure environment, to the best of our knowledge there are no such options available for in-vacuum operations. Enabling the synchronization of large droplets (of order 50+ microns) in vacuum would greatly reduce the gas background signals that make data processing remarkably difficult for solution scattering in particular. Here we develop and test 3D-printed gas dynamic virtual nozzles (GDVNs) that are designed to produce periodic droplets that may be synchronized with XFEL pulses. The experimental results are also compared with the numerical simulation results.

POSTER #57

Sabine Botha

Arizona State University, Tempe, AZ, USA

RETURN TO INDEX

Serial Macromolecular Crystallography at ALBA Synchrotron Light Source

Botha, S.,[1,2] and Martin-Garcia, J. M.,[1,5] Hu, H.,[2] Jernigan, R.,[1] Castellvi, A.,[3] Lisova, S.,[2] Gil, F.,[4] Calisto, B.,[4] Crespo, I.,[4] Roy-Chowdhury, S.,[1] Grieco, A.,[5] Ketawala, G.,[1] Weierstall, U.,[1,2] Spence, J.,[1,2] Fromme, P.,[1] Zatsepin, N.,[1,2,6] Boer, R.,[4] Carpena, X.,[4]

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The successful adaptation of serial crystallography at synchrotron radiation sources (Serial Synchrotron Crystallography, SSX) has been implemented at many synchrotrons around the world, facilitated by recent developments in hardware, faster detectors, brighter microfocus beams, as well as low sample consumption delivery methods such as the high-viscosity injectors, fixed targets, and hybrid devices. Here we present the first SSX experiments with viscous jets conducted at ALBA, beamline BL13-XALOC. Small crystals of five soluble proteins, lysozyme, proteinase K, phycocyanin, insulin, and the SH3 domain of α-Spectrin (α-Spectrin-SH3), were delivered to the X-ray beam with a high-viscosity injector developed at Arizona State University. Complete data sets were collected from all proteins and their high-resolution structures determined. The high quality of the diffraction data collected from all five samples, and the lack of specific radiation damage in the structures obtained in this study, confirms that the current capabilities at BL13-XALOC enable atomic resolution determination of protein structures from microcrystals as small as 15 µm using viscous jets at room temperature.

Acknowledgment

All experiments presented in this study were carried out at BL13-XALOC beamline at ALBA synchrotron, managed by the Consortium for the Construction, Equipment and Exploitation of the Synchrotron Light Source (CELLS). We would like to thank the entire BL13-XALOC staff and the floor coordinators for all their assistance in using the beamline. We would further like to thank Ana Cámara-Artigas (University of Almería, Spain) for providing us with the clone of the SH3 domain of α-Spectrin.

This work was funded by: The “Ayuda de Atracción y Retención de Talento Investigador” from the Community of Madrid, Spain (award No. 2019-T1/BMD-15552); The STC Program of the National Science Foundation through BioXFEL (award No. 1231306); the Centre for Applied Structural Discovery (CASD) at the Biodesign Institute at Arizona State University.

POSTER #58

Maximillian Wranik

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RETURN TO INDEX

Molecular snapshots of drug release over eleven orders of magnitude in time

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The dynamic interplay between proteins and their ligands is central to molecular biology, pharmacology, and drug development but is difficult to resolve experimentally. Using time-resolved serial crystallography, we studied the release of the photochemical affinity switch azo-combretastatin A4 from the colchicine site of the anti-cancer target tubulin. Thirteen logarithmically spaced temporal snapshots at near-atomic resolution, complemented by time-resolved spectroscopy and molecular dynamics simulations show how the cis to trans isomerization of the azobenzene bond stretches the ligand within its binding pocket in the picoseconds to nanoseconds range, followed by stepwise opening of a gating loop within microseconds, and completion of the unbinding reaction through col-lapse of the binding pocket within milliseconds. Ligand changes and unbinding are accompanied by global tubulin-backbone rearrangements. Our results have implications for the mechanism of action of anti-tubulin drugs and provide a general experimental framework to study protein-ligand interaction dynamics.