Ponce, Puerto Rico

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BioXFEL 2ND INTERNATIONAL CONFERENCE

PAUL WYN JENNINGS, NSF

HUMAN INTELLECTUAL CAPITAL: THE LEGACY OF THE BIOXFEL STC

The scientific research in this project is outstanding and will derive results that will reverberate around the scientific world for decades if not longer. Further, the results are likely to impact Society in a profound manner.

Results are generally assumed to be those coming from laboratories and computational confines. However, there is another, often silent, result that is derived from the same confines and can have a legacy that exceeds the former. These results are in human intellectual capital (i.e., the graduates from the program). This legacy perpetuates itself for long periods of time and expresses itself in an ever broadening scope.

Yes, the graduates derived from this project are receiving a marvelous education and will carry their knowledge forward. However, can you enhance their knowledge base beyond the norm to make them much better than the norm? If not, it will be a marvelous opportunity lost.

BIOGRAPHY

Dr. Jennings is the Program Director for the National Science Foundation's Education & Human Resources and Research on Learning in Formal and Informal Settings. He has been involved in Education Initiatives with the NSF since 2005. He earned a BA in Chemistry from the University of Colorado, a Ph.D. in Organic Chemistry from the University of Utah, and was a Postdoctoral Fellow at the California Institute of Technology. Dr. Jennings has taught courses in Organic, Inorganic, and Organometallic Chemistry at Montana State University. He has published over 60 scientific articles and authored more than 40 successful grants. He is a proud recipient of the Japan Society Award for Promotion of Science, Sigma Xi Award for Excellence in Research, The Charles and Nora L. Wiley Faculty Award for Meritorious Research, and named Visiting Scholar of the University of Washington.

Wednesday January 14th

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LEO CHAVAS, CFEL

POSSIBLITIES FOR SERIAL FEMTOSECOND CRYSTALLOGRAPHY AT FUTURE LIGHT SOURCES

Serial femtosecond crystallography (SFX) uses x-ray pulses from free-electron laser (FEL) sources to outrun radiation damages and thereby overcome long-standing limits in the structure determination of macromolecular crystals. Intense x-ray FEL pulses allow the collection of damage-free data at room temperature, and give the opportunity to study highly time-resolved reactions including irreversible events. This method may open the way to understanding the structure of biological molecules that fail to crystallize readily into large crystals.

By taking full advantage of a high repetition rate x-ray pulse delivery scheme, protein structures could be determined in just minutes of measurement time. Automation in sample delivery during SFX experiments could therefore induce a turnover of samples much higher than at today's third generation synchrotrons and automated macromolecular crystallography beamlines, as no crystal alignment or complex robotic motions are required. New challenges would emerge from this enormous amount of data that could be recorded at such facilities, at all levels of the experiment, from the beam delivery to the data management, through the sample environment and injection process.

Recently, a user consortium for SFX experiments (SFX-UC) has received funding from a number of sources to deliver instrumentation for enabling a fully automated SFX end-station. The development of the SFX-UC contributed instrumentation comes in complement of already scheduled efforts to deliver instruments for structural studies of biological materials, further adding to some extent the output of the FELs for structural biology and directly contributing to enabling more science with the unique properties of these future light sources.

EVGENY SALDIN, DESY

PERSPECTIVES OF IMAGING OF SINGLE PREOTEIN MOLECULES WITH THE PRESENT DESIGN OF THE EUROPEAN XFEL

The Single Particles, Clusters and Biomolecules (SPB) Instrument at the European XFEL is located behind the SASE1 undulator, and aims to support imaging and structure determination of biological specimens between about 0.1 µm and 1 µm in size. The instrument is designed to work at photon energies from 3keV up to 16keV. This wide operation range is a cause for challenges to the focusing optics. In particular, a long propagation distance of about 900m between X-ray source and sample leads to a large lateral photon beam size at the optics. The beam divergence is the most important parameter for the optical system, and is largest for the lowest photon energies and for the shortest pulse duration (corresponding to the lowest charge). Due to the large divergence of nominal X-ray pulses with duration shorter than 10 fs, one suffers diffraction from mirror aperture, leading to a 100-fold decrease influence at photon energies around 4keV, which are ideal for imaging of single biomolecules. The nominal SASE1 output power is about 50 GW. This is very far from the level required for single biomolecule imaging, even assuming perfect beamline and focusing efficiency. Here we demonstrate that the parameters of the accelerator complex and the SASE1 undulator offer an opportunity to optimize the SPB beamline for single biomolecule imaging with minimal additional costs and time. Start-to-end simulations from the electron injector at the beginning of the accelerator complex, up to the generation of diffraction data, indicate that one can achieve diffraction without destruction with about 0.5 photons per Shannon pixel at near atomic resolution with 10^{13} photons in a 4 fs pulse at 4keV photon energy and in a 100 nm focus, corresponding to a fluence of 10^{23}ph/cm^{2}. This result is exemplified using the RNA Pol II molecule as a case study.

FRANZ X. KÄRTNER, CFEL

LARGE-SCALE AND LONG-TERM STABLE TIMING DISTRIBUTION FOR FREE-ELECTRON LASERS

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We will review recent progress in scaling of rf-guns and accelerators towards the THz operating regime. This opens up new approaches towards compact coherent X-ray sources. Experimental results on efficient THz generation with lasers, THz driven guns and accelerators will be discussed and estimates for photon flux and brightness of a coherent X-ray source based on this technology will be presented.

MARIUS SCHMIDT, UWM

TIME-RESOLVED NANOCRYSTALLOGRAPHY OF PHOTOACTIVE YELLOW PROTEIN

J. Tenboer, S. Basu, N. Zatsepin, K. Pande, D. Milathianaki, M. Frank, M. Hunter, S. Boutet, G. Williams, J. E. Koglin, D. Oberthuer, M. Heymann, C. Kupitz, C. Conrad, J. Coe, S. Roy-Chowdhury, U. Weierstall, D. James, D. Wang, T. Grant, A. Barty, O. Yefanov, J. Scales, C. Gati, C. Seuring, V. Srajer, R. Henning, P. Schwander, R. Fromme, A. Ourmazd, K. Moffat, J. Van Thor, J. H. C. Spence, P. Fromme, H. Chapman, H. Ihee, N. Purwar, S. Tripathi, M. Schmidt

Time-resolved crystallography unifies structure with kinetics. The molecular structures of reaction intermediates as well as the temporal evolution of the associated concentrations can be extracted from a time-series of difference electron density maps alone. Powerful synchrotron beamlines such as BioCARS at the Advanced Photon Source (APS) make the collection of these time-series possible. Recent experiments with Photoactive Yellow Protein (PYP) demonstrate that *multiple* time series can be collected swiftly. An additional experimental parameter such as the temperature can be varied to extract new, hitherto inaccessible, information on the macromolecular reaction. Crystallography becomes 5-dimensional. Time-resolved serial femtosecond crystallography (TR-SFX) at an X-ray Free Electron Laser (FEL) such as the Linac Coherent Light Source (LCLS) has several advantages. (i) Small nano- and micro-crystals are utilized that can be easily and uniformly excited: laser pulses can be used to initiate reactions in photo-reactive proteins such as PYP, and reactions in enzymes may be started by diffusion of substrate. (ii) Cyclic reactions as the one in PYP and non-cyclic reaction such as those catalyzed by enzymes are conceptually on the same footing. According to the 'diffract-before-destroy' principle each tiny crystal is exposed to only one X-ray pulse and then discarded. (iii) The ultra-short, femtosecond X-ray pulses provide access to ultrafast time-scales beyond the pulse-limitations at the synchrotron. We show here the first difference maps determined from TR-SFX on PYP at beamline CXI at the LCLS. These results will pave the way to exciting, new experiments with photoreceptors and enzymes at X-ray FELs.

UWE WEIERSTALL, ASU

SERIAL CRYSTALLOGRAPHY IN LIPIDIC CUBIC PHASE

Uwe Weierstall[1], Daniel James[1], Dingjie Wang[1], John C.H. Spence[1], Petra Fromme[2], Vadim Cherezov[4]
[1] Arizona State University, Department of Physics, USA
[2] Arizona State University, Department of Chemistry and Biochemistry, USA
[4] Dept. of Molecular Biology, The Scripps Research Institute, USA

The progress in structure determination of Membrane Proteins and GPCRs in particular is limited by the difficulty of growing sufficiently large crystals for collecting high-resolution diffraction data at synchrotron microfocus beamlines. Ultimately the achievable resolution from small, well ordered protein crystals is limited by radiation damage. Serial femtosecond crystallography (SFX) at an XFEL has been shown to achieve atomic resolution from microcrystals in a liquid jet. This approach obviates the need for larger crystals and greatly reduces or even eliminates radiation damage, addressing the most significant barriers to high-resolution structure determination success with small crystals. Protein crystals grown in lipidic cubic phase (LCP) often have limited size and are therefore ideal for SFX experiments. Unfortunately, the GDVN-injector developed for SFX can only be used with low viscosity liquids. Therefore a new approach was developed to generate a stream of high viscosity liquid with tens of micrometer diameter. The injector has been used successfully at the LCLS to solve structures of several membrane proteins in LCP. It has also been used at synchrotron microfocus beamlines and the use of other high viscosity liquids is being explored. We present an overview of SFX experiments in LCP.

BRENDA HOGUE, ASU

XFEL DIFFRACTION OF ICOSAHEDRAL VIRUS NANOCRYSTALS FOR HIGH RESOLUTION STRUCTURE DETERMINATION

Brenda G. Hogue[1,2], Robert Lawrence[1,2], Shibom Basu[1,4], Haiguang Liu[3], Shatabdi Roy Chowdhury[1,4], Daniel James[3], Garrett Nelson[3], Sebastien Boutet[7], Petra Fromme[1,4], Edward Snell[6], Vijay S. Reddy[5]
[1]Biodesign Institute, [2]School of Life Sciences, [3]Departments of Physics and [4]Chemistry and Biochemistry, Arizona State University (ASU), Tempe, AZ; [5]Department of Integrative Structural and Molecular Biology, The Scripps Research Institute, La Jolla, CA; [6]Department of Structural Biology. State University of New York, Buffalo, NY; [7]Linac Coherent Light Source, SLAC National Accelerator Laboratory, Menlo Park, CA

Icosahedral virus structures represent some of the largest biomolecules studied by X-ray crystallography. Large dimensions of these molecules and the corresponding unit cells pose challenges with respect to obtaining high-resolution diffraction. During a recent screening beam time at the LCLS, we obtained diffraction to 2.5Å resolution from nanocrystals (< 20um) of empty virus-like particles (eVLPs) of Cowpea Mosaic Virus (CPMV) that are devoid of RNA. CPMV is a plant virus of ~30nm in diameter with *pseudo* T=3 icosahedral symmetry that is characteristic of picornaviruses, a large group of RNA viruses that also includes many important human and animal pathogens. CPMV eVLPs are being used for vaccine development, in vivo imaging, drug delivery and other nanotechnology applications. Since eVLPs of CPMV do not contain any RNA, the internal volume is available for a “payload” for delivery of drugs, or other molecules. The crystal structure of the infectious form of CPMV (whole virus crystallized at pH 8.0) is determined to 2.8Å resolution. Interestingly, the eVLPs of CPMV particles were crystallized in low pH 4.8, but not at pH 8.0. These results lay important groundwork for determining structures of other medically and environmentally relevant viruses using SFX. Continuing studies aim to determine the high resolution CPMV-eVLP structure, which will perhaps be the first virus capsid structure to be determined, employing serial femtosecond crystallography methods. This will allow us to compare the results with structures obtained using the conventional synchrotron sources.

BELINDA PASTRANA, UPR

AN ACCURATE AND REPRODUCIBLE ANALYSIS FOR PROTEIN AGGREGATION

Belinda Pastrana-Rios[1] and Aslin Rodríguez Nassif[1]

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One potential factor that may be responsible for the existing bottleneck in protein crystallization is aggregation. The presence of protein aggregates can hamper the success of crystallization and therefore a need exists in: **(1)** The evaluation of the protein sample prior to setting up the crystallization experiment, to obtain assurance of the high quality protein in an aggregate free state as a starting condition. **(2)** The evaluation of different buffer conditions that may induce unwanted type of aggregates which will not lead to an ordered oligomer that may crystallize. Currently, techniques such as dynamic light scattering, micro flow imaging (MFI), or light obscuration are being employed, yet they provide limited information only about the size of the aggregate without addressing the identity of the aggregate or the extent of the aggregation. We have developed a molecular biophysical approach which has been granted a USPTO patent using 2D IR correlation spectroscopy to determine the sequential order of events of the aggregation process. Consequently, this method also allows for the determination of the relative stability of the protein and the extent of aggregation simultaneously without the use of probes, resulting in a complete evaluation of the nature of the aggregate. The method also allows for the evaluation of reversibility of the process at the molecular level. Three case studies will be presented for high quality human recombinant proteins. The first is IgG2a, since most of the protein therapeutics being designed involve this type of molecule. Second, is the evaluation of the relative stability of related calcium-binding proteins known as centrins belonging to the EF-hand superfamily, currently comprised of more than 1,000 proteins within the human genome. Third is a Krr1 fragment comprised of a ssDNA binding site in the presence and absence of an excipient. We are convinced that this approach and its automated version currently being developed can contribute to the development of a rational process for protein crystallization.

Project supported by: Henry Dreyfus Teacher Scholar Award (BPR), R25 GM088023 (BPR, ARN)

RICHARD KIRIAN, ASU

IMPROVING AEROSOL INJECTORS FOR SINGLE-PARTICLE DIFFRACTIVE IMAGING

Richard Kirian-[1,2], Niko Eckerskorn[3], Salah Awel[2], Andrei Rode[3], Jochen Küpper[2], Henry Chapman[2]

1) Arizona State University

2) Center for Free-Electron Laser Science, DESY and Universität Hamburg

3) Australian National University

XFEL-based single-particle imaging methods aim to overcome the challenge of forming biomolecular crystals, which is often the critical bottleneck in structure discovery. There are several challenges associated with this goal: data-analysis algorithms must contend with the reality of imperfect detector response, background signal that evolves with time, and variations in the incident illumination. Progress is being made on all three of these fronts. In addition, experiments often suffer from low hit rates, the fraction of x-ray pulses that intercept a particle, and sample delivery efficiency, the fraction of particles that are intercepted. This problem can be severe for sub-micrometer-focused x-rays, where hit rates often fall below 0.1%, and delivery efficiency orders of magnitude lower. As a result, the scope of targets that are amenable to single-particle experiments is limited to those that can be obtained in high abundance. Some of the challenges that result from sample heterogeneity can be traced to sample delivery efficiency. In this presentation I will discuss some of the challenges associated with gas-phase particle injection for XFEL diffraction experiments. I will then describe our ongoing efforts to improve the present situation, which includes the implementation of aerosol-beam diagnostics, new gas-dynamic aerosol injectors, and our investigation of the use of optically induced forces for precision manipulation of particles.

Thursday January 15th

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LARS REDECKE, U OF HAMBURG & LÜBECK

IN VIVO PROTEIN CRYSTALLIZATION AND X-RAY FREE ELECTRON LASERS: NEW STRATEGIES FOR STRUCTURAL BIOLOGY

L. Redecke[1], M. Klinge[1], R. Schönherr[2], D. Rehders[1], D. Oberthür[3,4], B.P. Sommer[4,5], J. Martinez-Costas[6], R. Duden[2], M. Duszenko[5], C. Betzel[4]

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Crystal structure determinations of biological macromolecules are limited by the availability of sufficiently sized crystals and by the fact that crystal quality deteriorates during data collection due to radiation damage. Unfortunately, growing well-ordered crystals sufficiently large to efficiently diffract X-rays while withstanding radiation damage can be often difficult. Exploiting the ‘diffraction-before-destruction’ paradigm by using highly brilliant XFEL pulses of a few femtoseconds duration, serial femtosecond crystallography (SFX) has already shown it can overcome resolution limits imposed by radiation damage at conventional sources, allowing serial diffraction data collection from protein crystals down to the nanometer regime [1,2]. The validation of this XFEL-based approach turns *in vivo* grown crystals into valuable targets for structural biology, as we have recently shown [3,4]. If successful, spontaneous protein crystallization within living cells abolishes the need for time-dependent optimization of recombinant protein production and crystallization strategies. This process has been observed several times in nature so far, but *in vivo* crystal growth can also occur during gene over-expression, as we have particularly discovered in baculovirus-infected insect cells [3]. As a proof-of-principle, we collected single crystal diffraction pattern of cathepsin B and inosine monophosphate dehydro-genase, both from the parasite *Trypanosoma brucei*, applying SFX techniques at an XFEL, which yields high-resolution structural information of the associated proteins [3,4]. So far, the cellular mechanisms involved in the *in vivo* crystallization process remain to be understood, preventing a more successful application of this novel approach. Thus, our research aims at identifying the parameters that are crucial for optimal crystal growth within insect cells. Combining confocal microscopy with live-cell imaging techniques and compartment-specific staining methods, we systematically investigated the impact of the intracellular environment on *in vivo* crystallization by directing recombinant proteins into different cellular compartments using specific signal sequences. Moreover, the impact of cellular transport mechanisms and induced cellular stress on the quality and size of *in vivo* crystals was investigated in detail. The results will provide important insights into the process of protein crystallization within living cells and will therefore significantly contribute to increasing the success rate for spontaneous crystal growth of other proteins. Considering that *in vivo* crystals represent highly suitable targets for structural biology particularly at XFELs, this approach offers exciting new possibilities for proteins that do not form crystals suitable for conventional X-ray diffraction *in vitro*.

[1] H. Chapman *et al*. *Nature* (2011) **470**, 73-77.
[2] S. Boutet *et al*. *Science* (2012) **337**, 362-364.
[3] R. Koopmann*, K. Kupelli*, L. Redecke* *et al*. *Nat. Methods* (2012) **9**, 259-262.
[4] L. Redecke*, K. Nass* *et al*. *Science* (2013) **339**, 227-231.

BILL WEIS, STANFORD UNIVERSITY

XFEL DATA COLLECTION AND PROCESSING USING FEWER CRYSTALS

Femtosecond X-ray free-electron laser (XFEL) pulses enable the possibility of obtaining crystal structures free from radiation damage, which could be a decisive advantage in solving challenging macromolecular crystal structures. Because the illuminated volume diffracts and is destroyed within a single XFEL pulse, and is effectively stationary during the exposure, "still" diffraction patterns containing only partial reflections are obtained. To date, most structures solved from XFEL data have used a "Monte Carlo" method in which a large number of measurements of a given reflection are summed and averaged. This method requires a very large number of crystals to insure sufficient multiplicity, which could preclude the study of many systems, such as integral membrane proteins and large macromolecular complexes, which are difficult to prepare in sufficient quantity. We have developed a post-refinement procedure in which the partiality of each measurement is estimated and used to correct it to its full Bragg intensity. We have applied it to three data sets, two measured from frozen crystals mounted on a standard goniometer/detector setup at the LCLS XPP station, and the third from an electrospin injector setup at LCLS CXI. In all three cases we find that this procedure gives more accurate data, as assessed by several metrics, better electron density maps, and better models with significantly lower R/Rfree values. Importantly, we show that using this method complete and accurate data sets can be obtained from a very limited number of images. These findings demonstrate that it is feasible to measure complete XFEL data from systems where the number of crystals is limited.

GEBHARD SCHERTLER, ETH

2D CRYSTALLOGRAPHY AND PROGRESS TOWARDS UNDERSTANDING FUNDAMENTAL PROTEIN DYNAMICS

Membrane protein structure determination is dependent on the most advanced diffraction methodologies. The application of X-ray Free Electron Lasers (FEL) is developing rapidly. PSI has engaged in several successful beamline proposals at LCLS, the FEL in Stanford. The teams have developed solid support structures and were able to measure and evaluate diffraction from two-dimensional protein crystals. Only nanograms of crystals were necessary for these screening experiments. We are able to study very fast protein dynamics with wide angle scattering experiments (WAX) a consortium led by Neutze and Schertler carried out measurements with the visual pigment rhodopsin and the consortium observed very fast changing protein features between 5 and 15 picoseconds. Together with several international groups we have used fast liquid jets for serial femtosecond protein crystallography. For membrane proteins a PSI led team has explored slow material saving gel jets. They have carried out pump probe experiments on retinal protein crystals using femtosecond pump lasers. With fast jets and slower lipidic cubic phase (LCP) jets. All these methods will in the future enable us to study membrane protein dynamics in detail. The dynamics of membrane protein loops are important for the on and off rate of ligand binding. Therefore membrane protein dynamics will become more and more important for drug design projects.

SOICHI WAKATSUKI, LCLS

XFEL DATA COLLECTION AND PROCESSING USING FEWER CRYSTALS

A strategy for developing an accelerator based integrative bioscience at SLAC will be presented. The key for further progress in biological research is the successful integration of different methods with multiple length- and time scales. The goal of the SLAC Bioscience strategy is to establish an accelerator based Bioscience Hub for structural biology research using protein crystallography, spectroscopy, small angle scattering and imaging at LCLS and SSRL, and in the future, new core expertise on CryoEM/UEM/UED and super-resolution light microscopy. In parallel, several science areas will be identified and developed as science spokes at the Hub focusing on environmental/bioenergy and biomedical science areas including a structure-based drug discovery platform.
The second part of this presentation will focus on the two new developments for macromolecular crystallography using XFEL beams: (1) two-color beam for *de novo* phasing and (2) a new LCLS station, the Macromolecular Femtosecond Crystallography (MFX). Results of the recent LCLS experiment using two-color doubly self-seeded pulses for experimental *de novo* phasing will be presented. The MFX station project has been launched in mid April with a two-year timeline and will be optimized for in-air data collection with a variety of sample delivery schemes. Finally, efforts and progress towards building a roadmap for single particle imaging using XFELs will be described. These projects are being pursued in collaboration with many groups locally and globally with a goal to provide integrated general user facilities as part of the SLAC Bioscience Strategy for cutting edge structural biology research.

AXEL BRUNGER, STANFORD UNIVERSITY

TOWARDS XFEL CRYSTALLOGRAPHY FOR CHALLENGING BIOLOGICAL SYSTEMS

Departments of Molecular and Cellular Physiology, Neurology and Neurological Sciences,
Structural Biology, and Photon Science, Stanford University, Howard Hughes Medical Institute, Stanford, CA, USA

X-ray diffraction plays a pivotal role in revealing atomic structures of macromolecules and their complexes. Since crystals of large complexes or membrane proteins often diffract weakly, new experimental tools and computational methods need to be developed. For example, the X-ray free electron laser (XFEL) at the Linac Coherent Light Source (LCLS) produces ten orders of magnitude higher peak photon brilliance, compared to third generation light-sources. XFELs circumvent radiation damage by collecting a diffraction image before the molecular structure is affected by radiation damage. These experimental advances are complemented by new computational methods. As an example, the DEN (Deformable Elastic Network) refinement method uses reference model restraints, and allows global and local deformations of this reference model. Cross-validation with R_{free} determines the optimum deformation and influence of the reference model. The method improves a model refined at low resolution, as assessed by coordinate accuracy, the definition of secondary structure, and the quality of electron density maps. Examples will be discussed where XFELs and DEN refinement played a role in determining difficult crystal structures of biological interest.

BioXFEL 2ND INTERNATIONAL CONFERENCE

BRIAN ABBEY, UNIVERSITY OF MELBOURNE

UNDERSTANDING THE EFFECTS OF ELECTRON DYNAMICS AND STRUCTURAL DISORDER IN PROTEIN CRYSTALLOGRAPHY

Brian Abbey[1,*], Hannah Coughlan[1], Victor Streltsov[3], Ruben Dilanian[2], Nicholas Phillips[1], Keith Nugent[1], Harry Quiney[1], Connie Darmanin[1,ǂ]

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The availability of extremely bright sources of coherent X-rays such as the recently developed hard X-ray Free Electron Laser (XFEL) at Stanford are creating entirely new fields of research across all major disciplines. Femtosecond nanocrystallography, for example, is rapidly maturing into a major new technique for structural biology, enabling high-resolution structural data to be collected from nanocrystals which are too small to measure using conventional X-ray sources. As increasing amounts of data become available however, the need to understand and conduct research into the effects of non-equilibrium electron dynamics and structural disorder is ever more apparent.

Here we present recent results of imaging and diffraction experiments carried out at both XFELs and third generation synchrotrons. Experimental data have been collected from the Coherent X-ray Imaging (CXI) endstation at the LCLS - XFEL and beamline 34-ID-C at the Advanced Photon Source (APS). We find that the XFEL data contains evidence of electronic and structural disorder whilst the synchrotron data provides a window into the elastic strain properties of protein crystals that can influence the structure retrieval. Applying both crystallographic analysis and coherent imaging approaches to these data provide new insights into these effects, the results of which will be presented here. A more detailed understanding of how such phenomena influence the formation of crystallographic diffraction patterns will benefit both serial femtosecond nanocrystallography experiments as well as more conventional crystallography experiments at third generation synchrotron sources.

ANTHONY HUTCHINSON, BIOXFEL

RESEARCH AND EDUCATIONAL OPPORTUNITIES WITH BIOXFEL

Despite a century of progress using X-ray crystallography to study macromolecular structures, many important targets remain uncharacterized and little has been accomplished to visualize molecular movements. X-ray free electron lasers (XFELs) promise to break the barriers that keep us from attacking these problems. XFELs produce a stream of x-ray pulses tens of femtosecond in duration. Each pulse is so short and so intense that it can produce an observable scattering pattern from a nanometer-sized crystal before the sample is vaporized. Because the technology is so new, much work is needed to harness its capabilities for biological research. The BioXFEL Science and Technology Center is a consortium of scientists and students with backgrounds in physics, chemistry, biology, mathematics, and computer science working to devise new methods for conducting biological experiments using XFELs. How can nanocrystals be grown, detected and manipulated reliably? How can we deliver these samples to the XFEL beam? How does damage imparted by the beam to the sample affect the resulting image? How can we decode the image to understand the underlying molecular structure? How can XFELs be used to gain new insights on biological processes like photosynthesis or gene transcription? The answers to these questions will generate new techniques for visualizing the structures of single molecules and for creating “movies” of molecules in action during chemical reactions. A new workforce of specialized scientists is needed to carry this work into the next generation, and BioXFEL is committed to fostering the emergence of this group. Our graduate students prepare for leadership roles in the XFEL field at top American research universities, including the University at Buffalo, Arizona State University, University of Wisconsin-Milwaukee, Cornell, Stanford, Rice and the University of California-San Francisco. BioXFEL brings this geographically separated group together through regular in-person workshops as well as webconference colloquia for discussing XFEL-related publications. We prepare undergraduate students to enter our graduate programs through summer internships wherein they conduct research in XFEL-related areas and receive advanced training in experimental techniques and scientific communications. We envision that our students’ future careers will account for groundbreaking discoveries illuminating fundamental life processes, insights on human health, and new forms of clean, renewable energy. This session will introduce undergraduates to the channels for joining BioXFEL, with short research presentations provided by current BioXFEL graduate students.

DRAGANA BRZAKOVIC & JOHN COZZENS, NSF

RESEARCH PROPOSAL PREPARATION 101

This presentation will focus on the important do’s and don’ts of proposal preparation. It will show you how to search for funding opportunities, and things to consider when selecting an idea around which you plan to build a research proposal. Additional tips will include how to identify/seek help, learning more about the proposal review process, and learning how to use/benefit the feedback received from a declined proposal. Participants will have an opportunity, if interested, to have a follow-up ``hands on`` experience of preparing an abbreviated proposal and getting feedback.

ALI DASHTI, UWM

FRONTIERS IN CONFORMATIONAL MAPPING OF MOLECULAR MACHINES

Structural heterogeneity is pervasive. For example, the observation of a series of objects in reaction or interaction naturally leads to a collection of snapshots from non-identical objects. A primary goal of single-particle imaging is to gain access to the structural changes underlying the function of molecular machines. Recent advances in cryogenic electron microscopy (cryo-EM) have made it possible to map single-particle structure at the 0.3-0.5nm level, where the secondary structure of biological entities can be directly resolved. Using experimental cryo-EM snapshots of the ribosome as an example, I describe a new algorithmic platform for determining the energy landscape of molecular machines and mapping their continuous conformational changes along any chosen trajectory in the energy landscape, without recourse to timing information, supervision, or templates. The same approach can be applied to XFEL snapshots not dominated by extraneous factors.

* In collaboration with R. Fung, A. Hosseinizadeh, A. Ourmazd, P. Schwander, and J. Frank et al.

MAX HANTKE, UPPSALA

STUDIES ON SAMPLE HETEROGENEITY FROM THOUSANDS OF SINGLE-PARTICLE DIFFRACTION PATTERNS

Structural heterogeneity interferes with crystallization and causes systematic gaps in structural biology. Ultra-intense femtosecond pulses from X-ray lasers permit solving structures without crystals.[1,2] Every diffraction pattern is a unique structure measurement and high-throughput flash-diffractive imaging allows sampling the conformational space of heterogeneous structures. We demonstrate this in an experiment on carboxysomes.[3,4] Carboxysomes are heterogeneous, polyhedral cell organelles that facilitate 40% of Earth's carbon fixation.[5] A new aerosol sample injector allowed us to record 70,000 low-noise diffraction patterns in 12 minutes at the 120 Hz repetition rate of the LCLS. The diffraction data shows that the size distribution is preserved during injection. We computationally separate different structures directly from the diffraction data, automate phase retrieval, improve resolution, and avoid reconstruction artefacts. These advances lay foundations for accurate, high-throughput studies on structure and structural heterogeneity in biology and elsewhere.

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CHRISTOPH BOSTEDT, LCLS

THE LCLS SINGLE PARTICLE IMAGING INITIATIVE

Andy Aquila and Christoph Bostedt

Imaging single objects with single intense x-ray pulses is one of the visionary applications for free-electron laser sources. Since the start of LCLS operations in 2009, many imaging experiments following different strategies have been performed and the resolution of single-shot experiments obtained has been constantly pushed to a few ten nanometers. However, it has also become clear that for the ultimate goal of atomic resolution many technical and scientific hurdles have to be overcome. In 2014 LCLS has launched the Single Particle Imaging initiative in order to identify and solve the main challenges for reaching atomic resolution in single particle imaging experiments.

In my talk I will first present an overview of the very diverse imaging results obtained at LCLS so far, ranging from biological to physical and materials science. I will then describe the LCLS Single Particle Imaging initiative and introduce the four target areas of 1) Sample damage, 2) Algorithm development, 3) Instrumentation, and 4) Sample characterization and delivery that were identified in the workshops. I will conclude the talk with an outlook at the first steps to be taken within dedicated beamtimes at LCLS in spring and summer 2015.

Friday January 16th

A National Science Foundation Science and Technology Center

NICK SAUTER, LBNL

XFEL DATA PROCESSING FOR GOOD AND BAD CRYSTALS

Femtosecond-scale XFEL pulses can produce diffraction free from radiation damage, under functional physiological conditions where reaction dynamics can be studied for systems such as photosystem II. However, deriving accurate structure factors from a large number of crystals (10^3-10^5) presents numerous issues: 1) the resolution limit varies widely over the ensemble of crystals; 2) crystal orientations are difficult to determine accurately in the absence of rotation data; 3) multi-tiled detectors require very accurate spatial calibration to enable data reduction; and 4) sample injectors often deliver more than one crystal to the beam for each shot. Processing algorithms in *cctbx.xfel* and *DIALS* are adapted to account for these effects. Data are integrated with well-conforming descriptions of the Bragg spot shape and crystal mosaicity. Structure factor accuracy is improved by carefully discriminating between image pixels known to contain diffraction signal and the surrounding pixels containing only background noise, and by extending post refinement techniques that lead to a better crystal orientation. It is hoped that these developments will make it easier to measure small structure factor differences, such as those from anomalous scattering that will enable the *de novo* determination of macromolecular structure.

JOE LUFT, HAUPTMAN-WOODWARD INSTITUTE

AN INVESTIGATION OF THE FORMATION, FREQUENCY, AND EXCLUSIVITY OF SUBMICRON CRYSTALS AMIDST PROTEIN CRYSTALLIZATION ASSAYS

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X-ray crystallography requires crystals for structure determination; therefore crystallization screening assays occur at the earliest stages of the structure-determination pipeline. Increasing the success of this assay can lead to a corresponding increase in structural output. Improvements in crystallization assays include better crystallization methods, and more sensitive means to identify crystals. We describe the use of the latter, Second Harmonic Generation (SHG) and Ultraviolet Two-Photon Excited Fluorescence (UVTPEF) imaging to rapidly and very effectively detect submicron crystals produced from a high-throughput, chemically diverse 1536 cocktail assay. The combination of these methods can detect and verify chiral protein crystals that are visually obscured, or too small to detect by microscopy. The ability to rapidly detect submicron crystals identifies a greater number of crystallization conditions for individual proteins, with the potential to identify crystallization conditions for a greater number of proteins where the initial crystallization hits would otherwise be missed. It is important to note that this technology pipeline to identify submicron crystals in a rapid manner makes it possible to study their growth. These submicron crystals are valuable for time-resolved studies and it has been argued are of higher overall diffraction quality than larger crystals. Our initial data suggests that using a chemically well-designed assay, when submicron crystals are detected from conditions of high supersaturation, there are oftentimes visually detectable crystals in complementary conditions at lower levels of supersaturation. A simple protocol for the optimization of submicron crystals has been developed and tested to produce larger crystals when they are required for biophysical characterization.

SANG-KIL SON, CFEL

PHASING WITH ELECTRONIC RADIATION DAMAGE AT HIGH X-RAY INTENSITY

X-ray free-electron lasers (XFELs) show promise for revealing bio-macromolecular structure using serial femtosecond crystallography (SFX), but the associated phase problem remains largely unsolved. Many *ab initio* phasing methods with synchrotron radiation employ anomalous scattering from heavy atoms, for example, multiwavelength anomalous diffraction (MAD). Because of the extremely high intensity of XFELs, samples experience severe and unavoidable electronic radiation damage, especially to heavy atoms, which hinders direct implementation of those phasing techniques with XFELs. In this talk, I will present a theoretical model of electronic radiation damage dynamics during intense x-ray pulses. The XATOM toolkit is used to simulate electronic radiation damage dynamics - detailed x-ray ionization and relaxation dynamics - of heavy atoms. Then, I will discuss how to turn x-ray multiple ionization at high intensity into an advantage for SFX. It will be demonstrated that a key equation for MAD in the high-intensity regime exists in spite of the high degree of ionization and that novel high-intensity phasing methods are achievable utilizing the high degree of ionization. I will also discuss challenges towards new high-intensity phasing methods in SFX experiments.

LORENZO GALLI, UNIVERSITY OF HAMBURG

HIGH INTENSITY PHASING

It has been predicted that, while exposed to FEL radiation, a crystalline sample experiences severe electronic damage before coulomb explosion takes place. As a consequence, the scattering strength of the atoms contained in the protein crystal will be reduced due to loss of electrons. The heavy atoms in particular are more affected by the electronic damage, and their absorption edges at high intensity are shifted towards higher energies. This ionization process can prevent the use of standard anomalous methods at FEL sources, but it could also create the condition of a new *de novo* phasing approach that utilizes the specific electronic damage of the heavy atoms. A moderate reduction of the scattering signal has been experimentally observed on a high Z species inside a chemically modified protein and on sulfur atoms in a native protein. By accurately sorting the data according to the impinging photon flux, one could maximize the effective ionization of the heavy atoms, and retrieve the coordinates of the damaged substructure, as the first step to high intensity phasing methods.

CHRIS KUPITZ, ASU

TIME-RESOLVED FEMTOSECOND NANOCRYSTALLOGRAPHY OF PYP/PSII

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Membrane proteins are extremely difficult to crystallize, however they are highly important proteins for cellular function. Photosystem I, one of the most complex membrane proteins solved to date, took more than a decade to have the structure solved to molecular resolution. Large, well-ordered crystal growth is a major bottleneck in the structural determination of proteins by x-ray crystallography, due to the difficulty of making the “perfect” crystal. The development of femtosecond nanocrystallography, which uses a stream of fully hydrated nanocrystals to collect diffraction snapshots, effectively reduces this bottleneck (Chapman, *Nature*, **470**, (2011)).

Photosystem II changed our biosphere via splitting water and evolving oxygen 2.5 billion years ago. Using femtosecond nanocrystallography we are developing a time-resolved femtosecond crystallography method (Aquila, *Optics Express,* **20**, 2706 (2012)) to unravel the mechanism of water splitting by determining the conformational changes that take place during the oxygen evolution process. Multiple crystallization techniques were originally developed in order to make the nanocrystals necessary for femtosecond nanocrystallography. For Photosystem II nano/microcrystals, a free interface diffusion method is used to increase yield over traditional methods. Here we will discuss the crystallization techniques which we have developed, primarily using Photosystem II.

JUNKO YANO, LBNL

BIOLOGICAL X-RAY SPECTROSCOPY COMBINED WITH CRYSTALLOGRAPHY AT XFELS

Biological crystallography and spectroscopy are complementary methods that contribute to understanding the structure and function of enzymes. The free electron laser facility, the Linac Coherent Light Source (LCLS) at Stanford, has made possible the study of biological molecules at room temperature in real time, using both methods. We are designing various X-ray spectroscopic detection schemes that are suitable for collecting time-resolved data at XFELs from metalloenzymes, in addition to crystallographic data collection. [1-6] Such data sets will provide an unprecedented combination of correlated data between the entire protein and the co-factors, all of which are necessary for a complete understanding of the structure and mechanism. Spectroscopy includes both K-edge-emission and L-edge absorption/emission spectroscopy to get a complete understanding of the time-evolution of the electronic structure, while room temperature time-resolved X-ray crystallography would provide the time-evolution of the geometric structure of the overall protein complex. We are applying the techniques mentioned above to study the photo-induced water oxidation reaction that occurs in Photosystem II (PS II) using both crystals and solution samples. [2-4,7] Photosynthetic water oxidation proceeds through four oxidation steps operating in a cyclic fashion resulting from the successive absorption of four photons by the PS II reaction center. The reaction takes place at the Mn_4CaO_5 cluster in the oxygen-evolving complex (OEC) of PS II. The femtosecond X-ray pulses of the free electron laser make it possible to outrun X-ray induced damage, allowing studies at room temperature. This is essential for following the turnover of the catalytic reaction, and the time-evolution of the photo-induced reaction can be probed using a visible laser-pump followed by the X-ray-probe pulse.

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KAROL NASS, MPI HEIDELBERG

RADIATION DAMAGE IN SERIAL FEMTOSECOND CRYSTALLOGRAPHY

Serial femtosecond crystallography (SFX) offers the diffraction before destruction approach to X-ray crystallography by exposing tens of thousands of microcrystals, one at a time, in random orientations to FEL pulses. This allows three orders of magnitude higher dose per single shot and crystal than the tolerable room temperature dose limit at synchrotrons. While local damage has been predicted in SFX, no high-resolution experiments were performed to date studying this issue. Proteins that contain metal cofactors are highly radiation sensitive since the degree of X-ray absorption correlates with the presence of high atomic charge number elements and X-ray energy. To explore the effects of local damage in SFX, *Clostridium* ferredoxin was used as a model system. The protein contains two [4Fe-4S] clusters of slightly different geometry that serve as sensitive probes for radiation-induced electronic and structural changes. High dose SFX datasets were collected at the Linac Coherent Light Source of ferredoxin microcrystals above and below the iron K-shell absorption edge using 80 fs pulses of ~10^{19} W/cm^2 irradiance, conditions that ensure radiation damage. The SFX data show unusual cumulative intensity distributions, which are consistent with diffraction from a non-homogeneous distribution of crystals containing molecules displaying different degrees of local damage. Surprisingly, the electron density corresponding to the sulphur atoms of the [4Fe4S] clusters appear to be displaced from their positions as found in synchrotron structures, indicative of correlated displacements of the atoms occurring during the accumulation time of the Bragg signals. These observations differ from what would be expected of a “bleaching” of the iron atoms and are suggestive of an influence of the molecular bonding and geometry on the atomic displacement dynamics following initial photoionization.

STEFAN HAU-RIEGE, LLNL

RADIATION DAMAGE PROCESSES IN BIOIMAGING

X-ray free-electron lasers have enabled femtosecond protein nanocrystallography, a novel method to determine the structure of proteins. It allows time-resolved imaging of nanocrystals that are too small for conventional crystallography. The short pulse duration helps in overcoming the detrimental effects of radiation damage because x-rays are scattered before the sample has been significantly altered. However, radiation damage may remain an issue for a large class of protein crystals containing heavier atoms with a higher charge number Z, such as metalloproteins. Since the photoabsorption cross section depends superlinearly on Z, these atoms absorb x-rays very efficiently, which could lead to enhanced local damage. In this presentation, we discuss our simulation results of the evolution of nanocrystals of metalloproteins under x-ray irradiation, focusing specifically on the significance of high-Z hot spots.

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MATTEO LEVANTINO, UNIVERSITY OF PALERMO

MYOGLOBINI QUAKE-LIKE MOTION OBSERVED WITH XFEL RADIATION

The events following the photodissociation of the bond between myoglobin and its ligand have been extensively studied with a variety of experimental and computational methods. The results of these investigations have been rationalized in terms of a model that implies a protein quake-like motion, i.e. the propagation of the strain released upon photoexcitation through the protein similar to the propagation of acoustic waves during an earthquake. However, the experimental investigations performed so far have been based on spectroscopic measurements or did not have sufficient time-resolution to measure the timescale of such a "protein quake." I will report on direct experimental evidence of myoglobin protein quake that has been obtained through femtosecond X-ray solution scattering measurements performed at the LCLS X-ray free electron laser. Our data clearly show that the structural changes induced in heme upon photolysis are propagated through the polypeptide chain in the picosecond timescale.

ROBERT DODS, UNIVERSITY OF GOTHENBURG

TIME-RESOLVED WIDE ANGLE X-RAY SCATTERING AND SERIAL FEMTOSECOND CRYSTALLOGRAPHY OF PHOTOSYNTHETIC REACTION CENTRE

Excitation of the *Blastochloris viridis* photosynthetic reaction center has been used to demonstrate a method to measure ultrafast protein structural changes using time-resolved wide-angle X-ray scattering at an X-ray free-electron laser. Measuring the scattering of the protein solution in the picoseconds following excitation has revealed an ultrafast global conformational change that precedes the propagation of heat through the protein. This provides direct structural evidence for a 'protein quake': the hypothesis that proteins rapidly dissipate energy through quake-like structural motions. Further experiments at the free-electron laser have aimed to provide greater weight to these observations by using time-resolved serial femtosecond crystallography to obtain a snapshot of the protein structure in the midst of this quake.