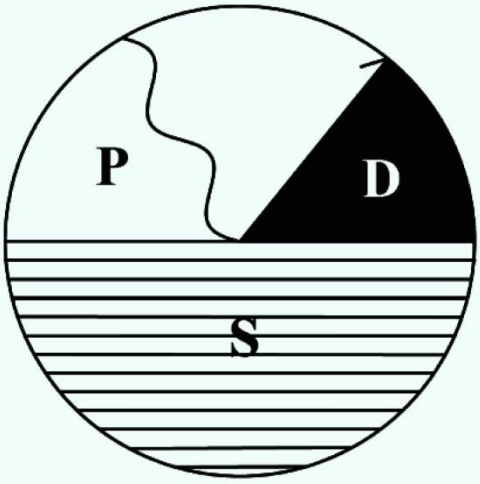
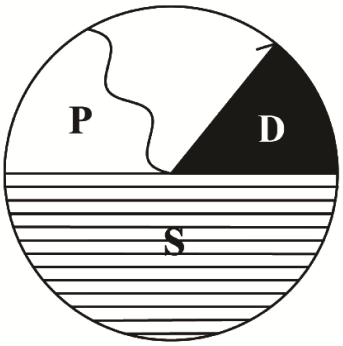
September 18-20, 2013

Buffalo, New York

The 71st Annual Pittsburgh Diffraction Conference



Hauptman-Woodward
Medical Research Institute



The 71st Annual Pittsburgh Diffraction Conference

Conference Chairman	Vivian Cody
Symposium Organizers	Edward Snell
	Joseph Luft
Poster Session Chairman	Vivian Cody
Local Arrangements Coordinators	Vivian Cody
	Jill Szczesek, Brianna Williams

2013 Officers of the Pittsburgh Diffraction Society

President-Elect	Vivian Cody (2013 Conference)
President-Elect	John Rose (2014 Conference)
President	Guillermo A. Calero
Past President	Vivien Yee
Treasurer	Matthias Zeller
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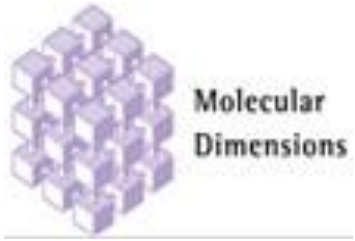
- 

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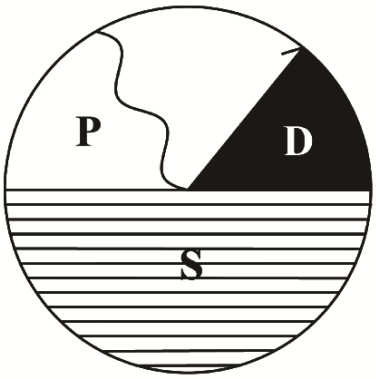
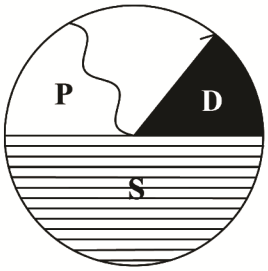


Sidhu Award

This award honors the memory of Professor Surhain Sidhu, who while Professor of Physics and Director of the X-ray Laboratory at the University of Pittsburgh was a founder of the Pittsburgh Diffraction Conference in 1942. Later, Professor Sidhu moved to Argonne National Laboratory, where he pioneered the use of the null matrix technique in neutron diffraction. This involves choosing isotopes of an element in the proportion that gives a zero net coherent scattering factor. The procedure has been widely used for studying biological materials in which the isotopic ratio of hydrogen to deuterium is appropriately adjusted.

The award recognizes an outstanding contribution to crystallographic or diffraction research by an investigator whose doctoral degree was conferred within five years before the award date. Previous winners of the award are:

1967	A. I. Bienenstock
1968	R.M. Nicklow
1969	T.O. Baldwin
1970	S.-H. Kim
1971	L.K. Walford
1972	D. E. Sayers
1974	B.C. Larson & N.C. Seeman
1975	P. Argos
1978	K. Hodgson & G. DeTitta
1980	G. Petsko
1985	D.C. Rees
1986	D. Agard & J.M. Newsam
1988	Q. Shen
1989	M. Luo
1990	L. Brammer
1992	R.C. Stevens
1993	M. Pressprich & T. Yeates
1994	A. Vrielink & J. Wang
1995	M. Georgiadis
1996	M.J. Regan
1999	C. Ban & M Wahl
2000	W.R. Wikoff
2001	L. Shapiro
2002	Y. Lee
2003	E. O. Saphire
2004	Y. Xiong
2005	C.-Y. Ruan
2006	P. Chupas

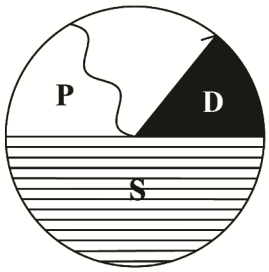


Chung Soo Yoo Award

Dr. Chung Soo Yoo, Adjunct Associate Professor in the Department of Medicinal Chemistry and Research Associate in the Department of Crystallography of the University of Pittsburgh, was killed in the Korean Airlines Flight 007 disaster of 31 August 1983. Dr. Yoo came to the U.S. from Korea in 1965; he obtained his M.S. Degree in Chemistry at Rice University in 1967 and his Ph. D. in Crystallography at the University of Pittsburgh in 1971, and became a U.S. citizen. He was a member of the Biocrystallography Laboratory of the Veterans Administration Medical Center in Pittsburgh.

Dr. Yoo was one of the most likeable crystallographers among students and colleagues in Pittsburgh, and was always very enthusiastic about the Pittsburgh Diffraction Conference.

The Chung Soo Yoo Award, established by the Pittsburgh Diffraction Society to honor Dr. Yoo's memory, is given to a graduate student presenting the best poster at the annual Pittsburgh Diffraction Conference.



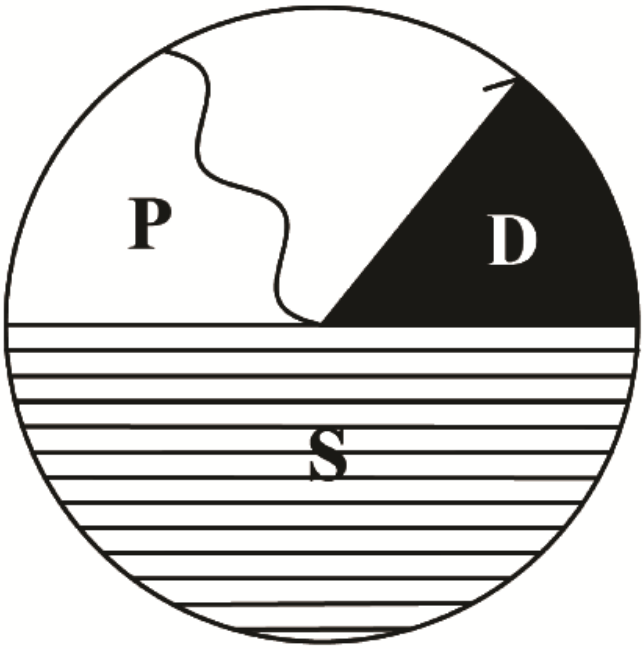
The PDS Award Funds

Over the years, the Pittsburgh Diffraction Society has created and bestowed awards to scientists and students involved in the many facets of diffraction study of matter. The first of these is the Sidhu Award, which recognizes the work of a young scientist who has made outstanding contributions to diffraction science within five years of earning a Ph.D. The second of these is the Chung SooYoo Award, which is given to the graduate student with the best poster presentation at a Pittsburgh Diffraction Conference. The most recent of these awards is the George A. Jeffrey Award given to meritorious graduate students who desire support to attend the triennial meeting of the International Union of Crystallography.

The three awards were established with generous gifts from family and friends of Sidhu, Chung Soo, and Jeff. Now we are seeking help to secure a more solid financial footing for the three PDS award funds. Please consider making a generous donation to the Pittsburgh Diffraction Society targeting one or more of the award funds.

Checks should be sent to the PDS Treasurer, Dr. Matthias Zeller, Youngstown State University, One University Plaza, Youngstown, Ohio 44555 (mzeller@ysu.edu)

All donations are tax deductible in the USA; check with your tax consultant in foreign countries.



The 71st Annual

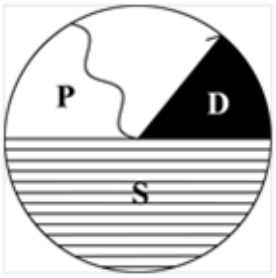
Pittsburgh Diffraction Conference

Hauptman-Woodward Medical Research Institute

Buffalo, New York

18-20 September 2013

Program and Abstracts



71st Pittsburgh Diffraction Conference 2013

Hauptman Woodward Institute, Buffalo, NY September 18-20, 2013

September 18th

From 4:30 pm	Conference Committee member present at hotel
5:30-7:30 pm	Opening reception at Bacchus Wine Bar and Restaurant

September 19th

8:00 am	Conference Registration opens, light breakfast available
8:45 am – 9:00 am	Opening introduction – Vivian Cody, Eaton Lattman, CEO
First session:	**RNA and DNA (Chair Edward Snell)**
9:00 am – 9:30 am	**Ned Seeman**, New York University "DNA: Not Merely the Secret of Life"
9:30 am – 10:00 am	**Joanne Yeh**, University of Pittsburgh “Binding of Damaged DNA Induces Dimerization of UV-damaged DNA-binding Protein (UV-DDB): Investigating the Role of Oligomerization in Chromatinized DNA Repair”
10:00 am – 10:30 am	**Guillermo Calero**, University of Pittsburgh "Structural Basis of Transcription: Crystal Structure of a RNA Polymerase II Transcribing Complex"
10:30 am – 10:50 am	Coffee
Second session:	**More RNA; Crystallization (Chair Clara Kielkopf)**
10:50 am – 11:20 am	**Joseph Wedekind**, University of Rochester "PreQ$_1$, Riboswitches Use Diverse Modes of Pyrrolopyrimidine Recognition in Gene Regulation”
11:20 am – 11:50 am	**John Hunt**, Columbia University "Rational Engineering of Improved Protein Crystallization"

11:50 am – 12:20	**Joseph R. Luft**, Hauptman Woodward Institute "Crystallization Screening: Technologies and Science"
12:20 am- 12:50 am	**Hilary Stevenson**, University of Pittsburgh “Nanocrystal Identification and Crystal Optimization by Electron Microscopy”
12:50 pm – 1:30 pm	**Lunch provided**
Third session:	**Small Molecule Crystallography (Chair Matt Zeller)**
1:30 pm – 2:00 pm	**Jesse Rowsell**, Oberlin College "A Common Supramolecular Motif in Compounds of “H-Acid”, featuring the Incommensurately Modulated Phase of the Commercially Important Sodium Hydrate"
2:00 pm – 2:30 pm	**Silvina Pagola**, College of William and Mary "Crystal Structure Analysis of Organic Mechanochemical Reaction Products from X-ray Powder Diffraction"
2:30 pm – 3:00 pm	**Yurij Mozharivskyj**, McMaster University "Elucidating Fine Structural Features and Physical Properties in Responsive Materials via Single Crystal X-ray Diffraction"
3:00 pm – 3:30 pm	Coffee
Fourth session:	**Short talks to be selected from submitted abstracts (Chair Joesph Luft)**
3:30 pm – 4:00 pm	**Joseph Ng**, University of Alabama, Huntsville “Challenges and Breakthroughs During the Pursuit of the Structure-based Catalytic Mechanism of Inorganic Pyrophosphatase from *Thermococcus thioreducins*
4:00 pm – 4:30 pm	**Ben Orlando**, University of Buffalo/HWI “Lipid Bilayer Nanodisc Incorporation Facilitates the Investigation of Membrane Bound Cyclooxygenase-2”
4:30 pm – 5:00 pm	**Oliver Ernst**, University of Toronto **“**Opsin, a Structural Model for Olfactory Receptors?**”**
5:30 pm – 6:30 pm	Poster sessions (posters will be available during dinner)
6:30 pm – 8:30 pm	Banquet – All participants; Poster prizes

September 20th

8:00 am -	Light breakfast available
Fifth session:	**New Diffraction Technologies (Chair John Rose)**
9:00 am – 9:30 am	**Aina Cohen**, SLAC National Accelerator Laboratory "Femtosecond High-resolution Diffraction Studies at LCLS"
9:30 am – 10:00 am	**Flora Meilleur**, Oak Ridge National Laboratory "Locating Hydrogen Atoms in Enzymes Using Neutron Protein Crystallography"
10:00 am – 10:30 am	**Marian Szebenyi**, Cornell University "Developments and Opportunities for Structural Biology at MacCHESS"
10:30 am – 11:00 am	Coffee
Sixth session:	**More Diffraction Technologies (Chair Edward Snell)**
11:00 am – 11:30 am	**Michael Murray**, Rigaku Americas Corporation "Hybrid Pixel Array Detectors in the Home Lab: Applications for Diffraction and SAXS on Rigaku X-ray Generators"
11:30 am – 12:00 noon	**Roger Durst**, Bruker AXS "Design and Characteristics of Metal Jet X-Ray Sources"
12:00 pm – 12:30 pm	**John Rose**, University of Georgia "The SER-CAT Virtual Beamline: Lessons Learned When Over 95% Of All Data Is Collected Remotely"
12:30 pm - 1:00 pm	Lunch provided (can be taken into the business meeting)

All member business meeting:

1:00 pm – 2:00 pm	Business meeting of Pittsburgh Diffraction Society
Closing:	Taxis will be called for those needing a ride to the airport (Recommend making arrangements ahead of time)

September 19th

Poster session: **Chair: Vivian Cody**

Number	Presenter	Eligible for Chung Soo Yoo Award
P1	**Benjamin Orlando** Lipid Bilayer Nanodisc Incorporation Facilitates the Investigation of Membrane Bound Cyclooxygenase-2	**Yes**
P2	**Ritwik Nandagiri** Investigating Substrate Binding in Fatty Acid Oxygenases of the Peroxidase-Cyclooxygenase Superfamily	**Yes**
P3	**Michael Lucido** Role of Phe-205 in Determining the Stereoselectivity of Arachidonate Oxygenation by Aspirin-acetylated Cyclooxygenase-2	**Yes**
P4	**Jarrod Bogue** "Structure and Function Analysis of Novel Class 1 $PreQ_1$ Riboswitches From Bacterial Pathogens"	**Yes**
P5	**Adam Wier** The Structural Basis for Spt5-mediated Recruitment of the Paf1 Complex to Chromatin	**Yes**
P6	**Sarah Loerch** The CAPERα: UHM Provides a Potential Means for Alternative Splice Site Regulation	**Yes**
P7	**Leigh Allen** Investigation of Non-Ribosomal Peptide Synthesis-Related Genes from An Uncharacterized Operon Implicated in *Acinotobacter baumannii* Motility	**Yes**
P8	**Christopher Barnes** Structural Basis of Transcription: Revealing the Structure of RNA Polymerase II Transcribing Complex	**Yes**
P9	**Thomas Grant** Combining Crystallography, SAXS, Bioinformatics and Molecular Dynamics to Assess the Structure and Dynamics of Eukaryotic GlnRS	**Yes**
P10	**Guowu Lin** Novel Approaches to Stabilize Large Protein Assemblies for Crystallization Experiments	**Yes**
P11	**Rick Roberts** Integration of *in situ* X-ray Diffraction Analysis with High-throughput Crystallization Screening	**Yes**
P12	**Marc Messerschmidt** LCLS Protein Crystal Screening	**No**
P13	**Matthias Zeller** Crystallography of the Ferromagnetic Shape Memory Alloy Ni-Mn-Ga: Single Crystal Structure Analysis of Five Layered Martensites	**No**
P14	**Ruthairat Nimthong** Synthesis and Characterization of Copper (I) Complexes with Thiourea and Related Ligands	**Yes**
P15	**Kristen Hernandez** Understanding the Selectivity of Hydrogen Bonding in Cocrystals of Pyridine-Imidazole Compounds	**Yes**
P16	**Jonathan Wagner** Structure of Mycobacterial MycP1 Protease, A Potential Drug Target	**Yes**

September 19th

Poster session: **Chair: Vivian Cody**

Number	Presenter	Eligible for Chung Soo Yoo Award
P17	**Ivan Belashov**	**Yes**
	Identification of a Human Cullin5 Peptide that Interacts with a Complex Comprising HIV-1 Vif/Elongin B/C/Core Binding Factor β	
P18	**Timothy Umland**	**No**
	Influenza A PB2 and Human MAVS Complex: Extending Crystal Structural Data to Identify Virus-host Protein-Protein Interaction Determinants of Host Tropism	
P19	**Vijay Kumar**	**Yes**
	Towards Improved Heme-mimetic Activation of Soluble Guanylyl Clyclase Using Structure-based Drug Design	
P20	**Derek Veith**	**Yes**
	Structural Studies of Interactions Between α-Thrombin and Clotting Factors	
P21	**Kristin Sutton**	**Yes**
	Structural Biology of the Shikimate Pathway in Multi-drug Resistant Gram Negative Bacteria	
P22	**Alex Bressler**	**No**
	Study of Lamprey TTR as a Model for Binding Site Function of Transthyretin-like Proteins	
P23	**Vivian Cody**	**No**
	Analysis of Human Transthyretin (hTTR) with the Potent Inhibitor VCP-6, 3',5'-Dichloro-2-carboxy-diphenylamine	
P24	**Jim Pace**	**No**
	Structure-activity Correlations for Three Pyrido[2,3-*d*]pyrimidine Antifolates Binding to Human and *Pneumocystis carinii* Dihydrofolate Reductase	

Invited Speakers

DNA: Not Merely the Secret of Life

Nadrian Seeman,Department of Chemistry, New York University, New York City, NY 10003, ned.seeman@nyu.edu

We build branched DNA species that can be joined using Watson-Crick base pairing to produce N-connected objects and lattices. We have used ligation to construct DNA topological targets, such as knots, polyhedral catenanes, Borromean rings and a Solomon's knot. Branched junctions with up to 12 arms have been made. Nanorobotics are a key area of application. We have made robust 2-state and 3-state sequence-dependent devices and bipedal walkers. We have constructed a molecular assembly line using a DNA origami layer and three 2-state devices, so that there are eight different states represented by their arrangements. We have demonstrated that all eight products can be built from this system. A central goal of DNA nanotechnology is the self-assembly of periodic matter. We have constructed 2-dimensional DNA arrays with designed patterns from many different motifs. We have used DNA scaffolding to organize active DNA components. We have used pairs of 2-state devices to capture a variety of different DNA targets. One of the key aims of DNA-based materials is to construct complex material patterns that can be reproduced. We have built such a system from bent TX molecules, which can reach 2 generations of replication. This system represents a first step in self-reproducing materials. Recently, we have self-assembled a 3D crystalline array and have solved its crystal structure to 4Å resolution, using unbiased crystallographic methods. We can use crystals with two molecules in the crystallographic repeat to control the color of crystals. Since we dictate the intermolecular contacts, we have been able to examine the impact on resolution of phosphorylation and of modifying the sticky ends.

This research has been supported by the NIGMS, NSF, ARO, ONR and DOE.

Binding of Damaged DNA Induces Dimerization of UV-damaged DNA-binding Protein (UV-DDB): Investigating the Role of Oligomerization in Chromatinized DNA Repair

Joanne Yeh, Shoucheng Du, Haibin Shi, Unmesh Chinte, Harshad Ghodke, Hong Wang, Ching Hsieh, James Conway, Bennett Van Houten, Vesna Rapic-Òtrin, University of Pittsburgh School of Medicine, Pittsburgh, PA. jiyeh@pitt.edu

UV light-induced photoproducts are recognized and removed by the nucleotide-excision repair (NER) pathway. In humans, the UV-damaged DNA-binding protein (UV:DDB) is part of a ubiquitin E3 ligase complex (DDB1-CUL4ADDB2) that initiates NER by recognizing damaged chromatin with concomitant ubiquitination of core histones at the lesion. UB-DBB is thought to play vital roles in maintaining genetic integrity, surveying the genome for UV-damaged DNA, initiating NER by recruiting repair enzymes and coordinating regulated protein degradation via the ubiquitin proteosome system (UPS) upon detecting UV-damaged DNA. In humans, a cancer prone genetic disorder, xerderma pigmentosum (XP), is caused by mutations in the uvddb gene, demonstrating UV-DDBs function as an essential sensor of DNA damage. A fundamental unanswered question in biology is how does tightly packed chromatin get inspected for DNA damage in an effective and efficient manner? Related to this is how are the proteins involved in NER and UPS organized at the damaged DNA lesion site, in a manner that enables repair reactions to proceed rapidly in conjunction with ubiquitination and proteosomal degradation, accommodating the multitude of proteins that assemble and disassemble dynamically to support both NER in tandem with UPS. Here, we present some of our findings from an expansive multidisciplinary study aimed at deciphering, from a structural and mechanistic perspective, the molecular changes involved in damage detection by UV-DDB. We reveal for the first time a distinct dimeric state of UV-DDB [i.e., (DDB1-DDB2)2] with two molecules of damaged DNA bound, forming novel intermolecular interactions to a previously structurally unresolvable NT domain of DDB2. The helical fold of DDB2s NT-domain allows extensive contacts to be formed to both DNA molecules complexed to the dimeric UV-DDB. To verify that damaged-DNA binding induces dimerization of UV-DDB, we applied electron microscopy, atomic force microscopy, solution biophysical, biochemical, and functional analyses to characterize the molecular states of UB-DDB as a function of assorted UV-damaged DNA oliogonucleotides and genomic DNA. Altogether, we have applied tangential experimental approaches to verify the damaged DNA binding dependency for the dimerization of UV-DDB. The consistency of molecular dimensions determined, despite differences in the methods of analysis, validates, and extends our original structural findings. Mechanistically, we propose that binding of damaged DNA induces domain folding in the context of the bound UV-damaged DNA substrate, favoring NT-helical ordering of DDB2, which in turn promotes dimerization as a function of nucleotide

binding in UV-DDB. The temporal and spatial interplay between domain ordering and dimerization provides an elegant molecular rationale for the unprecedented binding affinities and selectivities exhibited by UB-DDB for UV-damaged DNA. We surmise that a functional role of dimerization could be related to verification, to molecularly ensure that high-affinity intermolecular contacts are formed only when the correct substrates are bound. This oligomerization step in the NER pathway may represent a key checkpoint before repair proteins are recruited, a crucial junction before cellular resources are fully invested. In the context of chromatin modeling, the DDB1-CUL4ADDB2 nucleosome, according to the subunit alignment defined by our dimeric UB-DDB-substrate complex structure, results in spatially and functionally consistent intermolecular arrangement. Overall, our findings provide original insights into the molecular architechure of the DDB1-CUL4ADDB22 E3 ligase and new implications on how molecular organization of multiprotein complexes can regulate associations and modulate reactions in the NER and the UPS pathways.

Structural Basis of Transcription: Crystal Structure of a RNA Polymerase II Transcribing Complex

Christopher Barnes[1,2]†, Monica Calero[1]†, Indranil Malik[3], Aina Cohen[4], Guowu Lin[1], Ian S. Brown[1], Qiangmin Zhang[1], Filippo Pullara[1], Craig Kaplan[3] and **Guillermo Calero[1*]** gcalero@structbio.pitt.edu

[1]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA.[2]Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA.[3]Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843, USA. [4]Stanford Synchrotron Radiation Lightsource, Menlo Park, CA 94025, USA.

We present the first co-crystal structure of Pol II bound to a complete nucleic acid scaffold (NAS). The structure reveals the architecture of the upstream double helix, the non-template strand (NTS) and the full-transcriptional bubble (TB). The upstream double helix lies over a wedge-shaped loop from Rpb2 (residues 862-874) that engages the minor groove of the duplex providing part of the structural framework for DNA-tracking during transcript elongation. Locations of the rudder and fork loop-1 (FL1) at the upstream end of the TB, suggest that residues involved in the contacts may play a direct role in coordinating annealing of the template and non-template strands. At the downstream (or opening) end of the TB, the interaction between NTS and a stretch of Rpb1 residues (1103-1112) unwinds the downstream duplex DNA. Moreover, these Rpb1 residues form a rigid domain with the Trigger Loop (TL, Rpb1 1078-1092), that stabilizes the TL in the open state. These observations suggest that the conformational transition of TL (opening/closure) may be structurally linked to the downstream interactions with NTS, possibly in a synchronized ratcheting manner conducive to polymerase translocation.

$PreQ_1$ Riboswitches Use Diverse Modes of Pyrrolopyrimidine Recognition in Gene Regulation

Joseph E. Wedekind, Department of Biochemistry and Biophysics, Center for RNA Biology, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue Box 712, Rochester New York 14642 USA. joseph.wedekind@rochester.edu

In the past decade, >20 riboswitch classes have been identified that regulate bacterial, archaeal, plant and fungal genes. Frequently located in the 5´-leader sequences of mRNAs, riboswitches are organized as an aptamer followed by a downstream expression platform. Aptamers bind specific ligands, whereas expression platforms harbor signals [e.g. the ribosome-binding site (RBS)] that direct expression of coding sequences. When a cellular effector exceeds a threshold concentration, it binds the aptamer and induces expression platform conformations that mask or unfurl regulatory sequences, leading to gene “on” or “off” states. To elucidate riboswitch mechanisms of action, we investigated the structure and function of two phylogentically distinct bacterial riboswitches, called $preQ_1$-I (class 1) and $preQ_1$-II (class 2). Each “senses” $preQ_1$, the last soluble metabolite in the biosynthetic pathway for queuosine – a hypermodifed base in certain tRNAs. We showed that each riboswitch binds $preQ_1$ with comparably high affinity ($preQ_1$-I K_D 7.4 ± 2.3 nM and $preQ_1$-II K_D 17.9 ± 0.6 nM), and folds topologically into an H-type pseudoknot. This architecture sequesters the RBS via base pairing to the $preQ_1$-bound aptamer. Remarkably, the class 1 riboswitch achieves gene regulation via an economical 33-nucleotides, whereas the class 2 riboswitch comprises 77 nucleotides. Comparison of the respective crystal structures revealed no spatial conservation of the tertiary folds and distinct modes of $preQ_1$ recognition. SAXS analysis showed that each riboswitch is folded extensively prior to ligand recognition. Single-molecule FRET further suggested that the $preQ_1$-I riboswitch uses induced fit for $preQ_1$ binding. Implications for gene regulation will be discussed.

Rational Engineering of Improved Protein Crystallization

Victor Naumov (1), Helen Neely (1), W. Nicholson Price (1), Samuel K. Handelman (1), Alex Kuzin (1), Farhad Forouhar (1), Jayaraman Seetharaman (1), Sergey Vorobiev (1), Rong Xiao (2), Joe Luft (3), Angella Lauricella (3), George DeTitta (3), Gaetano T. Montelione (2), and **John F. Hunt** (1).

Northeast Structural Genomics Consortium (www.nesg.org); Columbia University Department of Biological Sciences, New York, NY (1); the Center for Advanced Biotechnology and Medicine of Rutgers University and the University of Medicine and Dentistry of the State of New Jersey, Piscataway, NJ (2); & the Hauptmann-Woodward Institute, Buffalo, NY (3).

More than 50 years after the solution of the first protein crystal structure, protein crystallization remains a hit-or-miss proposition. Structural genomics consortia have systematically confirmed that crystallization is the major obstacle to the determination of protein structures using diffraction methods. While numerous methods have been developed that have efficacy in improving protein crystallization properties, none work with high efficiency and reliability for the average crystallization-resistant protein. In previously published work from the Northeast Structural Genomics Consortium, analyses of large-scale experimental studies were used to show that the entropy of the surface-exposed side chains is a major determinant of protein crystallization propensity. These studies also identified a number of primary sequence properties that correlate with crystallization success, including the fractional content of several individual amino acids. In a subsequent publication, equivalent methods were used to assess correlations between protein sequence properties and expression/solubility results. These studies demonstrate that the individual amino acids that positively correlate with crystallization success negatively correlate with protein solubility, and vice versa. This effect severely limits the efficacy of single amino acid substitutions in improving protein crystallization because crystallization probability is low unless starting with a monodisperse soluble protein preparation. Therefore, more sophisticated approaches than single amino-acid substitutions are needed for efficient engineering of improved protein crystallization. We have developed such an approach based on introduction of more complex sequence epitopes that have already been observed to mediate high-quality packing contacts in crystal structures deposited into the Protein Data Bank (PDB). Using this "epitope-engineering" approach, we have obtained high resolution crystal structures for a series of proteins that either do not crystallize at all or do not crystallize well with their native sequences. The approach is efficient, with structures being produced for 80% of crystallization-resistant proteins after evaluating an average of four mutant variants. Notably, the thermodynamic solubility of the protein variants yielding high-quality structures is generally equal to or better than the native protein sequence. Therefore, our epitope-engineering methods decouple thermodynamic solubility from the propensity to form a well-ordered lattice under crystallization conditions. The entirety of this work was supported by grants from the Protein Structure Initiative of the US National Institutes of Health.

Crystallization Screening: Technologies and Science

Joseph Luft, George DeTitta, Eleanor Franks, Angela Lauricella, Raymond Nagel, Edward Snell and Jennifer Wolfley, Hauptman-Woodward Medical Research Institute, Buffalo, NY, 14203 luft@hwi.buffalo.edu

Technology is defined as the application of scientific knowledge for practical purposes. Stagnant technologies do not survive; they are surpassed by scientific advances and the development of better technologies. Crystallization screeing exemplifies the successful advancement of technology through science. High-throughput methods to set up, image and report outcomes from crystallization screening assays are ever-evolving, multi-stage, technological processes. There are continuous improvements generated by experimental results, analysis and the application of technological advances. An iterative scientific approach, to develop technologies and expertise, is the foundation of the high-throughput crystallization screening laboratory at the Hauptman-Woodward Medical Research Institute. This laboratory has assayed more than 15,000 biological macromolecules for crystallization since its inception in 2000. We will discuss the latest crystallization technologies available to the scientific community through this facility.

Nanocrystal Identification and Crystal Optimization by Electron Microscopye

Hilary Stevenson, Alexander Makhov, Monica Calero, Irimpan Mathews, Lin Guowu, Christopher Barns, Hugo Santamaria, James Conway, Aina Cohen, Guillermo Calero, Structural Biology Department, University of Pittsburgh, Pittsburgh, PA.
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The emergence of X-ray free electron laser (X-FEL) based serial femtosecond nano-crystallography allows nanometer-sized crystals (nano-crystals, NCs) to be used, for the first time, to solve high-resolution protein structures. Presently, however, NCs that are not derived from conditions yielding large crystals cannot be identified nor can their quality be evaluated. In order to identify protein NCs, brightfield microscopy, UV-tryptophan microscopy and dynamic light scattering (DLS) techniques were employed to provide prescreened samples for electron microscopy (EM). UV-positive conditions bearing nano-particles were further analyzed by EM using negatively stained samples. EM permitted detection of NCs for most samples tested, including challenging targets such as membrane proteins and multi-protein complexes. Moreover, crystal lattice visualization using EM allowed 1) corroboration of the proteic nature of the NCs, and 2) determination of NC quality by calculating the electron diffraction pattern of the NC lattice. In addition to the advantages that EM brings to the field of nano-crystallography, NC imaging can be highly beneficial to improve crystallization conditions during conventional crystallography screens. This makes EM a fundamental tool for evaluating NCs, as essential as brightfield microscopy is for evaluating traditional, large crystals.

A Common Supramolecular Motif in the Alkali Metal Salts of H-Acid, Including the Incommensurately Modulated Phase of the Commercially Important Sodium Hydrate.

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H-Acid (4-ammonio-5-hydroxynapthalene-2,7-disulfonate) is a coal tar derivative and important intermediate in the manufacture of hundreds of dyes and some common histology stains. Despite the fact that on the order of one million metric tons of the sodium salt have been manufactured in the past century, no crystal structures incorporating this organosulfonate have been reported. Diffraction quality needles of the hydrated sodium salt are easily prepared by slow cooling a concentrated aqueous solution under anaerobic conditions. Variable temperature X-ray diffraction studies revealed the structure exhibits positional disorder of donor atoms around aqua-bridged Na^+ dimers between room temperature and 180 K. Upon further cooling the structure undergoes a reversible phase transition to an ordered, incommensurately modulated structure with retention of crystal quality. Structure elucidation of other alkali metal salts of H-Acid revealed them to have variant packing with no modulations and no disorder between 100 K and their hydration temperatures. Although none are isostructural, the stacking motif of the H-Acid is conserved in all cases, and is reinforced by hydrogen bonds involving a keystone water molecule that is retained during the first stages of dehydration. Further hydration leads to deregulation of the crystal specimens in all cases.

Crystal Structure Analysis of Organic Mechanochemical Reaction Products from X-ray Powder Diffraction

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Mechanochemistry affords alternative "green chemistry" synthetic routes to inorganic, metal-organic and organic materials, avoiding or largely reducing the use of crystallization solvents. Grinding the reactants with small quantities of solvents, called liquid assisted grinding (LAG), gives rise to the selective formation of particular polymorphs of the products.[1] Our work is focused in the synthesis, crystal structure determination from powders and solid state characterization of polymorphs of charge transfer salts (CTS) of the electron donor tetrathiafulvalene (TTF). The differences in bulk physical properties (*e.g.* electrical conductivity) observed in polymorphic modifications of these materials are remarkable. TTF forms CTS with electron acceptors such as *p*-benzoquinone and its derivatives, and it has two polymorphs with monoclinic and triclinic crystal structures. The LAG products of CA (CA=chloranil or 2,3,5,6-tetrachloro-*p*-benzoquinone) with both TTF forms using six solvents of increasing polarity index (toluene, ethyl acetate, acetone, acetonitrile, dimethyl sulfoxide and water) yielded two polymorphs of TTF-CA.[2] The properties of the LAG solvents, such as polarity index, as well as the TTF polymorphism play a role in determining the TTF-CA polymorph obtained. At room temperature the green form of TTF-CA is a pseudo-neutral CTS, obtained by LAG with low polarity solvents; whereas the black TTF-CA polymorph is an ionic compound, synthesized by grinding either TTF form with water or dimethyl sulfoxide, the most polar solvents. Moreover, the black TTF-CA form was obtained by LAG with acetonitrile, only from the monoclinic TTF polymorph. At present *direct-space methods* allow the determination of the crystal structures of organic materials from powders almost as a routine procedure, using global optimization algorithms such as simulated annealing and the known molecular geometry of the fragments to locate in the unit cell. The methodology will be briefly summarized and a few examples will be discussed. For mechanochemical products such as the materials above, often size/strain peak broadening interferes with indexing and space group determination. The latter can be partially overcome by LAG, which tends to yield products of increased crystallinity, and thus more often amenable to crystal structure determination from powders. The crystal structure of the black (ionic) TTF-CA polymorph has been solved from high resolution room temperature X-ray powder diffraction data which will be discussed in detail. While the green TTF-CA polymorph undergoes a pseudo-neutral to pseudo-ionic phase transition at around 81K,[3] the crystal

structure of the black (ionic) TTF-CA polymorph remains essentially unchanged between room temperature and 20K.[4]

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Elucidating Fine Structural Features and Physical Properties in Responsive Materials Via Single Crystal X-ray Diffraction

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The talk will present examples of how single crystal X-ray diffraction techniques were used to understand the nature of the structural transition, twinning, and stacking faults in the RE_5X_4 magnetocaloric phases (*RE* is a rare-earth metal, *X* is a *p*-element). In these materials, the ferromagnetic ordering is concomitant with structural transition, and a detailed analysis of the phase transitions can provide valuable insights into the structure-property relationship and allow to formulate some guiding principles for the design of new magnetocaloric materials. The second part of the talk will discuss how the Sb/Bi disorder in the thermoelectric $RE_2(Sb,Bi)O_2$ phases can be related to the changes in the transport properties. We showed that by controlling the Sb/Bi disorder, we can improve the electrical conductivity and Seebeck coefficient simultaneously, while the number of charge carriers remains constant.

Challenges and Breakthroughs During the Pursuit of the Structure-based Catalytic Mechanism of Inorganic Pyrophosphatase from *Thermococcus thioreducins*

Joseph D. Ng, Ronny C. Hughes, Leighton Coates, Michelle Morris, Manavalan Gajapathy, Matthew P. Blakely, Xiang-Quiang Chu, Marc L. Pusey, and Juan M. Garcia-Ruiz, University of Alabama in Huntsville, Huntsville, AL ngj@uah.edu

Using a fluorescence-based approach to determine initial protein crystallization conditions coupled to the counter-diffusion crystallization process in capillary vessels, the soluble inorganic pyrophosphatase from *Thermococcus thioreducins* (IPPase) has been crystallized in several unique metal or substrate complexes within several different space groups for X-ray and Neutron Crystallography analyses. Over 10 crystallographic structures have been determined for IPPase revealing molecular snapshots of the protein positions with its substrates at the atomic level. Consequently, a structure-based enzymatic mechanism of the IPPase is proposed revealing a sequence of catalytic events associated with the hydrolysis of phosphate. The challenges and breakthroughs in the methods developed and employed concerning the crystallization screening and optimization procedures; X-ray and Neutron data collection and processing; and molecular modeling will be discussed.

Hughes, R.C., Coates, L., Blakeley, M.P., Tomanicek, S.J., Langan, P., Kovalesvsky, A., Garcia-Ruiz, J.M., and Ng, N.D. (2012). Inorganic Pyrophophospatase Crystals from *Thermococcus thioreducens* for X-ray and Neutron Diffraction. Acta Cryst. Sect F, Struct. Biol. Commun. 68: 1482-1487.

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Lipid Bilayer Nanodisc Incorporation Facilitates the Investigation of Membrane Bound Cyclooxygenase-2

Benjamin Orlando[1], Daniel McDougle[2], Michael Lucido[1], Ed Eng[3], David Stokes[3], Aditi Das[2], Michael Malkowski[1], [1]Hauptman-Woodward Medical Research Institute and Department of Structural Biology, The State University of New York, Buffalo, New York, 14203, [2]Department of Comparative Biosciences, University of Illinois Urbana-Champaign, Urbana, Il 61802, [3]New York Structural Biology Center, 89 Convent Ave. New York, NY 10027. orlando@hwi.buffalo.edu

Growing diffraction quality crystals is a substantial hurdle to elucidating membrane protein structure. Membrane proteins remain greatly underrepresented in the Protein Data Bank and monotopic membrane proteins that insert into only one leaflet of the lipid bilayer are even less structurally characterized than integral membrane proteins. Importantly, the confines of the crystal lattice may influence the structure and dynamics of solution techniques such as SAXS, FRET, and SDLS-EPR are attractive methods for obtaining distance and dynamics information outside of the crystal lattice. Crystals of membrane proteins are most often grown from detergent solubilized protein. Alterations to protein structure, dynamics and function caused by detergent mediated extraction from lipid membrane may often go unrecognized. To investigate these possible limitations, we have incorporated the monotopic membrane protein Cyclooxygenase-2 (COX-2) into lipid bilayer nanodiscs of controlled size and composition. A dual affinity purification approach was developed to ensure isolation of COX-2:nanodisc complexes with high purity and monodispersity. Size exclusion chromatography and negative-stane EM confirmed that COX-2:nanodisc complexes had been properly formed and purified. Compared to detergent solubilized enzyme, nanodiscs incorporated COX-2 had similar kinetic characteristics with various fatty acid substrates and COX inhibitors. The nanodisc incorporated COX-2 was also allosterically activated by palmitic acid in a similar fashion to detergent solubilized enzyme. We analyzed COX-2:nanodiscs formed with POPC, DOPC, POPS, or DOPS lipids and determined that lipid composition caused no significant alterations to COX catalysis or inhibition. Thus, the detergent and nanodiscs maintain COX-2 in a similar catalytically active state. However, functional reconstitution of COX-2 and other fatty acid oxygenase family members into lipid bilayer nanodiscs will serve as a future utility in investigating the structure, dynamics and function of these important enzymes in the lipid bilayer bound state. The controlled size, monodispersity, and lack of detergent micelles make nanodisc incorporated COX-2 an attractive candidate for future studies including SAXS, DLS, and SDSL-EPR which are difficult or impossible in detergent micelle or liposome solutions.

This work was supported by NIH NIGMS grant R01 GM77176.

Opsin, a Structural Model for Olfactory Receptors?

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The large family of G protein-coupled receptors (GPCRs) detects signaling molecules such as hormones, neurotransmitters and odorants. About half of GPCRs are olfactory receptors (ORs), for which high-resolution structures remain elusive. ORs belong to the family of rhodopsin-like GPCRs. Rhodopsins are the photoreceptors in vision and their ligand, retinal, shares high hydrophobicity with OR ligands. We determined a new crystal structure of the opsin apoprotein, i.e. retinal-free rhodopsin, from the disc membrane of vertebrate retinal rod cells. A molecule of octylglucoside was identified in the ligand-binding pocket, replacing retinal, and stabilizing the active receptor conformation. The surprisingly well-defined hydrogen-bond pattern which holds octyl glucoside in the ligand-binding pocket was reminiscent of the dynamic hydrogen-bond pattern proposed for OR/ligand interaction, in which the receptor offers changing side chains for bonding. Further, experiments with various detergents resulted in a defined capability to occupy opsin's ligand-binding pocket. These findings indicated that the interaction of detergents with opsin may provide a simple model that mimics OR activation. The study provides a possible way for hydrophobic odorants to enter ORs, supports odorant-receptor hydrogen bonding for OR activation and recommends opsin as a suitable basis for OR homology modeling.

Femtosecond High-resolution Diffraction Studies at LCLS

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A major challenge of structural investigations of metalloproteins at synchrotrons is the damaging effects of radiation exposure. Even small X-ray doses can reduce or initiate reactions at metal centers modifying the active site. For example, *in-situ* visible absorption spectroscopy measurements have demonstrated that the heme/copper active site in oxidized ba_3 cytochrome oxidase (ba_3) is compromised during a single X-ray diffraction exposure. The use of ultrashort X-ray pulses at LCLS provides a means to measure high resolution diffraction before these damage processes occur. To this end, experiments were conducted at LCLS using large multiple crystals (> 50 µm) of ba_3, hydrogenase and myoglobin. Crystals were mounted in 'grids' or loops and flash frozen. The grids hold up to 75 crystals in known locations and are compatible with the Stanford Automounter used to exchange them. Following a semi-automated grid alignment procedure, a fully automated routine was used to position each crystal and collect a series of diffraction images using a Mar325 detector and the Blu-Ice/DCS control system that coordinated with the LCLS EPICS system and XPP DAQ software. Single femtosecond X-ray pulses produced a 'damage free' still diffraction image from each crystal. To provide additional information about crystal orientation, a series of pseudo-oscillation images were collected +/- 5.5 degrees spanning the orientation of the still image. For each one degree oscillation image the crystal was exposed to 120 attenuated X-ray pulses. A hard X-ray spectrometer was used to measure the energy spectrum of each individual X-ray pulse. The first of these experiments were carried out in December 2012 when data were collected from over 1400 crystals. The details and results of these experiments will be presented.

Locating Hydrogen Atoms in Enzymes Using Neutron Protein Crystallography

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The potential of neutron crystallography to help resolve enzymatic mechanism questions will be illustrated with the study of the W186Q xylose isomerase (E186Q XI) mutant. The pH optimum of this mutant is displaced to lower pH, making it of interest in biofuel production. We have solved the neutron structure of cyclic glucose bound E186Q XI at pH 7.7. The structure reveals an extended hydrogen bonding network that connects conserved residues Lys289 to Lys183 through conserved water molecules and residue 186 and suggest a novel acid/base pair that promotes sugar linearization. We will also introduce IMAGINE, a new high intensity, quasi-Laue neutron crystallography beam line developed at the High Flux Isotope Reactor (HFIR) at Oak Ridge National Laboratory (ORNL), that enables neutron protein structures to be determined at near atomic resolutions (1.5Å) from crystals with volume <1 mm^3 and with unit cell edge of <100Å. We will present results from data collected on standard proteins on the instrument. IMAGINE is now accepting user proposals for beam time allocation starting January 2014.

The acquisition and installation of IMAGINE at the HFIR was funded by the National Science Foundation (NSF) under award No. 0922719. ORNL is managed by UT-Battelle, LLC for U.S. Department of Energy under Contract DEAC05-00OR22725.

Developments and Opportunities for Structural Biology at MacCHESS

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MacCHESS ("**Mac**romolecular diffraction at **CHESS**") is an NIH funded facility at the Cornell High Energy Synchrotron Source providing support for macromolecular crystallography and BioSAXS, including access to a unique pressure cryocooling system. Special MacCHESS strengths are exceptional user support and the development of new x-ray methods to benefit the entire structural biology community.

MacCHESS beamlines, currently fed by wigglers, will be upgraded over the next 2-3 years with undulators and new optics to enhance rapid collection of data from weakly diffracting samples.

Crystallographic stations are well equipped with data collection systems including high-performance goniometer, selectable 100-µm or 20-µm beam size, cryocooling, click-to-center crystal centering, automounter, etc. Large CCD-based area detectors are presently installed, and pixel-array detectors are in the works. BSL-2 biohazards can be handled. Methods for collecting and processing multi-crystal data sets are under development.

The BioSAXS station features a dual SAXS/WAXS setup using 2 Pilatus 100K detectors and an integrated computer-controlled flow system including robotic sample loading from 96-well trays, a capillary cell with a replaceable insert, and a convenient graphical interface. Associated equipment for sample preparation and monitoring is being upgraded and increasingly integrated with the data collection system. Microfluidic “lab-on-a-chip” units are under development. An annual workshop is held to educate users in the intricacies of BioSAXS.

Pressure cryocooling is a novel method for cryocooling crystals under pressure which reduces both cooling-induced degradation and the need for penetrating cryoprotectants, and can stabilize mobile ligands and possibly reaction intermediates. We offer pressure-cooling as a service to CHESS users, while continuing to develop the method. Several sample mounting techniques are now available, and the technique has promise for use with biological samples other than crystals.

Hybrid Pixel Array Detectors in the Home Lab: Applications for Diffraction and SAXS on Rigaku X-ray Generators

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The final years of the 20th century saw widespread use of integrating area detectors in the home lab. Most common technologies were based on imaging plates and CCD/CMOS chips. While each format had its own strengths and weaknesses these products almost universally lead to increases in data collection speed and accuracy. However, inherent limitations in the technologies prevented their use in some situations. First, all integrating detectors must build a signal over the course of the exposure, which is followed by a separate readout step when the shutter is closed. This dependency on opening and closing the shutter limits data collection speed even for the fast CCDs. Also, IPs and CCD/CMOS detectors cannot directly read X-rays. They must first convert the X-rays to visible light for readout. This introduces a source of noise and a decrease in signal. The recent introduction of the Pilatus hydrid pixel array detectors by Dectris to the synchrotron beamline has advanced data collection technology beyond what was possible for previous technologies. These instruments are capable of directly detecting X-rays and converting that to an electric signal. Real time data readout is now a reality. The pixel array detectors are faster, more sensitive, and have no electronic noise. By combining these with advanced X-ray sources from Rigaku we can see these advantages in the home lab. Data collection at home has never been more accurate, efficient, and cost effective.

Design and Characteristics of Metal Jet X-ray Sources

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The brilliance of laboratory-based X-ray sources has been fundamentally limited by the melting point of solid anodes. By employing a high speed liquid metal jet as a target it is possible to surpass this limitation and thus achieve brilliance orders of magnitude higher than conventional solid targets sources. The design, operational characteristics and applications of metal jet-based sources are described.

The SER-CAT Virtual Beamline: Lessons Learned When Over 95% of All Data Are Collected Remotely

John P. Rose, John Chrzas, Jim Fait, John Gonczy, Zheng-Qing "Albert" Fu, Zhongmin Jin, Rod Salazar, Gerold Rosenbaum & Bi-Cheng Wang. Southeast Regional Collaborative Access Team and the Department of Biochemistry and Molecular Biology University of Georgia, Athens, GA 30602. rose@bcl4.bmb.uga.edu

From its inception, SER-CAT has been working towards the goal of providing its users with “Light When YOU Need It!” via the concept of a “*virtual home synchrotron*” which could be integrated into their daily work much like the X-ray lab down the hall. SER-CAT began exploring automation of its beamlines shortly after the signing of MOU with APS in March 1999. Working with Oceaneering Space Systems a conceptual design for automated data collection robot (ASTRO) was developed in 2000. In 2003, using funds from the Georgia Research Alliance, automation of the SER-CAT beamlines began with the installation of a highly modified Berkeley/ALS automounter on SER-CAT's bending magnet beamline 22BM closely followed by a higher capacity version on its undulator beamline 22ID. SER-CAT’s web-based experimental control system *SERGUI* was also designed with remote access in mind. *SERGUI* follows the *Bul-Ice* tab format with each tab representing one of the key data collection steps. The web-based nature of the experimental control system means that remote users have full control of the beamline from their home lab including MAD/SAD data collection, beamline/goniometer optimization, wavelength selection, sample mounting, annealing, two-click or automated crystal screening, sample rastering, fluorescence scans and traditional, segmented or helical data collection. Today over 95% of SER-CAT members routinely collect data remotely. The high number of remote users means that the system must be both robust and user tolerant. The presentation will focus on the system as a whole and the measures taken to ensure that remote users can routinely collect high-quality data in an efficient manner.

Work supported by the SER-CAT Member Institutions (see www.ser-cat.org), University of Georgia Research Foundation and the Georgia Research Alliance.

Poster Presentations

P1

Lipid Bilayer Nanodisc Incorporation Facilitates the Investigation of Membrane Bound Cyclooxygenase-2

Benjamin Orlando[1], Daniel McDougle[2], Michael Lucido[1], Ed Eng[3], David Stokes[3], Aditi Das[2], Michael Malkowski[1], [1]Hauptman-Woodward Medical Research Institute and Department of Structural Biology, The State University of New York, Buffalo, New York, 14203, [2]Department of Comparative Biosciences, University of Illinois Urbana-Champaign, Urbana, Il 61802, [3]New York Structural Biology Center, 89 Convent Ave. New York, NY 10027. orlando@hwi.buffalo.edu

Growing diffraction quality crystals is a substantial hurdle to elucidating membrane protein structure. Membrane proteins remain greatly underrepresented in the Protein Data Bank and monotopic membrane proteins that insert into only one leaflet of the lipid bilayer are even less structurally characterized than integral membrane proteins. Importantly, the confines of the crystal lattice may influence the structure and dynamics of solution techniques such as SAXS, FRET, and SDLS-EPR are attractive methods for obtaining distance and dynamics information outside of the crystal lattice. Crystals of membrane proteins are most often grown from detergent solubilized protein. Alterations to protein structure, dynamics and function caused by detergent mediated extraction from lipid membrane may often go unrecognized. To investigate these possible limitations, we have incorporated the monotopic membrane protein Cyclooxygenase-2 (COX-2) into lipid bilayer nanodiscs of controlled size and composition. A dual affinity purification approach was developed to ensure isolation of COX-2:nanodisc complexes with high purity and monodispersity. Size exclusion chromatography and negative-stane EM confirmed that COX-2:nanodisc complexes had been properly formed and purified. Compared to detergent solubilized enzyme, nanodiscs incorporated COX-2 had similar kinetic characteristics with various fatty acid substrates and COX inhibitors. The nanodisc incorporated COX-2 was also allosterically activated by palmitic acid in a similar fashion to detergent solubilized enzyme. We analyzed COX-2:nanodiscs formed with POPC, DOPC, POPS, or DOPS lipids and determined that lipid composition caused no significant alterations to COX catalysis or inhibition. Thus, the detergent and nanodiscs maintain COX-2 in a similar catalytically active state. However, functional reconstitution of COX-2 and other fatty acid oxygenase family members into lipid bilayer nanodiscs will serve as a future utility in investigating the structure, dynamics and function of these important enzymes in the lipid bilayer bound state. The controlled size, monodispersity, and lack of detergent micelles make nanodisc incorporated COX-

2 an attractive candidate for future studies including SAXS, DLS, and SDSL-EPR which are difficult or impossible in detergent micelle or liposome solutions.

This work was supported by NIH NIGMS grant R01 GM77176.

P2

Investigating Substrate Binding in Fatty Acid Oxygenases of the Peroxidase-Cyclooxygenase Superfamily.

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The stereospecific oxygenation of unsaturated and polyunsaturated fatty acids (PUFAs) generates a diverse group of lipids with potent biological functions known as oxylipins. In animals, oxlylipins generated by Cyclooxygenase (COX) enzymes are responsible for modulating physiological processes such as cardiovascular homeostasis and reproduction. Similarly, oxylipin production by the α-Dioxygenase (α-DOX) enzymes in plants is part of the pathogen defense mechanism. COX and α-DOX belong to the Peroxidase-Cyclooxygenase (POX-COX) superfamily of heme-containing oxygenases and share similar catalytic and structural properties. Specifically, histidine residues required for heme binding, a catalytic tyrosine, as well as four helices involved in substrate binding are conserved in these enzymes. While oxylipin formation in animal and plant members of the POX-COX superfamily is well studied, substrate binding and turnover in non-animal cyclooxygenases and fungal heme oxygenases is still relatively unknown. The COX-like enzyme found in the marine red algae, *Gracilaria vermiculophylla* (*Gv*COX) produces oxygenated C-20 PUFA prostaglandins as part of a defense and wound healing mechanism. *Aspergillus funigatus* 5,8-Linoleast Diol Synthase (*Af*5,8-LDS) is a fungal enzyme found in a number of Aspergillus species. Oxygenation of fatty acids by this enzyme has been shown to play a role in the development of fungal infections as well as in fungal cell growth, quorum sensing, and apoptosis. Both *Gv*COX and A*f*5,8-LDS show several conserved structural and functional features to the POX-COX superfamily despite their low sequence identity to COX and α-DOX (~20-26%). Homology models of *Gv*COX and *Af*5,8-LDS, generated using murine COX-2 (mCOX-2; PDB ID 3HS5) and Arabidopsis thalina α-DOX (PDB ID 4HHS), predict the presence of several basic residues that may be involved in interacting with the carboxylate group of substrate fatty acids to promote high-affinity binding. To test the role of these residues in substrate binding, wild type and mutant constructs of *Gv*COX and *Af*5,8-LDS were expressed in E. coli. The 6X-His tagged proteins were purified using Ni-NTA chromatography followed by size exclusion chromatography. Oxygen consumption assays were utilized to determine kinetic parameters using different fatty acids and esters. A report of our progress to date is presented.

This work was supported by NIH NIGMS grant R01 GM077176.

P3

Role of Phe-205 in Determining the Stereoselectivity of Arachidonate Oxygenation by Aspirin-acetylated Cyclooxygenase-2

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Non-steroidal anti-inflammatory drugs (NSAIDs) are inhibitors of the cyclooxygenases (COX-1 and -2), which generate prostaglandins (PGs) from arachidonic acid (AA). Despite high sequence and structural similarity between the isozymes, research has shown that the classically "nonselective" inhibitor aspirin (ASA) exerts a differential effect. Ser-530 acetylation following ASA treatment leads to complete inhibition of COX-1; however, acetylation of COX-2 leads to a shift of the product profile from generation of primarily 15*S*-prostaglandin H_2 (PGH_2) to that of 15*R*-hydroxyeicosatetraenoic acid (15*R*-HETE). 15*R*-HETE, in turn, is a precursor in the production of 15-epi-lipoxins, which are inflammation-resolving lipid mediators. Molecular dynamics studies suggest that the product shift in stereochemistry and relative abundance may be the result of the steric environment within the active site. The covalently modified Ser-530 increases the steric bulk, forcing AA to adopt an altered conformation and enhancing the shielding on the side of AA opposite the catalytic Tyr-385 (i.e. antarafacial face). The altered AA conformation and local shielding from the acetyl group is such that an alternate oxygenation occurs, which utilizes a secondary oxygen-binding pocket located between Tyr-385 and Phe-205 (i.e. suprafacial face). Though the molecular dynamic simulations have provided a feasible explanation for the generation of monooxygenated, 15*R* stereoisomers, there is presently no experimental evidence to support the steric shielding hypothesis in COX-2. In order to address this, we have generated a set of mutant COX-2 proteins that have an altered secondary oxygen-binding pocket. By mutating Phe-205 to a set of smaller hydrophobic and hydrophilic residues, we intend to experimentally address the role this pocket may have in binding oxygen and providing an alternate attack trajectory in acetylated COX-2. A report of our progress to date is presented.

This work was supported by NIH NIGMS grant R01 GM077176.

P4

Structure and Function Analysis of Novel Class 1 $PreQ_1$ Riboswitches from Bacterial Pathogens

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Riboswitches are a class of *cis*-acting functional RNAs that regulate gene expression by directly binding to ligands. Typically found in the 5´-leader sequences of bacterial genes, riboswitches have great potential as novel antimicrobial targets. Already, knowledge of the structural basis of ligand recognition has facilitated the design of novel antimicrobial-like compounds[1]. Of special interest are riboswitches that bind to ligands unique to the bacterial metabolome. One such molecule is $preQ_1$, a modified guanine that interacts with a cognate riboswitch in >1060 bacterial species, including numerous pathogenic organisms. Thus far, two classes of $preQ_1$ riboswitches, dubbed $preQ_1$-I and $preQ_1$-II, have been identified and characterized functionally[2]; our lab and others have made progress in the determination of their crystal structures[3]. Recently, an atypical $preQ_1$-I riboswitch was discovered[4] that is characterized by a small aptamer-binding loop, and a genomic distribution confined mostly to a bacterial class that comprises numerous species that are prevalent in nosocomial infections and often resistant to standard antibiotic regimens. To gain insight into this unusual $preQ_1$-I riboswitch, we characterized its $preQ_1$ binding thermodynamics, which revealed affinities distinct from our prior measurements on $preQ_1$-I and $preQ_1$-II sequences. A comparative thermodynamic analysis will be presented as well as preliminary progress on crystallization.

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P5

The Structural Basis for Spt5-mediated Recruitment of the Paf1 Complex to Chromatin

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Polymerase Associated Factor 1 Complex (Paf1C) broadly influences gene expression by regulating chromatin structure and the recruitment of RNA processing factors during transcription elongation. The Plus3 domain of the Rtf1 subunit mediates Paf1C recruitment to genes by binding to a repeat domain within the elongation factor Spt5. Here we provide a molecular description of this interaction by reporting the structure of human Rtf1 Plus3 in complex with phosphorylated Spt5 repeat. We find that Spt5 binding is mediated by two interfaces, a phosphothreonine recognition interface and a hydrophobic interface that includes residues outside the Spt5 motif. Changes within these interfaces diminish binding of Spt5 in vitro and chromatin localization of Rtf1 in vivo. The structure reveals the basis for recognition of the repeat motif of Spt5, a key player in the recruitment of gene regulatory factors to RNA polymerase II.

P6

The CAPERα: UHM Provides a Potential Means for Alternative Splice Site Regulation.

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CAPERα is of major medical interest as it plays a crucial role in tumor progression in Ewing sarcoma1 and other cancers. In its physiological function, CAPERα is responsible for the alternative splicing of genes under the control of certain steroid receptor promoters. However, the molecular mechanisms and pathways of splice site regulation by CAPERα remain unknown. CAPERα contains a putative U2AF homology motif *UHM), a domain frequently found in early stage 3' splice site recognition factors. UHMs recognize a key tryptophan in short sequences termed "U2AF ligand mofits" (ULM)2. We determined structures of the CAPERα UHM bound to a prototypical ULM at 1.74A resolution, and for comparison, in the absence of ligand at 2.20A resolution. The binding pocket for the ULM key tryptophan appears pre-formed in the apo-structure (rms 0.667A). The UHM/ULM-interactions authenticate CAPERα as a bona fide member of the UHM-family of proteins and suggest possible regulation of UHM-interactions by phosphorylation within the ULM. Furthermore, we identified the splicesome subunit SF3b155 as a relevant binding partner for CAPERα. Because SF3b155 also interacts with UHMs of constitutive splicing factors, this work demonstrates a new interface for the regulation of alternative and constitutive pre-mRNA splicing.

P7

Investigation of Non-Ribosomal Peptide Synthesis-Related Genes from an Uncharacterized Operon Implicated in *Acinetobacter baumannii* Motility

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The gram-negative *Acinetobacter baumannii* microbe is gaining notoriety as a dangerous infectious agent particularly in immunocompromised individuals. Due to its persistence to disinfectants, *A. baumannii* can be particularly hard to eradicate, thus making it a risk for hospital-acquired infection. Although it has been historically classified as non-motile, recent studies have revealed that these bacteria are, in fact, motile. Moreover, multiple genes implicated in bacterial motility are part of an operon that encodes a non-ribosomal peptide synthetase (NRPS)-derived secondary metabolite (1). This operon contains eight genes with two known NRPS modules. A crystal structure has been obtained for the peptidyl carrier protein ABBFA_003404, and substrate specificity has been investigated for the adenylation domains ABBFA_003403 and ABBFA_003406. Initial biochemical reconstitution of this pathway is underway and aims to determine the product being synthesized by these genes. Taken together, this work may aid in the understanding of NRPS domain choreography and the role of bacterial secondary metabolites in motility.

P8

Structural Basis of Transcription: Revealing the Structure of RNA Polymerase II Transcribing Complex

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Over the last decade, structural studies of the eukaryotic transcriptional machinery have revealed the nature of the DNA-RNA hydrid; the mechanisms of nucleotide selection and catalysis; as well as the mechanism of polymerase backtracking. Notwithstanding numerous structures published to date, structural details of a completely transcribing complex, including upstream and downstream DNA duplexes, have yet to be revealed. Here we report for the first time the co-crystal structure of RNA Polymerase II (Pol II) in complex with a 40-nucleotide scaffold to 4Å, illustrating the complete nucleic acid architecture of a transcribing Pol II. The nucleic acid scaffold is supported by multiple contacts with Pol II: 1) the upstream DNA duplex is supported by a wedge shaped loop of Rpb2 that engages the minor groove, 2) fork loop-1 residues of Rpb1 spatially coordinate nascent RNA at the arch allowing for re-annealing of upstream DNA strands during translocation, 3) the non-template strand is stabilized by Rpb1 residues at the opening end of the transcription bubble, and 4) interactions with the downstream DNA duplex by Rpb5 jaw residues. Overall, our data suggests that structural elements involved in DNA tracking could play important roles in promoting interactions with initiation, elongation or termination factors.

P9

Combining Crystallography, SAXS, Bioinformatics and Molecular Dynamics to Assess the Structure and Dynamics of Eukaryotic GlnRS

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Eukaryotic glutaminyl-RNA synthetase (GlnRS) contains an appended N-terminal domain (NTD) whose precise function is unknown. Although GlnRS structures from two prokaryotic species are known, no eukaryotic GlnRS structure has been reported. Here we present the first crystallographic structure of yeast GlnRS, finding that the structure of the C-terminal domain (CTD) is highly similar to *E. coli* GlnRS, but that 214 residues, including the NTD, are crystallographically disordered. We solved the structure of the isolated NTD and discovered a previously unknown structural homology to a protein (GatCAB) involved in a distinct, but related mechanism of gln-tRNA formation found only in prokaryotes. We present a model of the full-length enzyme in solution, using the structure of the CTD, and the isolated NTD, with SAXS data of the full-length molecule. We proceed to model the enzyme bound to tRNA, using the crystallographic structures of GatCAB and GlnRS-tRNA complex from bacteria. We contrast the tRNA-bound model with the tRNA-free solution state and perform molecular dynamics on the full-length GlnRS tRNA complex, which suggests that tRNA binding involves the motion of a conserved hinge in the NTD.

P10

Novel Approaches to Stabilize Large Protein Assemblies for Crystallization Experiments

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Proteins are both building blocks and molecular motors for virtually all interacting heterogeneous assemblies (henceforth referred to as multi-protein complexes or MPCs) whose components exchange continuously. Therefore, MPCs could be seen as a continuously morphing protein conglomerate, where subunits are swapped in and out as the stages of a physiological process progress in time and space. Solving the atomic structures of multi-protein complexes is the biggest challenge that modern-day structural biologists (SB) face. MPCs are an enormous and unexplored resource to SB, and in many instances MPCs hold the key to comprehend human disease at atomic level. As a result of their transient nature, MPC components typically have low binding affinities; moreover, the harsh conditions of the crystallization process often results in complex disassembly or sub-stoichiometric samples. In order to stabilize MPC for crystallization experiments, we have developed novel approaches using cross-linking agents. The use of chemical cross-linkers in solution is a long-standing and well-established technique; however, their use in solution often leads to large protein aggregates resulting from cross-liming of several MPCs. In order to overcome MPC aggregation, we performed cross-linking experiments on MPCs pre-bound to affinity or charged matrices. On-column bound MPCs are effectively isolated from one another preventing inter-complex cross-linking and therefore formation of large aggregates. We corroborate the monomeric nature of cross-linked MPCs using size-exclusion chromatography and electron microscopy techniques. On-column cross-linking technique allows large-scale efficient MPC stabilization for crystallization experiments.

P11

Integration of *in situ* X-ray Diffraction Analysis with High-throughput Crystallization Screening

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The Hauptman-Woodward Medical Research Institute operates a high-throughput crystallization screening service against 1,536 different cocktails. These represent an incomplete factorial sampling of chemical space (694 cocktails) and commercially available crystallization cocktails (842 cocktails). The success rate in identifying a condition for future optimization historically approaches 50%. However, less than half of these lead to deposition into the PDB. Failure has multiple pathologies, e.g., failed optimization, cryocooling or handling that may destroy diffraction properties, etc. To overcome this we have incorporated *in situ* synchrotron data collection directly from our screening plates. *In situ* data collection is not new but we show that our 1,536-well plates can be safely transported, crystals observed in the screening lab are maintained on the beamline, diffraction data can be collected from multiple crystals in each well, and that structural information is attainable. Our initial trials include a freshly screened protein (N-Type ATP Pyrophosphatase from *Pyrococcus furiosus)* from the Northeast Structural Genomics (NESG) group which produced visual crystallization hits and *in situ* diffraction data. From one well it was possible to collect enough data of sufficient quality to identify a ligand present in the sample. The use of a standard optimization plate closes a gap in the pipeline from a protein going into screening to structure coming out in a highly efficient manner. Preliminary results and future directions are presented.

P12

LCLS Protein Crystal Screening

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The LCLS Protein Screening (PCS) program aims to enable new users to get their first access to LCLS beam time for biological structure determination and also to allow testing samples in preparation of a full LCLS proposal. The available time for PCS studies will be limited to 6 hours. This allocation was chosen as it can be sufficient for both short screening runs of different preparations to evaluate crystal quality, as well as collection of a full data set under good running conditions, while providing a maximum number of different groups access to LCLS beam time. To enable measurements within such a short time frame and maximize the chances of success and efficiency, these studies must be carried out with limited flexibility to minimize the time impact of setup changes. At this time, gas dynamic virtual nozzle (GDVN) experiments are possible at CXI and several possibilities for fixed targets can be explored with CXI. Currently, all PCS experiments are carried out inside the standard CXI vacuum setup using the 1μm or 100 nm focus and CSPAD detectors, but other options are evaluated on a case by case basis.

P13

Crystallography of the Ferromagnetic Shape Memory Alloy Ni-Mn-Ga: Single Crystal Structure Analysis of Five Layered Martensites

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Shape Memory Alloys, SMAS, are metallic materials that, after mechanical deformation, can "remember" an original, cold-forged shape. Upon heating above a transformation temperature they revert to their pre-deformed shape. Shape memory properties are associated with a crystallographic phase transformation from a high-temperature and high symmetry cubic “austenite” form to a lower symmetry low temperature “martensite” form with the ability to form twin domains related by the symmetry of the high symmetry austenite variant. Shift of twin domain boundaries allows for large deformations of the martensite. Upon heating above the phase transformation temperature the austenite (which has no ability to compensate for mechanical stress by twin domain boundary movement) reverts to the original shape of the cold forged martensite even after recooling below the phase transformation temperature.

A special form of SMAs is ferromagnetic shape memory alloys, which combine magnetic and shape memory properties in one compound. Upon application of an external magnetic field crystallographic domains (the martensite variants) align with this field grow at the expense of unfavorably oriented domains by twin boundary motion driven by the applied magnetic field, thus inducing a shape change through application of a magnetic field. One such family of compounds are nickel-manganese-gallium alloys with a near stoichiometric composition at or around Ni_2MnGa, which exhibit a large reversible linear strain of up to 10% under an applied magnetic field. As such, they are regarded as highly promising materials for magneto-mechanical actuators, energy absorbers, and other devices that make use of the magnetically controllable shape memory properties of these materials.

Upon cooling to the martensite transformation temperature the Ni-Mn-Ga Heusler alloys undergo a phase change form a cubic $Fm\bar{3}$ m L21 (Heusler) type into one of several different types of modulated martensite structures, with the type of structure formed depended on the composition of the material and possibly other factors. Commonly observed are fivefold and sevenfold modulations, and the modulation direction is one of

the face diagonals of the original cubic phase. While the structure of the high temperature austenite phase of Ni-Mn-Ga alloys is known to at least some degree, the situation for the low temperature martensite forms is sketchy at best. Structures were heavily investigated by electron diffraction techniques, by powder X-ray and neutron diffraction techniques, often in combination with superspace approaches to account for their modulated nature, but no full single crystal structure determinations are reported thus far. Twinning and partial occupancy of atomic sites were usually ignored in all previous structure analysis attempts.

Analysis of the Heusler type Ni-Mn-Ga alloys, has been, with the exception of one time-of-flight neutron study, limited to X-ray and neutron powder studies, often in combination with use of the superspace approach for the refinement of modulated structures. The limited quantity of data that can be obtained from multicrystalline samples severely limits the amount of structural detail that can be extracted. Thus, the picture that is available for the structures of these compounds is vague at best. With 20 micron single-crystals - obtained through spark erosion and annealing of the created particles - we were able to collect data with ten minutes exposure per frame on a conventional sealed tube diffractometer. The data obtained indicate five-fold modulation of the non-merohedrically twinned low temperature phase (twin lattices are aligned at 90° to each other). This can be successfully refined in both an orthorhombic F-centered setting in space group *F*mm2, and in a centrosymmetric C-centered setting in space group C2/c that is twinned by pseudomerohedry in addition to the martensite non-merohedral twinning. The two alternative solutions are similar, but not identical, and feature identical structure quality indicators (R values are 2.1 and 2.2%, residual electron density is negligible). The type of modulation differs between the two choices and the monoclinic setting features a center of symmetry that is absent in the orthorhombic setting. The structures are also solid solutions: all three crystallographically independent atom sites are shared between all types of atoms in varying ratios, creating substantial correlations between twinning ratios, occupancy rates, and atomic ADPs. Our preliminary study is the first thorough investigation of any Ni-Mn-Ga alloy by single crystal X-ray diffraction that accurately takes into account the effects of twinning.

P14

Synthesis and Characterization of Copper (I) Complexes With Thiourea and Related Ligands

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Three new series of dinuclear copper (I) complexes, $[Cu_2(dppm)_2(xtu)X_2]$ and $[Cu_2(dppm)(dmtu)_2X_2]$ [X= Cl, Br, and I; xtu = ettu (1-ethylthiourea) or ptu (N-phenylthiourea), dmtu (N,N-dimethylthiourea); dppm = bis(diphenylphosphino)methane] have been synthesized and were characterized using spectroscopic and single-crystal X-ray diffraction techniques. The nine structures each feature a neutral dinuclear Cu (I) species in which each metal center exhibits a distorted tetrahedral coordination. The dppm ligand is bidentate with a chelating tendency ideal for bridging two Cu(I) ions, with Cu-Cu separations of 3.28-3.48 Å in the nine complexes. The ettu and ptu ligands are S-bridging, while dmtu is both S-bridging and S-terminal. N-H-X intermolecular hydrogen bonds connect molecules in adjacent complexes. The effects of solvent molecules incorporated into the crystal structures are discussed.

P15

Understanding the Selectivity of Hydrogen Bonding in Cocrystals of Pyridine-Imidazole Compounds

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Chemical reactivity in nature is not random it is in fact highly selective. Different factors may result in specific compounds not being able to form or form exclusively. This selectivity appears to have several factors, which if understood, could help improve the efficiency of pharmaceutical drugs, agrochemicals, and electronics. Hydrogen bonding is one of the primary interactions within biological systems. Using Pyridine-Imidazole ligands as the basic building block, hydrogen bond preference was measured by using different carboxylic acids which had different potential charges and was compared to the success of cocrystal formation.

P16

Structure of Mycobacterial MycP1 Protease, A Potential Drug Target

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Pathogenic mycobacteria such as Mycobacterium tuberculosis secrete protein virulence factors via the ESX-1 secretion system. These secreted proteins are at the front lines of molecular warfare waged between *Tuberculosis bacilli* and macrophages within the human lung. The secreted effectors prevent lysosomal fusion and promote the escape of bacteria from phagolysosomes, but they are also highly antigenic and eventually bring on the adaptive immune response by activating surrounding T cells. Consequently, ESX-1 secretion is tightly regulated during infection, and drugs that target the ESX-1 machinery may provide new and improved tuberculosis antibiotics. We determined the crystal structure of Mycosin 1, a subtilisin-like serine protease that is an essential component of the ESX-1 system. We found that Mycosin 1 has a unique N-terminal extension, and in contrast with other subtilisin-like proteases it does not require a cleavable pro-peptide for activation. In addition, we developed an in vitro assay based on quenched fluorescent peptides that provides a useful tool for high-throughput screening of Mycosin 1 inhibitors. We plan to follow a structure-based approach to identify and optimize Mycosin 1 inhibitors, which might be used to up-regulate ESX-1 secretion and activate the immune system counterattack during the latent stage of tuberculosis infection.

P17

Identification of a Human Cullin5 Peptide that Interacts with a Complex Comprising HIV-1 Vif/Elongin B/C/Core Binding Factor β

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HIV-1 has evolved several mechanisms to sustain infectivity including co-option of the cellular ubiquitin ligase machinery to degrade host-defense factors, such as APOBEC3, which can mutate first-strand viral DNA[1]. The HIV-1 protein Vif (viral infectivity factor) plays a central role in this activity by serving as a substrate receptor that recruits APOBEC3 family members to an E3 ligase complex where they are targeted for degradation by the proteasome[2]. As part of the E3 ligase complex, Vif interacts with human proteins Elongin B, Elongin C (EloB/C), Core Binding Factor β (CBFβ) and Cullin 5 (Cul5). Despite the identification of key proteins, the molecular level details of this host-virus complex have been elusive, and no crystal structure has been reported for Vif. Leveraging the discovery of CBFβ as a stabilizer of Vif[3], we developed methods to produce milligram amounts of pure E3 ligase-like complexes. We confirmed by ITC that Cul5 binds Vif/EloB/C/CBFβ with a K_D of 5 ± 2 nM[4], and proceeded to investigate the binding interface. Using hydrogen-deuterium exchange mass spectrometry we identified a ~30 amino acid region of Cul5 that becomes protected from solvent exchange in the context of the Cul5/Vif/EloB/C/CBFβ complex. We then tested a series of synthetic, Cul5 peptides for binding to the Vif/EloB/C/CBFb complex. Our results revealed several nested Cul5 peptides that bind with apparent K_D values in the mM to nM range. Boundaries will be further refined for use in crystallization trials, and to devise inhibitors with the goal of blocking the host-virus interaction.

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P18

Influenza A PB2 and Human MAVS Complex: Extending Crystal Structural Data to Identify Virus-host Protein-Protein Interaction Determinantes of Host Tropism

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Influenza A virus is an important human pathogen accounting for widespread morbidity and mortality, with new strains emerging from animal reservoirs possessing the potential to cause pandemics. The influenza A RNA-dependent RNA polymerase complex consists of three subunits (PA, PB1 and PB2) and catalyzes viral RNA replication and transcription activities in the nuclei of infected host cells. Variations in the PB2 subunits sequence have been implicated in both pathogenicity and host adaptation. This includes the inhibition of type I interferon induction through interaction with the host mitochondrial antiviral signaling protein (MAVS), an adaptor molecule of RIG-I-like helicases. This study reports the identification of the cognate PB2 and MAVS interaction domains necessary for complex formation. Specifically, MAVS residues 1-150, containing both the CARD domain and the N-terminal portion of the proline rich-region, and PB2 residues 1-17 are essential for PB2-MAVS virus-host protein-protein complex formation. The three α-helices constituting PB2 (1-17) were tested to determine their relative influence in complex formation, and Helix 3 was observed to promote the primary interaction with MAVS. The PB2 MAVS-binding domain unexpectedly coincided with its PB1-binding domain, indicating an important dual functionality for this region of PB2. Analysis of these interaction domains suggests both virus and host properties that may contribute to host tropism. Additionally, the results of this study suggest a new strategy to develop influenza A therapeutics by simultaneously blocking PB2-MAVS and PB2-PB1 protein-protein interactions and their resulting activities.

P19

Towards Improved Heme-mimetic Activation of Soluble Guanylyl Clyclase Using Structure-based Drug Design

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BAY58-2667 (BAY58) has been a novel drug lead in clinical phase IIb trials for the treatment of acute decompensated heart failure, but is reported to have some issues with its dose-dependent hypotension development. Recently we solved the structures of BAY58 and BAY60-2770 (BAY60) bound to the H-NOX domain of Nostoc (Ns) sp. On the basis of this structural information, our goal was to design the new derivatives of BAY58 having better activity profiles at lower doses. While testing the effects of various derivatives on sGC activity, we observed that derivative 20 was the most potent among the ones synthesized and it activates sGC by 4.8-fold more than that of BAY58. In the current study, we present the 2.8Å resolution crystal structure of this new sGC activator 20 bound to the Ns H-NOX domain. This structure reveals that the increased conformational distortion at the carboxyl terminus region of important α helix containing 110-114 residues may contribute towards the higher activation of sGC triggered by derivative 20 in comparison to that of its parent BAY58.

P20

Structural Studies of Interactions Between α-Thrombin and Clotting Factors

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The coagulation cascade is a tightly regulated system of cleavage reactions involved in bleeding prevention and wound healing. α-thrombin is a 36 kDa protein produced from the cleavage of 70 kDa prothrombin by Factor Xa in the prothrombinase complex. This active form of thrombin is responsible for the creation of fibrin from fibrinogen in the penultimate step of the coagulation cascade. In addition to this highly essential reaction, α-thrombin cleaves various clotting factors, with effects ranging from amplified thrombin production to crosslinking fibrin monomers. While there is a wealth of structural information for the interactions of thrombin with small molecule inhibitors, detailed conformational views of how thrombin interacts with its protein substrates are limited. Here a combination of X-ray crystallography, computational modeling and molecular dynamics simulations are employed to characterize and explore the structural features of thrombin recognition of several protein clotting factor substrates.

P21

Structural Biology of the Shikimate Pathway in Multi-drug Resistant Gram Negative Bacteria

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Gram negative bacteria (GNB) are becoming increasingly resistant to our current arsenal of antibiotics with new strains of multi-drug resistant (MDR) bacteria arising with greater frequency. The need for new classes of antibiotics is immediate as is the need for new antibiotic targets. A genomic screen for new antibiotic targets in the MDR DNB *Acinetobacter baumanii* was performed and yielded several unrecognized or underexploited targets within *A. baumanii*. These targets were determined to be *in vivo* essential (i.e., essential for growth and survival in a host) using an animal model. All had annotated biological functions, including metabolic, structural, two-component signaling, DNA/RNA synthesis, and protein transport. Several of the *in vivo* essential targets were found in the shikimate pathway utilized by bacteria to create aromatic amino acids. Humans must ingest aromatic amino acids and therefore do not have homologues to the enzymes found in DNB, making the enzymes of the shikimate pathway attractive antibiotic targets. Shikimate kinase (SK), 5-enolpyruvylshikimate 3-phosphate synthase (EPSP), chorismate synthase (CS) and prephenate dehydrogenase (PD) were enzymes identified to be *in vivo* essential. The crystal structure of SK in complex with the substrate shikimic acid has been determined to 2.1Å resolution. The crystal structure of EPSP has been determined to 2.4Å resolution. Low-resolution data (3.5Å) have been collected for crystals of a dual function EPSP synthase-PD fusion protein native to *A. baumanii* and only a few other bacterial species.

P22

Study of Lamprey TTR as a Model for Binding Site Function of Transthyretin-like Proteins.

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Transthyretin (TTR) is a tetrameric protein that is responsible for the transport of thyroxine (3,5,3',5'-tetraiodothyronine; T_4) and related molecules. Recent studies have identified open reading frames in sequenced genomes of invertebrate organisms that were predicted to encode a putative transthyretin homolog called transthyretin-like protein (TLP) that shares a 60% sequence similarity with human TTR and is able to form tetrameric structures as TTR does. TLP genes have been observed in all kingdoms whereas TTR genes are only found in vertebrate genomes. However, TLPs are not able to bind thyroid hormones. These data suggest that the TTR gene may have evolved from the duplication of the TLP gene in early vertebrate evolution. Structural data for TLP from *Salmonella dublin* revealed a dimer of dimers with the TTR fold[1]. Like TTR, TLP has a quaternary structure characterized by a central channel that forms the ligand binding domains. Comparison of these two proteins revealed major differences exist at the dimer-dimer interface of *S. dublin* TLP that alter their physicochemical properties. Sequence changes between TLP and TTR have resulted in an increased negative charge for the TLP binding domain that does not bind thyroid hormone. Also, none of the charged residues in the binding domain of TLP are conserved in TTR. The only exception to this pattern is the recently identified TTR from lamprey (*Lethenteron reissneri)* that has characteristics of both TLP and TTR and is considered the closest protein in the divergence of TLP to TTR. To understand how the lamprey TTR differs from human, the cDNA sequence for lamprey was optimized for recombinant expression in *E. coli* cells and cloned into a pET-SUMO vector for expression and purification. Crystallization trials are underway for lamprey TTR and conditions that resulted in small needles are being optimized. Hormone binding studies from the Yamauchi lab indicate binding of 3,5,3'-triiodothyronine is greater than that of T_4. Other data show that lamprey metamorphosis is driven by a DECREASE in thyroid hormone levels in blood, rather than an INCREASE in hormone levels that occurs in all other vertebrates that undergo metamorphosis. Therefore, structural studies of lamprey TTR can provide insight into its evolutionary diverge from TLP.

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P23

Analysis of Human Transthyretin (hTTR) with the Potent Inhibitor VCP-6, 3',5'-Dichloro-2-carboxy-diphenylamine.

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Structure activity data reveal that thyroid hormone analogues have different binding affinities for human transthyretin (hTTR), a homotetrameric serum protein with two identical binding domains. Furthermore, binding data show that some hormone metabolites, as well as strong binding competitors, have affinities greater than that of the natural ligand, thyroxine (3,5,3',5'-tetraiodothyronine; T_4). Activity data also show that a series of analgesic phenylanthranilic acids such as flufenamic, mefanimic or fenclofenac acids have stronger binding affinities than T_4 for TTR. Based on these findings, a series of potent analogues were synthesized that revealed that VCP-6 was the most potent inhibitor with 523% the affinity of T_4 for hTTR. Data to 1.6Å resolution were collected at beamline 11-1 at SSRL for the hTTR-VCP-6 complex. The crystals were isomorphous to other $P2_12_12$ hTTR complexes that have two independent monomers in the asymmetric unit. An Fo-Fc difference electron density map contoured at 5σ indicates that the 3',5'-dichlorophenyl ring is bound toward the center of the channel along the two-fold axis. There is an intramolecular hydrogen bond between the amine hydrogen and the 2-carboxylate anion that fixes the orientation of the benzoic acid ring that places the 2-carboxylate in hydrogen bonding distance to the terminal amine of Lys15 in both domains. The remaining moiety of the VCP-6 analogue occupies two positions at 50% occupancy along the two-fold axis. Analysis of these data suggests that the enhanced binding affinity of VCP-6 is due to these tight interactions with the residues along the channel. This analogue also binds better to hTTR than Tafamidis, a potent kinetic stabilizer of TTR tetramer formation under clinical trials as an inhibitor of the amyloid cascade[1].

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P24

Structure-activity Correlations for Three Pyrido[2,3-*d*]pyrimidine Antifolates Binding to Human and *Pneumocystis carinii* Dihydrofolate Reductase.

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To define further interactions that enhance selectivity of antifolates against pathogenic dihydrofolate reductase (DHFR), in particular that of *Pneumocystis jirovecii* (pj) DHFR, the causative agent in HIV-AIDS pneumonia, a series of bicyclic pyrido[2,3-*d*]pyrimidine analogues of piritrexim (PTX), a potent but nonselective DHFR inhibitor, have been studied[1]. Of note are a series of analogues in which there was a transposition of the 5-methyl of PTX to the N9-position of the pyrido[2,3-*d*]pyrimidines. It was hypothesized that the N9-methyl group would interact with Ile123 of pjDHFR (and Ile123 of *Pneumocystis carinii* (pc) DHFR), but not with the shorter Val115 in human (h) DHFR. Structure activity data for this series of antifolates revealed that a trifluoro derivative (#**26**)[1] was the most selective against pjDHFR compared with mammalian DHFR (selectivity ratio 35), while compound **24**, a naphthyl analogue, was the most potent and selective for pcDHFR compared to rat liver DHFR (selectivity ratio 9.9), and compound **22,** an isopropyl analogue, ranked highly against both pj- and pcDHFR (selectivity ratio 21 against hDHFR vs 9.9 against rat liver DHFR). The crystal structures of compounds **22**, **24** and **26** were determined in complex with human (h) and pcDHFRs and are compared with structures of pcDHFR active site variants previously reported. Data were collected using the remote access robot at liquid N_2 temperatures on beam line 11-1 at the Stanford Synchrotron Radiation Laboratory imaging plate system for hDHFR-**22,** hDHFR-**26** and pcDHFR-**24** ternary complexes with NADPH. Comparison of the h- and pcDHFR ternary complexes with **26** reveals that the trifluorophenyl ring occupies two alternate conformations as is evident by the additional partial 3σ density for the phenyl ring. One conformer is similar to that observed in the pcDHFR which places the fluro ring in contact with residues Pro66 and Phe69, and another which is closer to residue Ser59 in the active site. As hypothesized, the contacts of the N9-methyl of **26** in pcDHFR are closer to Ile123 (4.8Å) and Thr61 (3.6Å) than in hDHFR (5.8Å, 3.9Å, respectively for Val115 and Thr56). Similar results were observed for the hDHFR-**22** and pcDHFR-F69N-**22** complexes. In the case of the pcDHFR-**24** complex, the contacts between the N9-methyl and Thr61 (3.5Å) and Ile123 (4.4Å) are even closer than observed in pcDHFR-**26** or **22** complexes. The unlike the other inhibitors, the naphthyl ring of **22** makes hydrophobic contacts with Ile33, Ile65 and Phe69. The added interactions are consistent with the similar potency against both pc- and pjDHFR.

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