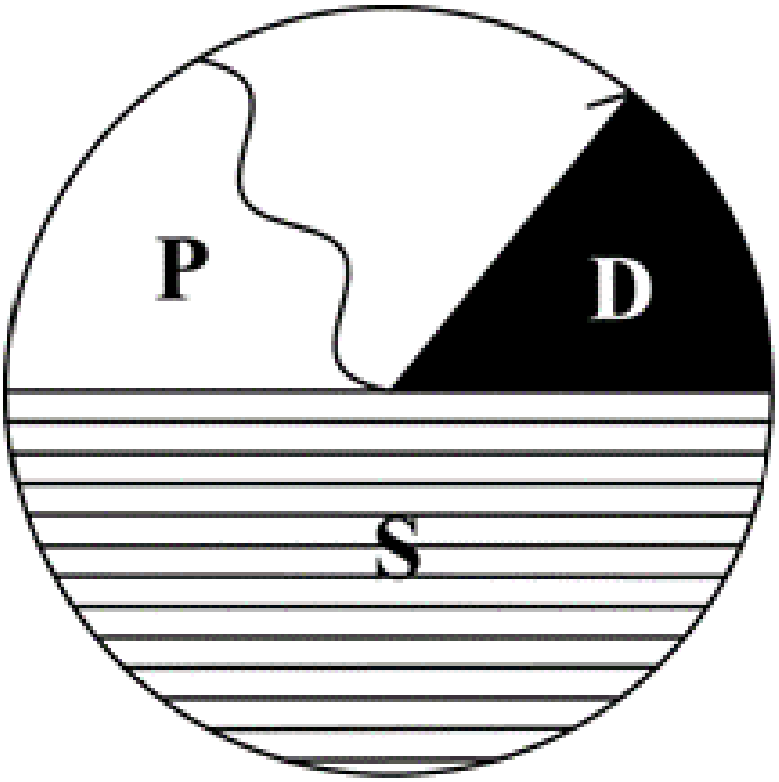
The 70th Annual

Pittsburgh Diffraction Conference



Conference Schedule and Poster Abstracts

Pittsburgh Diffraction Conference

Sept 30 – Oct. 2, 2012
Redwood Conference Rooms, SLAC building 48
Menlo Park, CA

Sunday, September 30, 2012

5:00 PM	Registration
6:00 PM	**Opening Reception**

Monday, October 1, 2012

7:30-8:00 AM	Registration / Coffee and pastries / Vendor exhibit
8:00-8:05	Introductory remarks
8:05-10:35	**Session 1: Macromolecular Crystallography**, Chair: Joanne Yeh
8:05-8:40	Keynote lecture: Approaches to G Protein Coupled Receptor Structure Determination. Brian Kobilka
8:40-9:05	Structural Studies of a Pol II Elongation Complex. Guillermo Calero
9:05-9:30	Structural Insights into Eukaryotic Transcription Initiation. David Bushnell
9:30-9:55	Uncovering Allostery in a Uniquely Folded Metalloprotein. Elizabeth Baxter
9:55-10:10	Coffee break/ refreshments
10:10-10:35	Crystallizing Our Understanding of Botulinum Neurotoxin. Rongsheng Jin
10:35-12:15	**Session 2: Hybrid Methods in Crystallography** Chair: Guillermo Calero
10:35-11:00	Providing a Time Axis to Structural Biology using Single-Molecule Spectroscopy. Jody Puglisi
11:00-11:25	A General Method for the Large Scale Solubilization of Cellular Proteins. Jennifer Guerrero
11:25-11:50	Structural Mechanism of HIV-1 Capsid Assembly: from Cryo-EM to All-Atom Molecular Dynamics Simulation. Peijun Zhang
11:50-12:15	Modeling the Assembly and Disassembly of Macromolecular Complexes: From Cryo-EM to X-ray. Carlos Camacho
12:15-2:15	**Poster Session, Vendor show and Lunch**

2:15-5:30	**Session 3: New Ideas in Crystallography**, Chair: Clyde Smith
2:15-2:50	Keynote lecture: Milestones in structural biology at the LCLS. John Spence
2:50-3:15	Revisiting and Improving Experimental Phasing Protocols, from Design to Processing. Gerard Bricogne
3:15-3:35	Understanding the structure and dynamics of eukaryotic glutaminyl-tRNA synthetase. Thomas Grant
3:35-3:50	Coffee break/refreshments
3:50-4:15	Automation at the MX beamlines of the Australian Synchrotron. Tom Caradoc-Davies
4:15-4:40	The MDS (Multiple-Dataset) Approach and its Implications for Dose Reduction. John Rose
4:40-5:05	Structural and Biophysical Characterization of Multicomponent Complexes. Joanne Yeh
5:05-5:30	Back to the Future: Multi-Conformer Contact Networks in Room Temperature PX Data Elucidate NMR Conformational Dynamics. Henry van den Bedem
5:35	**Bus leaves SLAC for Banquet** (The bus will leave the banquet at 8:30, first stop at the Cardinal Hotel at 8:40, stop at Sheraton Hotel 8:55, final stop at SLAC guest house 9:15)
6:00-10:00	**Banquet at Gordon Biersch** (640 Emerson Street, Palo Alto, CA 94301)

Tuesday, October 2, 2012

8:30-8:55 AM	Registration / Coffee and pastries / Vendor exhibit
8:55-9:00	Remarks/notices
9:00-10:40	**Session 4: Nanocrystallography** Chair: Garth Williams
9:00-9:25	Nanocrystal Jets, Aerosols & Single Molecules. Michael Bogan
9:25-9:50	Instrumentation for Protein Nanocrystallography at LCLS. Garth Williams
9:50-10:15	Serial Femtosecond X-ray Crystallography of *In Vivo* Crystallized Proteins Opens New Routes in Structural Biology. Lars Redeke
10:15-10:40	Re-thinking Data Processing for a World Where the Crystal Can't Rotate. James Holton
10:40-11:00	Coffee break and refreshments
11:00-12:15	**Session 5: Fast Science** Chair: James Holton
11:00-11:25	Femtosecond Nanocrystallography of Membrane Proteins. Petra Fromme
11:25-11:50	X-ray Spectroscopy and Femtosecond Diffraction of Photosystem II. Jan Kern
11:50-12:15	Measuring Distortion and Defects in Engineered Heterostructures. Apurva Mehta
12:15-1:30	**Lunch / Vendor Exhibit**
1:30-1:55	Time-resolved Synchrotron Studies of Metal-to Ligand Electron Transfer in Metaloorganic Complexes. Phillip Coppens
1:55-3:40	**Session 6: New Science with Femtosecond Diffraction** Chair: Michael Bogan
1:55-2:20	Aerosol morphology and x-ray free electron laser. Duane Loh
2:20-2:45	Probing the Structure of Water. Anders Nilsson
2:45-2:50	short break
2:50-3:15	Probing the Ultrafast Dynamics of Charge and Magnetic Order in Complex Oxides. Michael Foerst
3:15-3:40	Ultrafast high-resolution three dimensional imaging of compression waves in gold Nanocrystals. Jesse Clark
3:40-5:00	Trace Fluorescent Labeling for Macromolecule Crystallization Screening. Marc Pusey
5:00-5:10	Concluding remarks, vendor prize ceremony
5:10-5:30	**Pittsburgh Diffraction Society business meeting** (all welcome)

Poster Abstracts

The poster session will be held on October 1st from 12:15 to 2:15 in the Redwood Rooms. Lunch is included.

P1*

A Neutron Diffraction Investigation of the Solid-Solution $Na_2(Zn,Co)SiO_4$.

Rebecca L. Beadling and Charles H. Lake
Department of Chemistry, Indiana University of Pennsylvania, Indiana, PA 15701

The quaternary adamantine solid-solution $Na_2(Zn_{(1-x)}, Co_x)SiO_4$ ($0<x<0.50$) was investigated with neutron powder diffraction to address two questions. First, did the Zn^{2+} and Co^{2+} ions form a complete solid-solution or was there phase separation between Na_2CoSiO_4 and Na_2ZnSiO_4. Secondly, did a structure transition accompany the antiferromagnetic transition determined at 5.7 K? To answer these questions, high resolution neutron diffraction data were collected at 100 K, 10 K and 2 K with the POWGEN diffractometer at the Spallation Neutron Source, Oak Ridge National Laboratory using the orange crysostat. Refinements of the neutron data converged with $X^2 = 1.501$ and $R_P = 4.97$ %. Analysis of the results revealed that no phase-separation could be detected. The Co^{2+} ions appear to be randomly distributed at the divalent sites in the host structure making a complete solid-solution. Comparison of the 100 K and 2 K data revealed little evidence that a structure transition accompanied the magnetic transition in this material.

P2*

Insights into the Substrate Specificity and Mechanism of the Macrolide Sugar O-Methyltransferase, MycF

Steffen Bernard, David Akey, Sung Park, Shengying Li, David Sherman, Janet Smith
Chemical Biology Doctoral Program, University of Michigan

Natural products represent a significant source of pharmacologically active compounds. Heteroatoms are frequently methylated in natural product biosynthesis pathways. Mycinamicin is a potent macrolide antibiotic produced by the soil bacterium Micromonospora griseorubida. In the biosynthesis of mycinamicin, a 6-deoxyallose sugar is appended to the macrolactone core and subsequently methylated first at the 2′ hydroxyl to create javose and then at the 3′ hydroxyl group to create mycinose. MycE and MycF are S-adenosylmethionine (SAM)- and metal-dependent methyltransferases responsible for these 2′ and and 3′ methylations. Despite their similar substrates and cofactor dependence, MycE and MycF exhibit no meaningful sequence identity. Additionally the enyzymes have different oligomeric states. The structure of MycE (Akey et al, JMB, 2011) and MycF in complex with substrates and cofactors provide an opportunity to examine why two catalysts are required for similar chemistry. Crystal structures capture MycF in three states; MycF:Mg2+, MycF:Mg2+:S-adenosyl homocysteine (SAH), and MycF:Mg2+:SAH mycinamicin III. The three crystals belong to different space groups. Interestingly, crystals of MycF:Mg2+:SAH and MycF:Mg2+:SAH:mycinamicin III demonstrate the unique characteristic of having nearly identical unit cell constants and different space groups. This relationship results from a translocation that disrupts a crystallographic screw axis while forming a new crystallographic two-fold axis. The structure of MycF in complex with Mg2+, SAH and the substrate mycinamicin III suggests that the catalytic base is most likely Asp191. Mutagenesis results confirm that Asp191 is necessary for activity. In the absence of substrate, Asp191 is a Mg2+ ligand. The structure supports a mechanism where Mg2+ plays a critical role in substrate binding and stabilization of the hydroxylate intermediate. The enzyme forms no specific interactions with the substrate macrolactone core, suggesting that MycF may accept a variety of substrates bearing the javose moiety. Consistent with this hypothesis, MycF methylates javose-bearing intermediates from the tylosin biosynthetic pathway. Active site lids and Mg2+ constrain substrate positions and dictate the sites of methylation by MycE and MycF. These differences in active site geometry force the substrate sugar moiety to adopt different chair conformations in the two enzymes. These results provide insight into the mechanism of site-specific, ordered methylation of a macrolide natural product and demonstrate the utility of these enzymes in bioengineering efforts.

This work was supported by NIH grants DK42303 and GM078553 and a Chemistry Biology Interface Training Grant.

P3

Unfolding Anthrax: Molecular Mechanisms of Protein Transport

Geoffrey Feld, Katie Thoren, Alex Kintzer, Jennifer Colby, Bryan Krantz, Brent Segelke, Mark Hunter, Matt Coleman, Matthias Frank
Biosciences and Biotechnology Division, Lawrence Livermore National Laboratory

The transmembrane transporter anthrax lethal toxin is comprised of two proteins, protective antigen (PA) and lethal factor (LF). PA forms ring-shaped homooligomers on the surface of mammalian cells capable of binding multiple copies of the Zn2+-dependent protease LF. Following internalization, acidic conditions within the endosome induce PA to form a transmembrane channel through which LF unfolds and translocates into the host cell cytosol. Once in the cell, LF inactivates MAPKK signaling molecules, leading to cell death. Cytotoxicity is strictly dependent on the ability of PA to assemble with and subsequently translocate LF. We solved the X-ray structure of the core of a lethal toxin complex containing a PA octamer bound to four PA-binding domains from LF (LFN) to 3.1-Å resolution. The structure revealed that upon binding, the α1 helix and β1 strand of each LF unfold and dock into a novel cleft on the PA surface, called the α clamp.[1] To our knowledge, this is the first structure to capture an unfolding machine in complex with its substrate in a stable, partially-unfolded conformation. Using biophysical methods and electrophysiology, we extensively characterized the α clamp and detailed its function in toxin assembly, substrate binding, unfolding, and translocation. We demonstrated that the α clamp binds polypeptide in a largely sequence-nonspecific manner. Hydrophobic/aromatic residues not involved in initial binding to LFN catalyse translocation at the a-clamp subsite, especially for the multi-domain LF. Using polyprolinated and racemized substrates, we probed the essential role helical structure plays in PA-dependent translocation. We propose a translocation mechanism in which clamping sites, including the α clamp and the downstream ϕ clamp, interact nonspecifically with unfolding substrates via shape recognition of α helical structure.[2] We propose to further elucidate a mechanism for translocation-coupled protein unfolding by determining a series of structures using the Linac Coherent Light Source (LCLS). By making use of photocleavable cross-linkers, we envision imaging intermediates of unfolding and translocation on both the PA prechannel and channel complexes. These structures will provide a detailed description of the unfolding and translocation processes and substructures that are present in transmembrane channels, and should apply broadly across biology.

[1] Feld et al. (2010) Nature Struct Mol Biol., 17, 1383-1390
[2] Feld, Brown and Krantz. (2012) Protein Sci, 21, 606-624

P4

First Results from Imaging Two-Dimensional Protein Crystals with LCLS

Matthias Frank[1,2,*], David B. Carlson[3], Mark S. Hunter[1,2], Garth J. Williams[4], Marc Messerschmidt[4], Nadia A. Zatsepin[5], Anton Barty[2,6], W. Henry Benner[1], Kaiqin Chu[2], Alexander Graf[1], Stefan Hau-Riege[1], Richard Kirian[5], Tommaso Pardini[1], Bill Pedrini[8], Brent Segelke[1], M. Marvin Seibert[4], John C. H. Spence[5], Ching-Ju Tsai[7], Stephen M. Lane[2], Xiao-Dan Li[7], Gebhard Schertler[7], Sébastien Boutet[4], Matthew Coleman[1,2], and James E. Evans[2,3,9,**]

[1] Physical and Life Sciences Directorate, Lawrence Livermore National Lab, Livermore, CA 94551, USA
[2] Center for Biophotonics Science & Technology, University of California Davis, Sacramento, CA USA
[3] Department of Molecular and Cellular Biology, University of California Davis, Davis, CA USA
[4] Linac Coherent Light Source, SLAC National Accelerator Center, Menlo Park, CA, USA
[5] Department of Physics, Arizona State University, Tempe, AZ, USA
[6] Center for Free Electron Laser Science (CFEL), University of Hamburg and DESY, Hamburg, Germany
[7] Laboratory of Biomolecular Research, Paul Scherrer Institut, Villigen, Switzerland
[8] SwissFEL, Paul Scherrer Institut, Villigen, Switzerland
[9] Environmental Molecular Sciences Lab, Pacific Northwest National Lab, Richland, WA 99354, USA
** frank1@llnl.gov, ** james.evans@pnnl.gov*

Membrane proteins represent nearly two-thirds of current pharmaceutical drug targets, yet less than 2% of all known structures belong to this large and highly important class of proteins [1]. Unfortunately, not all membrane proteins readily form well-ordered three-dimensional (3D) nanocrystals or microcrystals suitable for analysis using LCLS [2] or conventional synchrotron light sources. Two-dimensional (2D) crystals of membrane proteins, although only one-layer thick, have the benefit of being surrounded by a near-native environment devoid of detergents or precipitating/nucleating agents. Electron crystallography has proven highly capable of solving structures of membrane proteins to near atomic resolution from 2D crystals. However, that approach continues to suffer from bottlenecks in crystallization, screening and data collection that sometimes require upwards of five years to determine a single atomic structure [3].

Our goal is to integrate coherent X-ray diffraction imaging (CXDI) with electron crystallography to solve protein structures from 2D rather than 3D crystals. This combined approach may allow atomic scale resolution to be achieved faster than with either approach alone. Here we present first results from preparing and conducting a recent LCLS beam time (Feb./May 2012) with 2D protein crystal samples in the following areas: 1) adaptation and optimization of sample preparation of 2D crystals for high-hit-rate imaging using fixed targets; 2) simulation of scattering patterns for streptavidin 2D crystals based upon realistic beam parameters; 3) observation of diffraction patterns from those samples and comparison to model predictions.

This Work was performed under the auspices of the US Department of Energy by Lawrence Livermore National Laboratory under contract DE-AC52-07NA27344 and Pacific Northwest National Laboratory (operated by Battelle Memorial Institute) under Contract DE-AC05-76RL01830. Support was provided by the UCOP Lab Fee Program (award no.

118036), NIH grant number 5RC1GM091755, NSF award MCB-1021557, and LLNL Lab-Directed Research and Development Project 12-ERD-031. Portions of this research were carried out at the Linac Coherent Light Source (LCLS) at SLAC National Accelerator Laboratory. LCLS is an Office of Science User Facility operated for the U.S. Department of Energy Office of Science by Stanford University. A portion of this work was funded by the Center for Biophotonics Science and Technology, a designated NSF Science and Technology Center managed by the University of California, Davis, under Cooperative Agreement No. PHY0120999.

References

[1] Arinaminpathy, Y., Khurana, E., Engelman, D. M. & Gerstein, M. B. Computational analysis of membrane proteins: the largest class of drug targets. Drug Discovery Today 14, 1130-1135 (2009).

[2] Chapman, H. N. et al. Femtosecond X-ray protein nanocrystallography. Nature 470, 73-77 (2011).

[3] Renault, L. et al. Milestones in electron crystallography. J Comput Aided Mol Des 20, 519-527 (2006).

P5

The PSI Tech Portal: A Web Resource for Protein Crystallographers

Lida Gifford, Helen Berman and Paul Adams
Lawrence Berkeley National Laboratory

The Technology Portal of the Protein Structure Initiatives Structural Biology Knowledgebase (PSI SBKB) is a web resource providing the latest technologies and methods to enable all areas of protein structure determination and annotation (http://technology.sbkb.org/portal/). The web site allows users to search or browse the continually updated database of over 300 technological advances via keyword search or experimental step, access videos of new methods on YouTube, and link to web-based resources that can be used to design, predict, or model results. There are opportunities for protein crystallographers to connect, ask questions, get news, and develop collaborations through our Facebook page and Nature Network technology forum. A dynamic article, Featured Technology, offers an in-depth profile of useful tools for furthering protein structure determination, modeling, and function prediction. Every month, the Center Profile highlights a different PSI:Biology-funded Center or Biology Partner to increase awareness of the types of work being done by each group. The Technology Portal is a component of the PSI SBKB, which presents integrated genomic, structural, and functional information for protein sequence targets and editorial content from the Nature Publishing Group (http://sbkb.org). Sample pages and searches will illustrate the tools, information, and opportunities for scientific interaction available through the Tech Portal.

The PSI SBKB Technology Portal is funded by the NIGMS.

P6

Fixed target protein crystallography using X-ray free electron lasers

Mark Hunter, Brent Segelke, Nadia Zatsepin, Matt Coleman, Stefan Hau-Riege, Bill Pedrini, Ching-Ju Tsai, Xiao-Dan Li, Gebhard Schertler, Matthias Frank
Physics and Life Sciences Directorate, Lawrence Livermore National Laboratory

Fixed-target protein crystallography was tested at X-ray free-electron laser (XFEL) sources and shown to be a alternate approach to liquid-jet based protein crystallography. Protein crystals were mounted on Si3N4 windows lithographically etched into a silicon crystal. Crystals of REP24, a 24kDa soluble protein, which were embedded in oils to protect against dehydration and damage during the in vacuo experiments, were shown to diffract beyond 2Å resolution using the CXI instrument at the Linac Coherent Light Source at SLAC National Accelerator Laboratory. Data collection rates of up to 10Hz were achieved. The approach also allowed for diffraction patterns to be recorded from 2D protein crystals such as bR in purple membrane. Although much work remains to be done, fixed-target experiments show promise for reduced sample consumption as well as novel paths to doing pump-probe crystallography at XFEL sources. Extension of the method to cryo-crystallography and membrane proteins is currently being explored.

P7*

Cytochrome P450cin substrate-free and Nitrosyl Crystal Structures Provide Clues for Oxygen Activation

Yarrow Madrona, Sarvind Tripathi, Huiying Li, Thomas Poulos
MBB Dept, UC Irvine

Cytochrome p450 enzymes are iron containing monooxygenases that use oxygen to catalyze substrate hydroxylation. Oxygen binds the ferrous iron forming an oxy-complex intermediate before subsequent cleavage and substrate hydroxylation. Most of what we know about the mechanism of oxygen activation comes from P450cam from the soil bacterium, Pseudomonas putida. P450cam contains an almost universally conserved Thr252 as well as a glycine (gly248) carbonyl that contribute to oxy-complex stabilization. Additionally, a recent substrate-free crystal structure of P450cam shows that the oxy-complex appears to be an intermediate between the substrate-bound and substrate-free structures.

P450cin from the bacterium Citrobacter brakki is unique among P450 s in that it contains Asn242 in place of the highly conserved Thr252. Lacking the ability to obtain a bonifide oxy-complex we solved the substrate-free structure. We show that there is a large change in moving from the substrate-bound to free form that allows the carbonyl atom of Gly238 (the analogue to Gly248 in P450cam) to move even further towards the heme iron in the P450cin-substrate free structure compared to P450cam. We anticipate that as in P450cam the oxy-intermediate is halfway between the substrate-free and substrate-bound crystal structures.

To support our hypothesis we present nitrosyl-P450cin and P450cam complex structures as geometric mimics for oxygen binding. We find that the bulkier side chain of Asn242 in P450cin is likely to exclude a hydrogen bond intermediate between Asn242 and the oxy-complex. A superposition of the NO-P450cin complex and the open structure shows that Gly238 is likely to be alone in forming a tighter interaction with the oxy complex than what is observed in P450cam. This tighter interaction may be what allows a single residue to stabilize the oxy-complex encouraging protonation and subsequent cleavage to produce the active ferryl species.

P8

Methylenetetrahydrofolate binding site of FDTS and the mechanistic implications

Irimpan I. Mathews, Eric M. Koehn, Amnon Kohen, and Scott A. Lesley.
Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, 2575 Sand Hill Road, Menlo Park, CA 94025-7015

Thymidylate synthesis is the terminal step in the sole de novo synthetic pathway to deoxythymidine monophosphate (dTMP), a nucleotide essential for the synthesis of DNA. Thymidylate synthase (TS) catalyzes this crucial reaction. TS inhibition stops DNA production, arresting the cell cycle and eventually leading to "thymineless" cell death. Thymidylate synthases use N5,N10-methylene-5,6,7,8-tetrahydrofolate (CH2H4folate) to reductively methylate 2'-deoxyuridine-5'-monophosphate (dUMP) producing dTMP. Flavin-dependent Thymidylate synthase (FDTS) are encoded by the thy1/thyX gene and are not homologous to classical TS encoded by thyA and thyB genes. FDTSs are essential for cell survival of many pathogenic organisms (Treponema pallidum (syphilis), Bacillus anthracis (anthrax), Mycobacterium tuberculosis (tuberculosis), Mycobacterium leprae (leprosy), Borrelia burgdorferi (Lyme disease), Helicobacter pilori (gastric ulcer), Clostridium botulinum (botulism), Rickettsia prowazekii (epidemic typhus), and Chlamydia pneumoniae (pneumonia) are FDTS family members). FDTSs provide a unique alternative for the development of antimicrobials capable of simultaneously targeting a wide range of organisms with potential use as biological weapons. In contrast to classical TSase where the cofactor CH2H4folate provides both the H- and methylene, in the FDTS reaction the H- is provided by the FADH2 and CH2H4folate is used only as a source for the methylene moiety. The absence of homology between FDTS and classical thymidylate synthase offers the possibility of developing specific inhibitors for deadly pathogenic microbes. Although several crystal structures of FDTSs have been reported, the absence of a structure with folate(s) limits understanding of the molecular mechanism and the scope of drug design for these enzymes. This work presents the first structures of FDTS with folate and folate mimics.

P9*

Decrease in carbapenem susceptibility due to carbapenem hydrolysis driven by oxacillinases in Acinetobacter baumannii

Lyudmila Novikova, Chris Milianta, Sergei Vakulenko, Clyde Smith
Colorado State University

Acinetobacter baumannii is a nosocomial pathogen that causes Acinetobacter infections, weakening the immune system. Currently, these infections are treated with the antimicrobials imipenem and meropenem, as both are unaffected by most bacterial defense mechanisms. These drugs are members of the carbapenem family of β-lactam antibiotics, commonly viewed as a last line of defense treatment against multi-drug resistant pathogens. Biologically, carbapenems function by inactivating peptidoglycan transpeptidases in bacteria such that there is inhibition of polymerization of the peptidoglycan polymers and ultimately, bacterial cell death.

Unfortunately some strains of A. baumannii have now become resistant to most types of antibiotics including the carbapenems, due to the development of a variety of resistance mechanisms, such as reduced affinity of penicillin binding proteins for antibiotics, decreased permeability of the outer membrane, and overexpression of efflux pumps.1 One of the most predominant resistance mechanisms involves the production of carbapenem-hydrolyzing enzymes. These enzymes, known as oxacillinases, are classified as class D β-lactamases, and decrease carbapenem susceptibility by opening the β-lactam ring of the drug and rendering it inactive. A potential goal in treating Acinetobacter infections is to study this mechanism of resistance to carbapenems. The class D β-lactamases, along with the class A and C enzymes, deactivate the β-lactam antibiotics by hydrolyzing the β-lactam ring via serine-based covalent catalysis2. More specifically, a serine residue in the active site of the β-lactamase is activated by a carbamylated lysine residue and forms a covalent acyl-enzyme intermediate with the β-lactam substrate as the first step in an acylation-deacylation mechanism. The β-lactam ring is opened during acylation and the antibiotic is subsequently released during the water-activated deacylation step as the inactive open-ring penicillinoic acid form.

To aid in the understanding of how these enzymes have evolved, we have undertaken the X-ray crystallographic analysis of one of these oxacillinases, OXA23. Originally it was identified in 1985 in Scotland and said to be the first β-lactamase of class D to be discovered. We have determined the structure of OXA23 in several forms, two substrate-free forms at low and neutral pH, and an acyl-enzyme complex with meropenem. X-ray diffraction data was collected from flash cooled OXA23 crystals using SSRL beam line BL12-2. The structure of the low pH form of OXA23 was solved by molecular replacement using the known structure of OXA24 (64% sequence identity) and refined with the PHENIX suite

of programs. The structures of the neutral pH form and the OXA23-meropenem complex were solved by molecular replacement using the low pH OXA23 structure.

In contrast to OXA24 and other oxacillinases, a 14-residue surface loop undergoes a major pH-dependent conformational change. In the neutral pH form, this loop is closed over the entrance to the active site in a configuration similar to that observed in other oxacillinases. However, at low pH the loop has swung aside and a short internal loop adjacent to the active site also adopts a different conformation. In the OXA23-meropenem complex, the surface loop adopts the closed conformation similar to the neutral pH form. The OXA23-meropenem complex has bound meropenem in an inactive tautomeric form2 such that the acyl-enzyme intermediate acts as an inhibitor. This is of interest because we are looking to find methods of deactivating carbapenamases to create therapeutic strategies for treating A. baumannii infections, since oxacillinases are evolving rapidly and becoming more prevalent each year.

1. Walther-Rasmussen J, Høiby, N (2006). J. Antimicrob. Chemother. 57, 373 383.
2. Schneider KD, Ortega CJ, Renck NA, Bonomo RA, Powers RA, Leonard DA (2011). J. Mol. Biol. 406, 583-594.

P10

The AFF4 scaffold binds human P-TEFb adjacent to HIV Tat

Ursula Schulze-Gahmen, Heather Upton, Andrew Birnberg, Nevan Krogan, Qiang Zhou, Tom Alber UC Berkeley

Human positive transcription elongation factor b (P-TEFb) phosphorylates RNA polymerase II and regulatory proteins to trigger elongation of specific gene transcripts. At key genes—including the integrated HIV genome—P-TEFb functions as part of a super elongation complex (SEC), a large assembly organized on a flexible scaffold in the AF4 family. The HIV-1 Tat protein selectively recruits SECs containing AF4 proteins AFF1 or AFF4. To explore the basis for this specificity and determine if scaffold binding alters P-TEFb conformation, we determined the cocrystal structure of a tripartite complex containing the functional recognition regions of P-TEFb and AFF4. AFF4 meanders over the surface of the cyclin T1 (CycT1) subunit of P-TEFb, making no stable contacts with the CDK9 kinase subunit. Amino acid substitutions in the interface reduce CycT1 binding and AFF4-dependent transcription. Unexpectedly, AFF4 is positioned to make direct contacts with HIV Tat, and Tat enhances P-TEFb affinity for AFF4. These studies define the mechanism of scaffold recognition by P-TEFb and reveal an unanticipated intersubunit pocket on the AFF4 SEC that potentially represents a target for therapeutic intervention against HIV/AIDS.

P11*

Serial Femtosecond Crystallography with Micrograms

Raymond G. Sierra, and Michael J. Bogan
Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory

We developed an electrospun liquid microjet that delivered 140 μg of thermolysin microcrystals at flow rates of 0.17 μl/min to perform serial femtosecond crystallography (SFX) studies with X-ray lasers. The state of the art for diffraction studies of biomolecules, like protein crystals, at current hard x-ray sources, like the Linac Coherent Light Source (LCLS) at SLAC National Accelerator Lab (SLAC), use aerosolized droplet streams or hydrodynamically focused liquid jets. These delivery methods have numerous advantages; however, they are prohibitive to use with precious samples due to their high sample consumption rates. The flow rates range from 1-20 μl/min and thus require milliliters of sample just to load the apparatus once; elaborate sample loading apparatuses are also required to load these volumes and to mitigate sample settling. Our method has 10x-100x lower sample consumption rates and only requires a microcentrifuge tube loaded with a minimum of 100 μl of sample solution to be loaded each time. The solution need only be comprised of the crystal of interest with the crystallizing buffer, salt and polymer, as well as glycerol, which helps the jet function properly in the vacuous sample chamber and helps mitigate the sample settling rate. Diffraction of the thermolysin crystals were observed with better than 3 Å resolution from 14000 indexable diffraction patterns. Our nanoflow electrospinning technique presented here extends diffraction studies, like SFX, to biological samples that necessitate minimal sample consumption.

P12

AutoDrug: An Automated Pipeline for Drug Discovery at the Stanford Synchrotron Radiation Lightsource

Yingssu Tsai[1], Ana González[1], Tim M. McPhillips[1], Scott E. McPhillips[1], S. Michael Soltis[1]
[1]Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, 2575 Sand Hill Road, Menlo Park, CA 94025-7015

High-throughput structural biology is necessary for functional genomics, mutagenesis studies, and drug discovery. Automation of data collection and data processing has taken place in the last decade. *AutoDrug* is a command line software program that fully automates crystal screening, data collection, data processing, and molecular replacement. Existing systems at the Stanford Synchrotron Radiation Lightsource (SSRL), such as the Stanford Automatic Mounting (SAM) system for sample mounting and Blu-Ice, the interface for beamline control, are fundamental components in the automated pipeline. *AutoDrug* is implemented using RestFlow, a scientific workflow automation framework, consisting of a programming language and a software engine. *AutoDrug* runs external programs and scripts, and renders and parses text. An input file specifies user-defined experimental parameters and collection criteria. *AutoDrug* is able to collect and process data from multiple samples without human intervention. Output directories are automatically structured, and reports summarize selected statistics. Input and output files retain information that gives the users the ability to scrutinize and analyze collection criteria for future data collection. The automated pipeline maximizes efficiency at the beamline and offers the opportunity for rational and systematic modification of procedures to improve the overall process.