KEYNOTE SPEAKER

Rajini Rao, Johns Hopkins University

The Matilda Effect: Addressing Structural and Institutional Bias in STEM

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Coined by science historian Margaret Rossiter in 1993 and named after 19th century US activist Matilda Gage who first described this phenomenon, the Matilda effect defines the common bias against recognition of women in science. It is related to the Matthew effect, which describes how prominent scientists get more credit for the same work done by less well-known researchers. Although women outnumber men in receiving undergraduate degrees, and there is near parity at the doctoral level, their numbers fall off drastically as they climb academic or corporate ladders. The National Science Foundation reports a decline from 49% of women with doctoral degrees, to 39% in postdoctoral positions, and 32% in full time faculty positions. Of these, only 12% are promoted to full professor and a mere 6% are National Academy of Science members. In physical sciences, math and engineering fields, representation by women has actually fallen since 1990 and is not showing significant gains. Dr. Rao will discuss many of the issues underlying these troubling statistics, including structural problems in academic hiring, the role of stereotype threat and unconscious bias. She will present steps that institutions can take to promote gender equity, diversity and inclusion in STEM, and discuss how we can change the culture and climate of sexism in society, how professional organizations can help, and how men can be allies.

Biography

Rajini Rao is professor of Physiology at the Johns Hopkins University School of Medicine in Baltimore, Maryland. Her laboratory investigates ion transporters, including proton and calcium pumps and exchangers, with recent publications focusing on the role of these transporters in brain and breast cancer, autism and Alzheimer disease. Her discovery driven research program exemplifies forward-looking Precision Medicine initiatives. Dr. Rao is an active educator and mentor, both within and outside Johns Hopkins. In her role as principal investigator of the NIGMS-funded T32 training grant and director of the graduate program in Cellular & Molecular Medicine, she oversees a multi-departmental program that includes 125 faculty and 130 Ph.D. students. Dr. Rao has been a long standing advocate for women in science, having chaired the Committee on Professional Opportunities for Women at the Biophysical Society for nearly a decade, and co-founded stemwomen.net, a site that raises awareness for gender disparity in STEM. She has held multiple elected leadership roles in the Biophysical Society, chaired internationally recognized FASEB and Gordon conferences, and served on journal editorial boards and review boards at the NIH, DOD, AHA and HHMI.

Bill Pedrini, SwissFEL

The Hard X-ray Beamline at the Swiss Free Electron Laser

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The Swiss free electron laser (SwissFEL) is foreseen to provide the X-ray light for the first commissioning experiments in Fall 2017, and to start normal user operation in 2018. We describe the expected beam parameters at the hard X-ray beamline and the infrastructure that will be available at the experimental stations, and discuss the experimental possibilities with a particular eye on protein crystallography applications.

Robert Schoenlein, LCLS-II

Recent Developments at LCLS and Science Opportunities and Plans for LCLS-II and LCLS-II-HE

Robert Schoenlein[1]
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The unique capabilities of LCLS, the world's first hard X-ray FEL, have had significant impact on advancing our understanding of biology at the molecular level. Key applications include single-particle imaging, serial femtosecond nanocrystallography, time-resolved crystallography, time-resolved small-angle X-ray scattering, and time-resolved X-ray spectroscopy. This talk will outline some of the ongoing developments at LCLS that will further advance the impact of this facility on biological science.

A major upgrade of the LCLS facility (LCLS□II project) is now underway. LCLS-II is being developed as a high-repetition rate X-ray laser with two simultaneously operating, independently tunable FELs. The baseline design features a 4 GeV continuous wave superconducting linac (CW-SCRF) that is capable of producing uniformly spaced (or programmable) ultrafast X-ray laser pulses at a repetition rate up to ~1 MHz spanning the energy range from 0.25 to 5 keV. The superconducting linac will be installed in the first third of the SLAC linac tunnel. The final third of the SLAC linac will continue to operate as a warm Cu accelerator at energies up to 15 GeV, providing tunable X-rays with photon energy up to 25 keV at 120 Hz. Four new instruments are planned to exploit the new capabilities of LCLS-II. One instrument will support AMO science, strong-field science, and a new dynamic reaction microscope. Two instruments will rely on a monochromator to support high-resolution and moderate-resolution soft X-ray spectroscopy at close to the Fourier transform limit. A fourth instrument will operate in the tender X-ray range (1-7 keV) and will be capable of combining pulses from both the soft X-ray and hard X-ray FELs.

Looking to the future, there is a compelling opportunity to upgrade the energy of LCLS-II (LCLS-II-HE). By adding CW-SCRF cryomodules, the electron beam energy can be doubled to 8 GeV, thus increasing the spectral reach of the hard X-ray undulator (HXU) to more than 12 keV. Anticipated improvements in electron beam emittance will extend the energy reach to 20 keV. This will enable the study of atomic-scale dynamics with the penetrating power and pulse structure needed for *in situ* and *operando* time-resolved studies of real-world materials, functioning assemblies, and biological systems.

This talk will present some of the important science opportunities and instrumentation
being planned for LCSL-II and LCLS-II-HE, focusing on future capabilities that will be particularly relevant for advancing our understanding of biology at the molecular level.

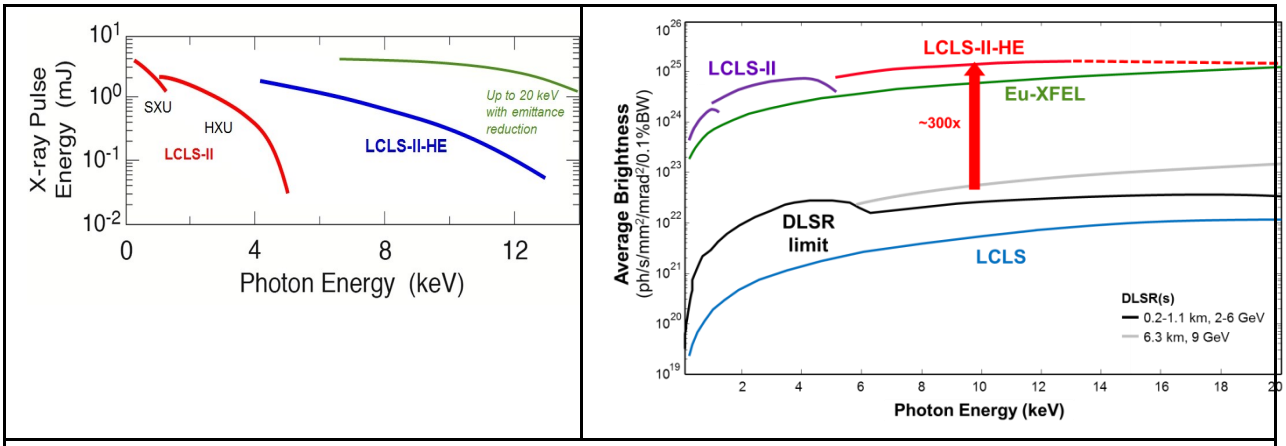

Figure 1. Calculated energy per pulse (left) and average brightness (right) for LCLS-II and proposed LCLS-II-HE, including future X-ray facilities: the European XFEL and diffraction-limited storage rings (DLSRs).

MENG LIANG, SLAC

SERIAL FEMTOSECOND CRYSTALLOGRAPHY OF AMYLOID CRYSTALLINE FIBERS

M. N. Liang[1], V. T. Forsyth[2], E. Mossou[2], D. Wojtas[3], K. Ayyer[4], R.P. Millane[3], M. Dubrovin[1], A. Mitraki[5], and H. N. Chapman[4,6]

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Amyloid systems are fibrous aggregates consisting of peptides that self-assemble into long filamentous structures. They are of fundamental scientific interest from the perspectives of health and molecular/cellular biology. Despite the fact that amyloid and amyloid-type systems have been studied using a vast array of techniques since they were first identified in the 1930s, major questions remain about their structure and formation.

Traditional X-ray fiber diffraction acquires data from mechanically aligned fiber bundles to obtain averaged data. The resolution and ability to determine structure is limited by the averaging of imperfectly aligned fibers. Serial Femtosecond Crystallography from individual amyloid crystalline fibers can reveal the structure of amyloid systems far beyond what can be learned from traditional fiber diffraction.

Following in the success of Serial Femtosecond Crystallography from protein crystals and the ability to obtain single diffraction data from submicron crystals, we obtained a dataset from single crystalline amyloid fibers. The sparsity of the diffraction intensities due to the small unit cell and fiber size represents a challenge for standard SFX indexing programs. We will present work to analyze sparse amyloid diffraction data utilizing fiber techniques and partial flow alignments constraints.

Jessica Thomaston, UC-San Francisco

XFEL Structures of the Influenza M2 Proton Channel at 1.4Å: Room Temperature Water Networks and Insights into Proton Conduction

Jessica L. Thomaston,[1] Rahel A. Woldeyes,[2] Takanori Nakane,[3] Kotaro Koiwai,[4] Ayumi Yamashita,[5] Tomoyuki Tanaka,[5] Toshi Arima,[5] Jun Kobayashi,[5] Tetsuya Masuda,[6] Mamoru Suzuki,[7] Michihiro Sugahara,[5] Rie Tanaka,[5] Eriko Nango,[5] So Iwata,[5] Fumiaki Yumoto,[4] James S. Fraser,[2] William F. DeGrado[1]

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The influenza M2 protein is the smallest proton selective, pH activated transmembrane channel and is also the target of two of the four FDA approved anti-flu drugs. During the viral replication cycle, it is necessary for protons to travel through M2's hydrophobic N-terminal pore, pass through gating His37 residues, then exit through the channel's C-terminus. This causes acidification of the flu virus interior, which allows flu virus RNA to uncoat from viral ribonucleoproteins so that the newly uncoated RNA can be released into the host cell and propagate the virus. The N-terminal pore of the M2 channel contains water molecules that play a role in both the conduction of protons to the gating His 37 residues and the stabilization of positive charges within the channel. However, previous attempts to structurally characterize this channel at a conventional synchrotron x-ray source yielded results that were confounded by the effects of cryogenic cooling and, when using room temperature data collection techniques, the effects of radiation damage [1]. Microcrystals of M2 were optimized to an appropriately high concentration and a variety of sample injection techniques were attempted. The most successful sample delivery method was an LCP injection system. XFEL structures of the M2 channel at 1.4 Å were collected at SACLA to reveal images of the channel's water filled pore at room temperature and in the absence of radiation damage. Structures of the M2 channel at pH 5.5, 6.5, and 8.0 reveal a pore water network with solvent ordering that is dependent on pH.

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Wei Liu, Arizona State University

Structure-Based Drug Design Platform for GPCRs in LCP

Wei Liu

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The Center for Applied Structural Discovery (CASD) aims at establishing a structure-based drug design (SBDD) platform for G protein-coupled receptors (GPCR) using our recently developed technique of serial femtosecond crystallography with crystals embedded in lipidic cubic phase (LCP-SFX). SBDD has proven successful for many soluble protein targets, such as kinases and proteases; however its application to GPCRs is limited by the difficulty of preparing large amounts of homogenous and stable samples, and growing sufficiently large crystals for many different receptor/ligand complexes that are needed to collect high-resolution diffraction data at synchrotron beamlines. The LCP-SFX approach obviates the need for larger crystals and requires only sub-milligram quantities of purified protein, addressing the most significant barriers to success. X-ray structure analysis will be based on hundreds of thousands diffraction patterns, collected from a continuous stream of fully hydrated GPCR microcrystals embedded in LCP. This platform builds on results from our successful structure determination of GPCRs by SFX using high resolution data collected at Linac Coherent Light Source (LCLS), providing strong evidence that the approach will revolutionize membrane protein structural biology. Our future studies will attempt to address and optimize workflow processes and collected data will be used to solve several receptors/ligand co-crystal structures at atomic resolution and thus provide a blueprint for establishing an SFX-SBDD platform.

Thomas Barends, MPI-Heidelberg

Observing Protein Dynamics with (Sub) Picosecond Time-Resolution Serial Femtosecond Crystallography

Thomas R. M. Barends

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Synchrotron radiation sources have revolutionized structural biology, by enabling the structure determination of thousands of proteins and so giving detailed insight into their working mechanisms. However, synchrotron-based crystallography is limited by its requirement for macroscopic crystals, as well as by the often severe radiation damage caused by X-rays, in particular when small crystals are being used.

Now, the advent of X-ray free-electron lasers (FELs) which provide femtosecond X-ray pulses allows the acquisition of high resolution diffraction data from micron-sized macromolecular crystals at room temperature while outrunning radiation damage to a large extent. Moreover, the extremely short pulses afforded by XFELs allow time-resolved studies that reach the chemical time scale of femtoseconds. We will discuss recent results from our high time resolution structural studies on proteins using XFEL-based crystallography.

Nadia Zatsepin, Arizona State University

Serial Femtosecond X-ray Crystallography of Carbon Monoxide-bound Cytochrome c Oxidase

Izumi Ishigami[1], Nadia Zatsepin[2,3], Chelsie Conrad[3,4], Garrett Nelson[2], Jesse Coe[3,4], Thomas Grant[5], Mark S. Hunter[6], Petra Fromme[3,4], Raimund Fromme[3,4], Syun-Ru Yeh[1] and Denis L. Rousseau[1]

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Cytochrome *c* oxidase (C*c*O), the terminal enzyme in the electron transfer chain, translocates protons across the inner mitochondrial membrane by harnessing the free energy generated by the reduction of oxygen to water. Several mechanisms for this redox coupling have been proposed but they lack confirmation, in part from uncertainties in the reported crystal structures due to radiation damage effects caused by the intense synchrotron radiation. Here we report the damage-free structure of the carbon monoxide (CO) bound derivative of bovine C*c*O (CO-bC*c*O) obtained by serial femtosecond X-ray crystallography (SFX) with an X-ray free electron laser and we compare it to a CO-photodissociated derivative obtained at a synchrotron light source. In the SFX structure resolved at 2.3Å, the CO is coordinated to the heme *a*3 iron atom and is bent to 1340 from the heme plane, whereas in the structure of CO-bC*c*O obtained by synchrotron radiation, at a resolution of 1.95Å, the Fe-CO bond is cleaved and the CO has moved to a position near CuB. Associated with CO dissociation from heme *a*3, the distance between the heme *a*3 iron atom and CuB changed from 5.27 to 4.91Å, primarily owing to a change in position of CuB. Surprisingly, an allosteric transition involving a large movement of a section of the Helix- X polypeptide that lies between the two hemes, is triggered by the change in ligation state. This transition plays a critical role in postulated mechanisms of proton translocation in mammalian C*c*Os.

Please see the corresponding poster for references.

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Oleksandr Yefanov, DESY

3D Merging: Getting More from Protein Crystallography

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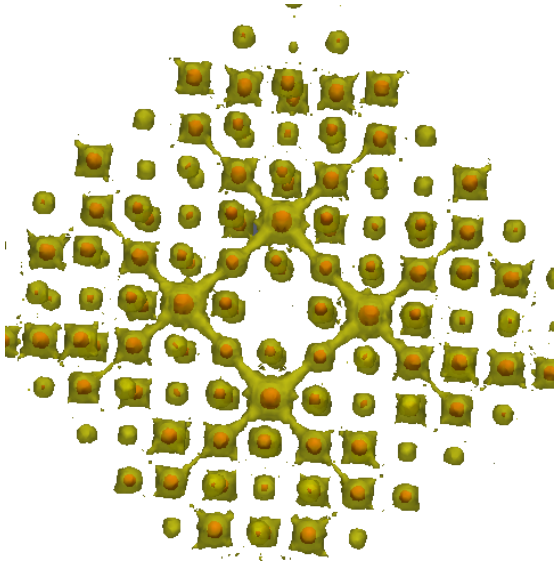
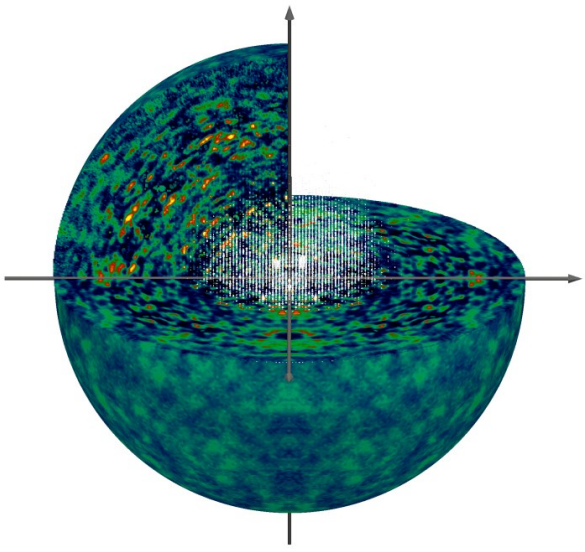
For decades protein crystallography was one of the best methods for biological structure determination. Its popularity is governed by the fact that highly ordered structures produce intensive Bragg peaks which are easy to detect and measure. At the same time the scattering between Bragg peaks is usually very weak, therefore it was usually neglected. Modern sources, like Free Electron Lasers (FELs) and 3rd generation synchrotrons combined with the new techniques such as Serial Femtosecond Crystallography (SFX) allow to increase the dynamical range of measured signal and therefore measure scattering between Bragg peaks.

In SFX at FELs individual crystals are introduced one after another into the X-ray beam and exposed to a single X-ray pulse. In this way thousands of measurements of crystal in random orientations are measured. All those measurements are merged in three dimensions. This method reveals the underlying continuous three-dimensional reciprocal space intensity distribution. For example Bragg peaks asymmetry and intensities between Bragg peaks (left figure) [1] as well as continuous diffraction at resolution beyond Bragg peaks (right figure) [2]. The analysis of such 3D intensity distribution can reveal additional structure information, improve resolution, and can potentially be used for ab initio structure reconstruction.

One of the issues for success of such experiments is removing background from solution which is usually much higher than the useful signal. Therefore we've developed a method to remove radially symmetrical background based on Willson statistics [3].





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Joe Chen, Arizona State University

Shape Transform Phasing

J. P. J. Chen[1], O. M. Yefanov[2], R. J. Bean[2], A. J. Morgan[2], K. R. Beyerlein[2], R. D. Arnal[3], R. P. Millane[3], H. N. Chapman[2], R.A. Kirian[1]

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Shape transform phasing, is a method that is particularly pleasing. No similar structures are needed, nor does the XFEL have to be seeded. The only thing that we require, are samples between Bragg peaks due to the coherence of the beam entire. Iterative projection algorithms can then be used to give the desired structure without fussing*.

*Conditions apply. See talk for details.

Acknowledgment

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Michael Thompson, UC-San Francisco

Infrared Laser-Induced Temperature-Jump: A General Pertubation Method for Studying Protein Dynamics with Time-Resolved X-ray Scattering and Diffraction

Thompson, M.C.,1 Barad, B.A,2, Cho, H.S.,3 Schotte, F.,3 Wolff, A.M.,2 Sierra, R.G.,4 Gonzalez, A.,5 Brewster, A.S.,6 Young, I.D.,6 Carbajo, S.,4 Demirchi, H.,7 Kim, S.J.,1 Poss, E.M.,8 Sali, A.,1 Boutet, S.,4 Sauter, N.K.,6 van den Bedem, H.,5,7 Anfinrud, P.A.,3 Fraser, J.S.1

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Time-resolved X-ray scattering and diffraction are among the most information-rich experimental techniques in structural biology. To date, systems that have been successfully studied are those in which a protein conformational change is coupled to excitation of a photoactive ligand molecule, because the conformational change can be initiated with an ultrafast laser pulse. Unfortunately, the number of proteins that undergo photochemistry as part of their functional cycle is small, and there is a fundamental need to develop generalized methods that can be used to synchronously excite conformational transitions in *any* protein molecule, even in the absence of specific photochemistry. A recent "multi-temperature" crystallographic study of a model enzyme, cyclophilin A (CypA), demonstrated that temperature perturbation is an effective way to experimentally manipulate a conformational ensemble of protein molecules for structural studies. Our current goal is to exploit CypA as a model system for the development of time-resolved X-ray experiments that utilize laser-induced temperature-jump (T-jump) methods to synchronize conformational dynamics. Initial time-resolved SAXS/WAXS experiments performed at a synchrotron demonstrated that even modest T-jumps produce a measurable change in X-ray scattering by the protein. Subsequent fitting of kinetic models to time-resolved differences in solution scattering curves allowed us to probe the timescales of underlying conformational changes. Additional, crystallographic T-jump experiments, performed at the Linac Coherent Light Source (LCLS), offer further insight into the atomic details of conformational transitions. Because laser T-jump methods exploit photochemistry of the solvent, and not the protein molecules, we hope they will be universally applicable as a tool for studying protein dynamics at both synchrotron and XFEL light sources.

CHELSIE CONRAD, NATIONAL INSTITUTES OF HEALTH

REDUCTION OF SAMPLE CONSUMPTION FOR SERIAL CRYSTALLOGRAPHY BY VISCOUS SAMPLE DELIVERY

Conrad, C.E.[1,2,3], Garcia, J.M.[1,3], Basu, S.[1,3], Nelson, G.[3,4], Coe, J.[1,3], James, D.[3,4], Wang, D.[4], Zatesepin, N.A.[3,4], Roy-Chowdhury, S.[1,3], Aquila, A.[7], Beyerlein, K.[5], Chen, J.[3,4], Gati, C.[5], Grant, T.[9], Hunter, M.[7], Ishigami, I.[8]; Kupitz, C.[1,6], Messershmidt, M.[9], Oberthüer, D.[5], Ogata, C.[10], Rousseau, D.L., Schaffer, A.[1,3], Subramanian, G.[3,4], Yeh, S.R.[8]; Zhao, Y.[3,4], Zook, J.[1,3], Fischetti, R.[10], Boutet, S.[7], Cherezov, V.[11], Spence, J.C.H.[3,4], Fromme, R.[1,3], Weierstall, U.[3,4], Wang, Y.X.[2], Fromme, P.[1,3]

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Most serial crystallography experiments have relied on delivering sample in the mother liquor focused into a stream by compressed gas. This liquid stream moves at a fast rate, wasting most of the valuable sample. For this reason, the liquid jet can require 10-100 milligrams of sample for a complete data set and it has been estimated that only 1 in 10,000 crystals intersect with the X-ray pulse.[1] In addition, the fast repetition rate of the liquid jet is not amenable for serial millisecond crystallography experiments which require millisecond exposure times. Serial crystallography sample delivery using lipidic cubic phase (LCP) allows for a slower moving jet due to its high viscosity and thus less sample is wasted between X-ray shots. However, LCP is not suitable as a general delivery system for membrane proteins as mixing with LCP is suspected to remove the detergent micelles used to stabilize the protein.[2] Aiming for a universal viscous sample carrier, two viscous mediums, agarose and poly(ethylene oxide) have been developed to decrease sample consumption and waste. Crystals can be mixed post-crystallization into either of these mediums allowing crystals to be grown via any method such as vapor diffusion, dialysis, batch etc. Because both of these mediums are hydrogels, the mother liquor solution can be used to make the hydrogel resulting in a similar environment to which the crystals are grown.[2] Additionally, the hydrogels are mainly composed of water (>80%) resulting in low background scattering, mainly from the solvent. The agarose delivery method has been shown to be compatible with a wide variety of crystal systems including, membrane proteins,[2] multi-protein complexes,[2] viruses,[3] and ribonucleic acids[4]. Unlike other viscous mediums used for sample delivery, the agarose stream provides low background, no Debye-Scherrer rings, and is compatible for sample delivery in vacuum environments.[2] Poly(ethylene oxide) has also been explored as a sample carrier for in atmosphere experiments, primarily for serial millisecond crystallography. Recently three data sets were collected using poly(ethylene oxide) at the Advanced Photon Source. Preliminary results indicate that poly (ethylene oxide) is also compatible with membrane proteins. But very limited X-ray free electron laser beamtime is needed for this experiment. Thus hydrogels haven been shown to allow sample limited proteins of difficult to crystallize systems to be investigated by serial crystallography.

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Alexandra Ros, Arizona State University

Microfluidic Tools for Serial Crystallography

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Serial femtosecond crystallography (SFX) with X-Ray Free Electron Lasers (XFEL) has evolved as a powerful technique for crystallography of large protein complexes over the past years. Several limitations of this emerging method have however prevented its general applicability. Among those limitations are the restrictions in growing protein crystals sufficiently small in size (ideally sub-µm) for SFX, the requirement of highly concentrated crystal suspensions of several mL volumes, the lack of tools for substrate based time-resolved crystallography studies as well as the lack of de novo phasing approaches. The field of microfluidics has developed in the past decade as a tool kit allowing prototype device design suitable for providing solutions to current challenges in SFX. To address the loss of precious protein crystals in liquidjet injection technology typically achieved with gas dynamic virtual nozzles (GDVNs), we propose microfluidic droplet generation intermitting the aqueous crystal suspension stream with an oil phase to dramatically reduce sample waste. We demonstrate that microfluidic droplet generation can be coupled to traditional GDVNs and applied this approach to SFX of granulovirus. This approach can also be fully integrated on a microfluidic chip. In addition, we developed a microfluidic device capable of separating protein crystal suspensions into sub-µm size fractions. This concept is based on dielectrophoresis at integrated constrictions allowing to guide crystals of different size into various outlets. Our latest work optimized the sorting principle for high throughput applications required for SFX studies and we demonstrate a yield of up to 500 µL per hour highly concentrated photosystem I crystal suspension which could be directly injected with GDVNs in SFX experiments. Crystal quality after sorting remained excellent and yielded hit rates up to 40%. The data set is currently investigated for novel phasing approaches. Further, we explore microfluidic mixing based on hydrodynamic focusing and fast diffusive mixing for SFX. Mixing devices were developed both with photolithography as well as 3D printing approaches achieving sub-ms mixing times at flow rates compatible with GDVNs. Geometrical optimization of device geometry and channel volume allow the measurement of reaction time points ranging from several ms up to seconds. This mixing approach has been applied to study the reaction of the enzyme 3-deoxy-D-manno- 2-octulosonate-8 phosphate synthase with its substrates phosphoenolpyruvate and arabinose-5-phosphate.

Athina Zouni, FU-Berlin

Insights into the Native-like Photosystem II Structure Using FS X-ray Free Electron Laser Diffraction

Zouni, A.,[1], Ibrahim, M.,[1], Chatterjee, R.,[2], Young, I. D.,[2], Kern, J.,[2,3], Yano, J,[2], Yachandra, V. K.,[2] Müh, F.,[4], Dobbek, H.,[1].

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Photosynthetic water oxidation is catalyzed by a Mn_4CaO_5 cluster (oxygen-evolving complex, OEC) associated with the dimeric Photosystem II core complex (dPSIIcc). The OEC cycles through five states (S_0 to S_4), where S_1 is dark-stable and S_3 is the last semi-stable state before dioxygen evolution. The conventional synchrotron X-ray radiation crystallography used to determine the structure of dPSIIcc from cyanobacteria[1, 2] causes radiation damage of the OEC. Although the recent 1.95 Å resolution structure at cryogenic temperature using an XFEL[3] provided a damage-free view of the S_1 state, measurements at room temperature (RT) are required to unravel the dynamic mechanism of water oxidation[4]. This goal can be achieved through femtosecond X-ray diffraction on PSIIcc microcrystals[5, 6]. Recently, a new crystal form of cyanobacterial dPSIIcc was obtained employing a dehydration protocol that results in the extraction not only of water but also of detergent from the crystals[2]. This transformation induces a hitherto unobserved transformation of membrane protein crystals from type II to type I packing and results in a resolution of 2.44 Å. In the new crystal form, dPSIIcc is packed in rows akin to dPSIIcc arrays observed in the native thylakoid membrane of *T. elongatus*. At the current dynamic LCLS experiments[8], we are continuously improving our seeding protocol in order to produce high quality dPSIIcc microcrystals in high amounts[7]. New insights into the water oxidation reaction will be given by presenting the XFEL diffraction data at RT in the S_3 state at 2.25 Å resolution[8] compared to the S_1 state.

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George Calvey, Cornell

Mixing Injector for Time-Resolved Studies of Chemically-Induced Structural Changes at X-ray Free Electron Lasers

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The past decade has seen breakthroughs in time-resolved crystallographic studies of light-activated biomolecular reactions at both synchrotrons and XFELs. However, chemically activated structural changes are far more prevalent and remain largely unprobed in crystalline systems, mostly as a result of the long soaking times required to initiate reactions in crystals that are appropriately sized for synchrotron studies. The long diffusion times of activating ligands preclude observation of short lived (millisecond) transient states. On the other hand, high energy, short pulses from X-ray free electron lasers uniquely enable structural determination from micron scale crystals. In these smaller crystals, the relevant diffusion times can be reduced to milliseconds. This type of experiment has the potential to provide key insights into the structural basis for chemically induced biomolecular reactions with implications ranging from fundamental understanding to structure-based drug design. In this presentation, I will discuss a new injector developed for the time-resolved study of chemically activated structural changes. It rapidly mixes ligands with microcrystals before injecting the reacting crystals into the x-ray beam. This mixing injector can access timepoints from milliseconds to seconds using a robust design that achieves a high hit rate. Preliminary XFEL results will be discussed.

Bill Graves, Arizona State University

Compact XFEL Light Sources

W.S. Graves, ASU

We are pursuing development of a very compact XFEL based on inverse Compton scattering (ICS) from a nanopatterned electron beam. CXFEL depends on a novel method to produce transform-limited x-ray output in all dimensions, i.e., with all photons in a single degenerate quantum state. This method avoids the noise amplification of SASE by imprinting a well-defined coherent modulation on the electrons via diffraction in a thin crystal grating. The spatial pattern in the diffracted electrons is then converted to a temporal pattern using sophisticated electron optics that exchange the transverse and temporal phase space dimensions. The result is a nano-patterned electron beam that can be tuned for wide range of applications. The method allows for coherent control of the phase, frequency, bandwidth, pulse length and amplitude of the x-ray pulses, and enables a variety of 2-color or multi-color experiments with precisely tunable femtosecond delays for pump-probe experiments, and perhaps even sub-cycle phase-locking of the multiple colors. The output will cover the photon energy range from 100 eV to 8 keV.

KATERINA DÖRNER, EUROPEAN XFEL

SAMPLE DELIVERY METHODS AT THE EUROPEAN XFEL

Katerina Dörner[1], Johan Bielecki[1,2], Carsten Deiter[1], Rita Graceffa[1], Matthäus Kitel[1], Kristina Lorenzen[1], James Moore[1], Dennis Ropers[1], Prasad Thute[1], David Watts[1] and Joachim Schulz[1]

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The European X-ray free-electron laser (XFEL) facility will be in operation and open for users in the second half of 2017. The initial operation mode will provide bunch trains (600 µs in length every 100 ms) with a pulse repetition rate of 100 kHz which will be later increased to 4.5 MHz. Together with the high peak brilliance, small wavelength and short pulse duration this opens new possibilities in high resolution and time-resolved structural studies of biological systems. In accordance with the high repetition rate, sample delivery methods are in demand which ensure quick and efficient sample renewal. The sample environment group provides support for biological sample delivery by way of liquid and aerosol injection as well as fixed targets. User laboratories with set-ups for nozzle manufacturing and test chambers for liquid and aerosol injection will be provided. A fast solid sample scanner and related pre-diagnostics for fixed targets will be available. Biology laboratories, for the most part equipped by the XBI user consortium, will be open for all users and allow the complete process of sample preparation and characterization on-site.

BioXFEL 4TH INTERNATIONAL CONFERENCE

TAISA GORKHOVER, SLAC

OVERCOMING THE PHASE PROBLEM WITH IN-FLIGHT X-RAY FOURIER HOLOGRAPHY

Tais Gorkhover[1,2,3], Anatoli Ulmer[1], Ken Ferguson [2,3], Max Bucher[1,2], Filipe Maia [4], Johan Bielecki[4], Tomas Ekeberg[4], Max Hantke[4], Benedikt Daurer[4], Carl Nettelblad [4], Jakob Andreasson[4,5], Gyula Faigel[6], Anton Barty[7], Petr Bruza[5], Sebastian Carron[2], Daniel Deponte[2], Dirk Hasse[4], Jacek Krzywinski[2], Daniel Larsson[4], Andrew Morgan[7], Kerstin Mühlig[4], Maria Müller[1], Kenta Okamoto[4], Alberto Pietrini[4], Daniela Rupp[1], Mario Sauppe[1], Marvin Seibert[4], Jonas Sellberg[4], Gijs van der Schot[4], Martin Svenda[4], Michelle Swiggers[2], Nicusor Timneanu[4], Daniel Westphal[4], Garth Williams[2,8], Alessandro Zani[4], Henry Chapman [7], Thomas Möller[1], Janos Hajdu [4] & Christoph Bostedt[2,3,9,10]

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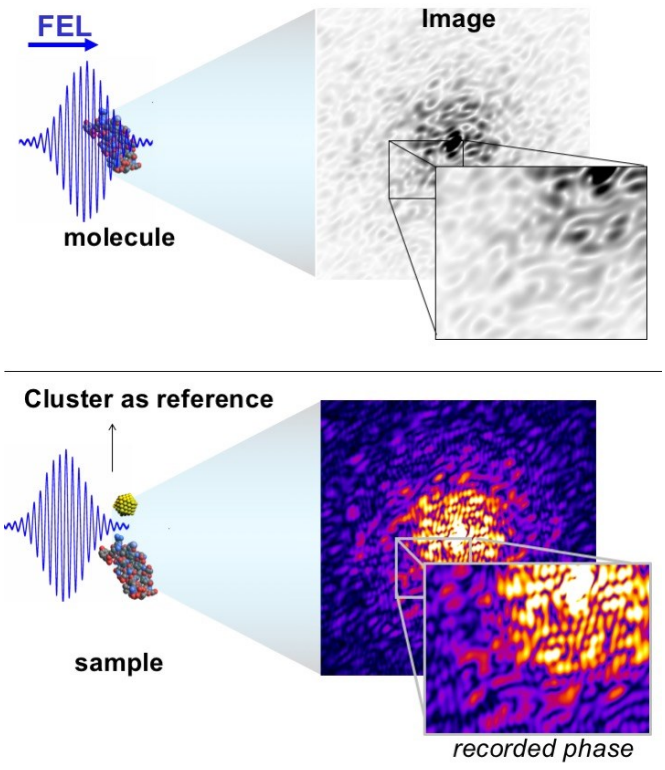
One of the ground breaking ideas behind X-ray free-electron lasers is to gain unprecedented insights into non-crystallin biosamples with single femtosecond x-ray pulses [1]. Examples of previous successfull X-ray diffraction studies include imaging of isolated viruses, bacteriophages, organelles, and cyanobacteria [2]. However, structure reconstruction from diffraction patterns is still challenging due to inherent phase loss. Despite advanced development of phase retrieval algorithms, structure determination from diffraction patterns alone still suffers from stagnation problems, sensitivity to noise and ambiguity of the reconstructed phase while the reconstruction itself is time consuming and often non-convex. X-ray Fourier holography can overcome many of these limitations by encoding the phase directly into the image [3]. The core element of this approach is a reference wave front which intereferences with the sample´s exit wave and thus, imprints relative phase information into diffraction patterns. We applied the holographic principle to single particle imaging and recorded holograms of the Mimi virus “in-flight”. The reference signal was generated from scattering on free xenon clusters. The first results and the potential of “in-flight” holography will be discussed in the talk.



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Wah Chiu, Baylor College of Medicine

Atomic Resolution Cryo-EM of Single Particles

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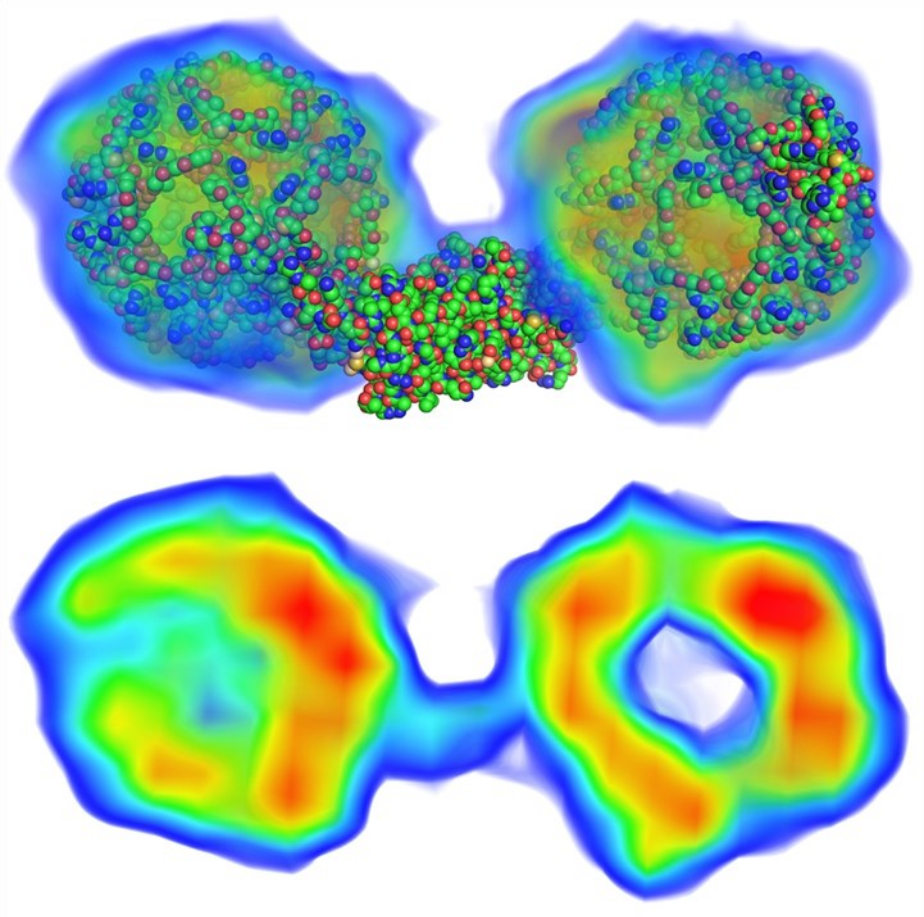
Electron cryo-microscopy (cryoEM) is in the midst of a rapid advance in resolving the biological structures of molecular machines previously either difficult or impossible to attain, at atomic resolutions. In our Center, we have solved cryoEM structures of molecular machines, including viruses, chaperonins, protein complexes and membrane proteins, to the level where either partial or full-atom models of the protein components can be derived from our cryoEM maps. Examples of membrane proteins will be presented illustrating how cryoEM can extract novel biochemical knowledge from their structures.

Thomas Grant, SUNY Buffalo

The Rapid Determination of Electron Density Maps Directly from Solution Scattering Data

Thomas D. Grant

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



Chuck Yoon, LCLS

Machine Learning Based Data Analysis at the Linac Coherent Light Source

Yoon, C. H.,[1]
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Sorting detector images into buckets of useful groups is often a crucial step in the data analysis pipeline for many experiments at the Linac Coherent Light Source (LCLS). Image classification in reciprocal space is however a challenging task where feature engineering often feels unnatural/cumbersome and manual human labeling does not scale beyond a few thousand images.

Psocake (pronounced piece of cake) is a psana-based graphical user interface (GUI), which can perform image classification tasks using machine learning. Examples will be discussed where unsupervised, semi-supervised, and supervised learning can be successfully applied to FEL data.

Christopher Kupitz, University of Wisconsin-Milwaukee

Mix and Inject: A βLaC Story

Kupitz, C.[1], Olmos, J.[2], Phillips, G.N.[2], Schmidt, M.[1], et al *
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ß-lactam antibiotics are antibiotics containing a lactam ring, many of which are used to treat diseases worldwide including tuberculosis, syphilis, and staphylococcus. Unfortunately, the bacteria responsible for these diseases are becoming resistant to such forms of antibiotics. Bacteria have developed an effective defense mechanism using special enzymes known as ß-lactamases. Regrettably, this defense mechanism is causing ß-lactams to rapidly become less effective and in some cases, almost obsolete. For example, tuberculosis, despite significant advancements in treatment, remains a major cause of human morbidity, killing over 1.5 million people worldwide in 2014. Successful time-resolved experiments with ß-lactamases could revolutionize the treatment of this disease and other deadly diseases by providing critical information of protein dynamics. These insights could lead to the creation of inhibitors that block ß-lactamases or antibiotics that avoid the need for blockage altogether. Time-resolved serial femtosecond crystallography (TR-SFX) offers an approach to determine the uncharacterized structural intermediates of enzymes during these irreversible reactions by employing the extremely brilliant femtosecond X-ray pulses produced by free electron lasers (FELs), protein crystals on the micrometer length scale (microcrystals), and the mix-and-inject technique.

Mix-and-inject crystallography (MIC) is an emerging method being developed specifically for studying the reactions catalyzed by enzymes in which microcrystals are mixed with a substrate just prior to being probed by an ultrafast X-ray pulse. By comparison with mixing cell solution-scattering studies at synchrotrons, this new approach offers three advantages – it gives atomic resolution images, while providing adequate time resolution, and a homogeneous reaction, through the use of micron-sized crystals, throughout which diffusion of the substrate is rapid compared with the reaction time. Finally the method out-runs radiation damage by using femtosecond X-ray pulses. We present results that demonstrate time-resolved structure-based enzymology is feasible using MIC and FELs. In this TR-SFX experiment we studied the reaction of ß-lactamase microcrystals from *M. tuberculosis* mixed with a ceftriaxone antibiotic solution. Electron density maps at 2.4 Å resolution of the ß-lactamase, ceftriaxone product were determined, showing there is an additional electron density feature visible in the binding pockets of ß-lactamase. This difference electron density feature corresponds with models of ceftriaxone, demonstrating that mixing was successful. These results pave the way to further studies of irreversible enzymatic reactions and
represent an entirely new field of time-resolved structural dynamics for enzymological systems.

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Jason Stagno, National Cancer Institute

Structures of Riboswitch RNA Reaction States by Mix-and-Inject XFEL Serial Crystallography

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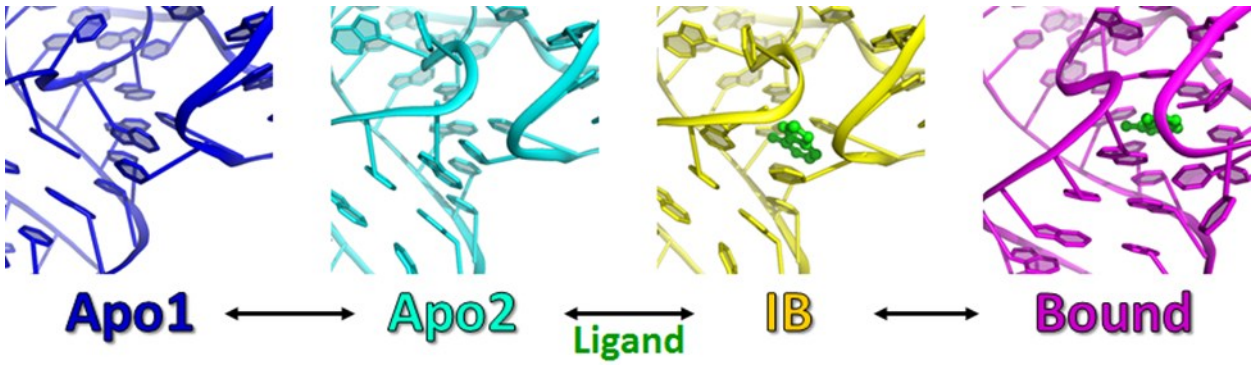
Riboswitches is RNA structural elements generally located in the 5ˈuntranslated region (3ˈUTR) of mRNA. In the genetic regulation, ligand binding to the aptamer domain of a riboswitch triggers a signal to the downstream expression platform. A complete understanding of the structural basis for this mechanism requires the ability to study structural changes over time. We apply femtosecond X-ray free electron laser (XFEL) pulses to obtain structural measurements from crystals so small that diffusion of a ligand can be timed to initiate a reaction prior to diffraction. We demonstrate this approach by determining four structures of the adenine riboswitch aptamer domain during the course of a reaction involving two apo, one ligand-bound intermediate, and the final bound states. These structures support a reaction mechanism model with at least four states and illustrate the structural basis for signal transmission. The two apo conformers differ significantly in the three-way junction and the P1 switch helix relative to the ligand-bound conformation. Our time-resolved crystallographic measurements with a 10-second delay captured the structure of an intermediate with changes in the binding pocket that accommodate the ligand. With a >10-minute delay, the RNA molecules were fully converted to the bound state, in which the substantial conformational changes resulted in conversion of the space group. Such drastic changes *in crystallo* highlight the important opportunities that micro/nanocrystals may offer in these and similar time-resolved diffraction studies. These results all together demonstrate the potential of "mix-and-inject" time-resolved serial crystallography to study biochemically important interactions between biomacromolecules and ligands, including those involving large conformational changes.



Arwen Pearson, University of Hamburg

Enabling the Time-Resolved Experiment: Developing Generally Applicable Tools for Reaction Initiation

Sans M., Monteiro D.C.F., Yorke B.A., Doyle J.J., Pearson A.R.

Hamburg Centre for Ultrafast Imaging & Institute for Nanostructure and Solid State Physics, Universitaet Hamburg, 22761 Hamburg, Germany.

Time-resolved structural biology has undergone a renaissance in recent years with the advent of high brilliance and short pulse length X-ray free electron lasers. These, as well as developments at synchrotron sources mean we now have the instrumentation to make time-resolved structural measurements over time-scales from femtoseconds to seconds. However, there are still only a handful of biological systems which have been structurally characterised with time-resolutions better than milliseconds. This is because a key requirement of any time-resolved experiment is the ability to, ideally, synchronously and uniformly initiate the biochemical reaction of interest. The most effective and fastest way to achieve this is to use short, intense laser light pulses to trigger a photochemical reaction. However, the applicability of this approach is limited by the fact that most biomacromolecules are not inherently sensitive to light. To address this challenge we are developing a range of photolabile protecting groups that can be bound to macromolecules in order to provide spatial and temporal control over reaction initiation. We are particularly interested in modifying the photocage moieties themselves to shift their absorption properties and decaging kinetics, as well as in defining new approaches to caging that do not rely on directly blocking a substrate or active site residue. Our ultimate aim is a library of light-activated compounds that will be of general use to the time-resolved structural biology community.

Nora Berrah, University of Connecticut

Time-Resolved Fullerene Ionization Dynamics Induced by Intense LCLS X-ray Pulses

Nora Berrah, Physics Department, University of Connecticut

Intense free electron lasers (FELs) have opened up new opportunities to study molecular dynamics with femtosecond temporal resolution. The detail understanding of physical and chemical changes at an atomic spatial scale and on the time scale of atomic motion is crucial for a broad range of scientific fields.

We will report on the time-resolved photoionization and fragmentation dynamics of gas phase C_{60} using intense femtosecond LCLS x-ray pulses. We will also discuss the photoionization dynamics of doped fullerenes, $Ho_3N@C_{80}$ carried out with x-rays from the LCLS [1].

This *spectroscopic* work addresses *directly* the dynamics of electronic damage, a primary limiting factor [2] induced by x-ray FELs, relevant to fs structure determination on metalloproteins [3]. Furthermore, the investigations of the ionization and fragmentation dynamics of nano-size fullerenes subjected to femtosecond strong x-ray lasers are important for understanding quantitatively [4] the mechanisms of radiation damage which will benefit the understanding of bio-imaging.

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Petra Edlund, Gothenburg University

The Dark Form Crystal Structure of a Photoactive Bacterial Phytochrome Determined by Serial Femtosecond Crystallography

Edlund. P.[1], Claesson E.[1], Takala. H[1,2,3], Henry. L.[1], Dods. R.[1], Lehtivuori. H.[4], Panman. M.[1], Pande. K.[5], White. T.[5], Nakane. T.[6], Berntsson. O.[1], Gustavsson. E.[1], Båth.P.[1], Modi. V.[7], Roy-Chowdhury.S.[8], Zook.J.[8], Berntsen. P.[9],Pandey. S.[10], Poudyal. I.[10], Tenboer. J.[10], Kupitz. C.[10], Barty. A.[5], Fromme.P.[8], Koralek J. D.[11], Tanaka. T.[12], Spence. J.[13,14,15], Liang. M.[11], Hunter. M. S.[11], Boutet. S.[11], Nango. E.[12], Moffat .K[16,17], Groenhof. G.[7], Ihalainen J. A.[18], Stojković E. A.[19], Schmidt. M.[10], and Westenhoff. S[1].

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Phytochromes are photosensory proteins ubiquitous in plants, fungi and bacteria and vital to all forms of life. They are sensitive to red and far-red light and trigger intracellular signaling cascades, which regulate many light-dependent phenotypes. The signaling is initiated by light responsive chromophore activation which induces structural changes that are propagated within the protein to the output domain, often a histidine kinase. The earliest steps in the structural activation is still not clear but serial femtosecond crystallography (SFX) enables the possibility to resolve the sub-picosecond time resolved structural evolution of these events at room temperature. As a first step, two resting state, room temperature SFX crystal structures of the chromophore binding domain (CBD), from a phytochrome from the bacterium *Deinococcus Radiodurans*, were obtained at Linac Coherent Light Source (LCLS) and SACLA at a 2.1Å and 2.2Å resolution respectively.[1] The structures are presented together with and compared to a steady state structure at cryogenic temperatures derived from conventional crystallography with the highest resolution (1.35Å) reported so far for a wild-type CBD fragment.

The investigation required efforts in optimizing crystal quality, first for conventional macro crystals and then in producing large quantities of microcrystals suitable for the two different injection systems at the two free electron lasers (XFELs). The structures at cryogenic and ambient temperatures shows overall very good agreement. However the thioether linkage between protein and chromophore is subjected to positional ambiguity in the synchrotron structure whereas it is fully resolved with SFX. These results and method development paves the way for time-resolved structural studies with SFX and are important steps in unravelling the dynamics connected with the phytochrome photocycle.

Edlund, P. et al. *The room temperature crystal structure of a bacterial phytochrome determined by serial femtosecond crystallography.* Scientific Reports 6, Article number: 35279 (**2016**) doi:10.1038/srep35279

Hasan DeMirci, Stanford PULSE Institute

Structure-Based Antibiotic Development Driven by Ambient-Temperature Serial Crystallography of Small and Large Ribosomal Subunits at High Resolution

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High-resolution ribosome structures determined by cryo X-ray crystallography have provided important insights into the mechanism of translation. Such studies have thus far relied on large ribosome crystals kept at cryogenic temperatures to reduce radiation damage. Here we describe the application of serial femtosecond X-ray crystallography (SFX) using an X-ray free-electron laser (XFEL) to obtain diffraction data from ribosome microcrystals in liquid suspension at ambient temperature. 30S ribosomal subunit microcrystals programmed with decoding complexes and bound to either antibiotic compounds or their next-generation derivatives diffracted to beyond 3.4 Å resolution. Our results demonstrate the feasibility of using SFX to better understand the structural mechanisms underpinning the interactions between ribosomes and other substrates such as antibiotics and decoding complexes. We have also collected a 3.9 Å full dataset from the dimer of large (50S) ribosomal subunit in 47 minutes of beamtime at the CXI instrument using less than 50 microliter of sample. This structure is the largest one solved to date by any FEL source to near atomic resolution (3 MDa). We expect that these results will enable routine structural studies, at near-physiological temperatures, of the large ribosomal subunit bound to clinically-relevant classes of antibiotics targeting it, *e.g.* macrolides and ketolides, also with the goal of aiding development of the next generation of these classes of antibiotics. Overall, the ability to collect diffraction data at near-physiological temperatures promises to provide new fundamental insights into the structural dynamics of the ribosome and its functional complexes.

ERIKO NANGO, SACLA

CAPTURING BACTERIORHODOPSIN STRUCTURES IN ACTION: THIRTEEN SNAPSHOTS BY TIME-RESOLVED SERIAL FEMTOSECOND CRYSTALLOGRAPHY

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Recent advent of intense, femtosecond X-ray pulses from X-ray free electron laser (XFEL) has enabled to acquire diffraction patterns from protein microcrystals before the onset of radiation damage. In serial femtosecond crystallography (SFX) with XFEL, intact microcrystals are continuous delivered with buffer or carrier media by an injector since a single-shot exposure by XFEL will destroy a tiny crystal, allowing “damage free” structure at physiological temperature. This technique is of great advantage to time-resolved experiment combined with an optical pump laser for light-sensitive proteins due to following reasons: observable ultra-fast reaction by femtosecond X-ray lasers and high photoconversion because microcrystals are easily excited.

We have performed time-resolved SFX to probe light-driven conformational changes in bacteriorhodopsin known as a light-driven proton pump on a time-scale from nanoseconds to milliseconds at the SPring-8 Angstrom Compact Free Electron Laser (SACLA). Our data reveal how an initially twisted retinal chromophore displaces a conserved tryptophan residue of helix F on the cytoplasmic side of the protein while dislodging a key water molecule on the extracellular side. The resulting cascade of structural changes throughout the protein provide unprecedented insight into how structural changes in bacteriorhodopsin conspire to achieve unidirectional proton transport.

Max Wiedorn, CFEL

Time-Resolved Serial Crystallography at FELs and 3rd Generation Synchrotron Sources

Kanupriya Pande

Time-resolved structural information of biological molecules is key to understanding the mechanism of biological functions, such as enzymatic catalysis and photo-induced signaling. Pump probe studies on light activated molecules, like photoactive yellow protein (PYP), have been successfully performed up to 100 ps time resolution at 3rd generation synchrotrons. With the availability of hard X-ray pulses on the femtosecond (fs) time scale emitted by free-electron laser (FEL) sources, the ultrafast fs to ps timescale has become experimentally accessible. In a previous experiment on PYP we demonstrated that time-resolved serial femtosecond crystallography (TR-SFX) could be successfully carried out at X-ray FELs on the nanosecond to microsecond time scales. In this talk I will discuss the results of TR-SFX experiments on PYP covering the time range from 100 fs to 3 ps, and identify the structural changes associated with the earliest steps in the trans-to-cis isomerization of the chromophore. I will also briefly talk about recent developments on enzymatic reactions using mix-and-inject serial crystallography at PETRA III.