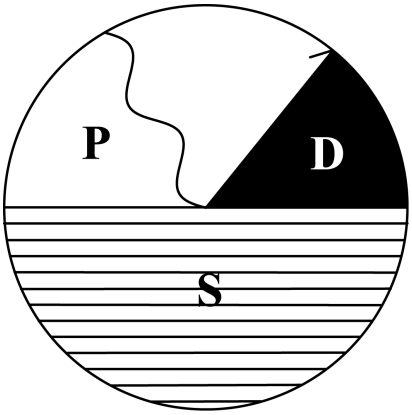


The 65th Annual Pittsburgh Diffraction Conference

Hauptman-Woodward Medical Research Institute
Buffalo, New York

25-27 October 2007

Program and Abstracts

Symposia

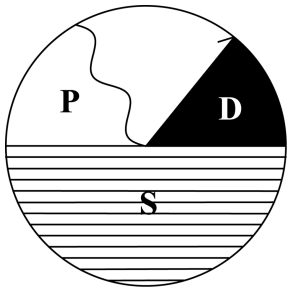
Biomacromolecular Crystallization and Crystal Growth

Biocrystallography with Synchrotron X-rays
and Spallation Neutrons

Biomolecular Dynamics in Crystals

Small Angle Scattering Studies of Biomolecular Structures

Crystal Structures by Powder XRD
and XRD Phasing by Charge Density Flipping



The 65th Annual Pittsburgh Diffraction Conference

Conference Chairman	Robert Blessing
Symposium Organizers	Joseph Luft Edward Snell George Phillips Wayne Schultz Robert Von Dreele John Spence
Poster Session Chairman	Timothy Umland
Local Arrangements Coordinators	Jane Griffin Walter Pangborn

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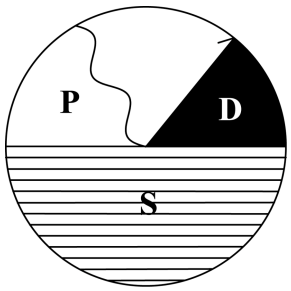


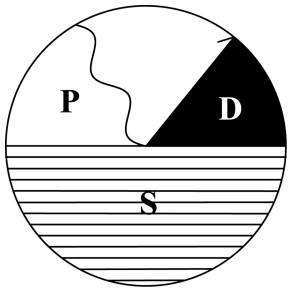
Sidhu Award

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

Very regrettably, no nominations were received this year for the Sidhu Award.

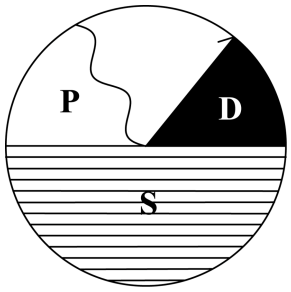


Chung Soo Yoo Award

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 Pittsburg 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 Soo Yoo 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 your 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. Charles Lake, Department of Chemistry, Indiana University of Pennsylvania, Indiana, PA 15705.

All donations are tax deductible in the US; check with your tax consultant in foreign climes

Program

Thursday, 25th October 2007

Morning Session A

High Throughput Biomolecular Crystallization and Crystal Growth Optimization

Joseph Luft (HWI, luft@hwi.buffalo.edu), Chairman Organizer

08:45 **Welcome and Introduction**
Robert H. Blessing (HWI)

09:00 **A.1. Screening for new ligands: A key to higher protein crystallization success rate**
Masoud Vedadi (U. of Toronto) *et al.*

09:30 **A.2. Creating and Milking a Macromolecular Crystallization Database: A View from the Trench**
George DeTitta (HWI) *et al.*

10:00 **A.3. Order from chaos: The design and interpretation of high-throughput crystallization screens to guide optimization**
Edward H. Snell (HWI) *et al.*

10:30-11:00 **Morning Break**

11:00 **A.4. The Utilization of Detergent Phase Boundary Information for Crystallizing Membrane Proteins**
Michael G. Malkowski (HWI) *et al.*

11:30 **A.5. A New Crystallization Plate Design for High Throughput Crystallization and *In Situ* X-ray Evaluation**
Robert E. Thorne (Cornell)

12:00 **A.6. *In-situ* X-ray inspection of protein crystals in multi-well plates: Latest results**
Chris Ceccarelli (Oxford Diffraction, Inc.)

12:30-14:00 **Lunch**

Afternoon Session B

Making X-Rays and Neutrons Work for You
(Technical Advances in Biocrystallography with Synchrotron X-rays and Spallation Neutrons)

Edward Snell (HWI, esnell@hwi.buffalo.edu), Chairman Organizer

14:00 **B.1.** **Combining Small Angle X-ray Scattering and X-ray Diffraction: Studies of the Anti-HIV Enzyme APOBEC3G and a Small Catalytic RNA Motif**
Joseph E. Wedekind (U. of Rochester)

14:30 **B.2.** **Modulated Crystals of Profilin:Actin**
Gloria E.O. Borgstahl (Nebraska Med. Center)

15:00 **B.3.** **How to Get Diffraction from Almost Nothing: Microcrystals and Microbeams at MacCHESS**
R.E. Gillilan (Cornell) *et al.*

15:30-16:00 **Afternoon Break**

16:00 **B.4.** **Building a user-friendly beam line**
Aina Cohen (SSRL) *et al.*

16:30 **B.5.** **Pushing the Limits of Neutron Structural Biology**
Dean Myles (ORNL)

17:00 **PDS General Membership Meeting**

19:00, Thursday evening, 25th October 2007

Poster Session and Conference Mixer

Timothy Umland (HWI, umland@hwi.buffalo.edu)
Poster Session Chairman

Posters should be mounted on Thursday morning or afternoon and left on display throughout the conference. Student poster presenters who are candidates for the Chung Soo Yoo Award must be present during the Thursday evening poster session to meet with the poster judges. The Award and its $200 prize will be presented at the Conference Banquet on Friday evening.

Biomacromolecular Crystallization Posters

P.1. Technology development at the Center for High Throughput Structural Biology (CHTSB)
Joseph R. Luft (HWI) *et al.*

P.2. Glycerol concentrations required for the successful vitrification of cocktail conditions in a high -throughput crystallization screen
Robin Kempkes (HWI) *et al.*

P.3. Interactions of tryptophan, tryptophan peptides and tryptophan alkyl esters at curved membrane interfaces
Wei Liu & Martin Caffrey (Ohio State U. and U. Limerick)

Biocrystallographic Diffraction Posters

P.4. Neutron Diffraction and Molecular Dynamics Study of Andrographolide – An Active Diterpenoid Natural Product
Thomas F. Koetzle (IPNS/ANL) *et al.*

P.5. Are X-rays damaging to structural biology? A case study with xylose isomerase
Kristin Wunsch (HWI) *et al.*

Molecular Structural Biology and Biochemistry Posters

P.6. Crystal structures and SAXS analysis of *S. aureus* FhuD2 provide insight into the mechanism of iron-siderophore transport
Brian H. Shilton (U. Western Ontario) *et al.*

P.7. Substrate specificity in the *E coli* ABC maltose transporter and the structure of a mutant periplasmic sucrose binding protein
Alister Gould (U. Western Ontario) *et al.*

P.8. Structure and function of the isolated DEAD motor domains of SecA
Stanley Nithianantham (U. Western Ontario) *et al.*

P.9. Characterization of the C-terminal domain of a potassium channel from Streptomyces lividans (KcsA)
Victor P. T. Pau (McMaster U.) *et al.*

P.10. The Molecular Basis for Aspirin-Triggered Lipoxin Formation by Cyclooxygenase-2
Danielle M. Simmons (HWI) *et al.*

P.11. Structural Insights Into Endocannabinoid Metabolism by Cyclooxygenase-2.
Alex J. Vecchiio (HWI) *et al.*

P.12. Crystal Structure of HoxA9 and Pbx Homeodomains Bound to the Renin PPE *In Vivo* Derived DNA Target
W. J. Bauer (HWI) *et al.*

P.13. Open Reading Frames and Codon Bias in Streptomyces coelicolor and the Evolution of the Genetic Code
R. Huether (HWI) *et al.*

P.14. Structure of acyl adenylate synthetase: a novel enzyme in the "wrong" conformation
Manish B. Shah (HWI) *et al.*

P.15. The Adenylate-forming Enzyme 4-Chlorobenzoate CoA Ligase/Synthetase Performs Two Unique Half-Reactions Using a 140^{o} C-terminal Domain Alternation
Albert S. Reger (HWI) *et al.*

Inorganic Materials Science Posters

P.16. Synthesis of $AA'(WO_4)_3$ Materials Using the Non-Hydrolytic Sol-Gel Method
Tamam Baiz and Cora Lind (U. Toledo, Ohio)

P.17. Temperature and Pressure Induced Polymorphism of $Y_2Mo_3O_{12}$
Stacy Gates and Cora Lind (U. Toledo, Ohio)

P.18. Variable Temperature Neutron and X-Ray Diffraction Studies of the $A^{2+}A'^{4+}W_3O_{12}$ Family
Amy Gindhart and Cora Lind (U. Toledo, Ohio)

P.19. NTE Zirconium Tungstate Materials
Leah Kozy (U. Toledo, Ohio) *et al.*

P.20. Non-Hydrolytic Sol-Gel Route for Preparing Titanium Sulfide
Tayseer Morcos and Cora Lind (U. Toledo, Ohio)

P.21. Non-hydrolytic sol-gel synthesis of iron and copper sulfides
Nathalie Pedoussaut and Cora Lind (U. Toledo, Ohio)

P.22. Twinning in the Adamantine-Like Quaternary Chalcogenide Li_2ZnSnS_4
Beth M. Leverett and Charles H. Lake (IUP)
Jonathan Lekse and Jennifer Aitken (Duquesne)

Friday, 26th October 2007

Morning Session C

Biomolecular Dynamics in Crystals

George Phillips (U. Wisc., phillips@biochem.wisc.edu),
Chairman Organizer

09:00 **C.1.** **Dynamics of Proteins in Crystals**
George N. Phillips, Jr. (U. Wisc. – Madison)

09:45 **C.2.** **Protein conformational flexibility in crystallography and in coarse-grained normal mode analysis**
Dmitry Kondrashov (U. Wisc. – Madison) *et al.*

10:30-11:00 **Morning Break**

11:00 **C.3.** **Surveying structural flexibility on a proteomic scale**
Mark Gerstein (Yale)

11:45 **C.4.** **Time-resolved Macromolecular Crystallography at BioCARS**
Vukica Šrajer (U. Chicago)

12:30-14:00 **Lunch**

Afternoon Session D

Small Angle Scattering Analysis of Biomolecular Structures

Wayne Schultz (HWI, schultz@hwi.buffalo.edu), Chairman Organizer

13:45 **D.1.** **Biomolecular Signaling and Regulation: A Solution Scattering View**
Jill Trewhella (U. Sydney, Bragg Inst. ANSTO, U. Utah)

14:20 **D.2.** **Protein Solution SAXS and XRD Protein Crystallography**
Gregory L. Hura (ALS/LBNL)

14:55 **D.3.** **The role of inter-subunit crosslinks in the maturation kinetics of HK97 bacteriophage**
Hiro Tsuruta (SSRL)

15:30-16:00 **Afternoon Break**

16:00 **D.4.** **Solution Structure of Human von Willebrand Factor Studied Using Small Angle Scattering**
Pachalis Alexandridis (SUNY Buffalo)

16:35 **D.5.** **Reconstructing static and time-resolved data from SAXS of macromolecules**
Jessica Lamb (Cornell) *et al.*

18:30, Friday evening, 26th October 2007

Conference Banquet

The Jacobs Center
Delaware Avenue and North Street

The Jacobs Center (http://mgt.buffalo.edu/jacobs/index.shtm), a SUNY-Buffalo facility, is housed in a beautiful turn-of-the-century Buffalo mansion designed by Stanford White. For those PDC participants whose roots are deep in the Crystallography Lab and Department in Thaw Hall at Pitt, the banquet setting has a special significance. Stanford White (1853-1906, http://en.wikipedia.org/wiki/Stanford_White), designer of the mansion that is the site for the 2007 PDC banquet, was a most prominent American *Beaux Arts* architect, and a notorious womanizer. He actively fancied the very beautiful Evelyn Nesbit (1884-1967, http://en.wikipedia.org/wiki/Evelyn_Nesbit), an artists' model and chorus girl, who was also fancied, and was being wooed, by White's fellow chorus-girl aficionado, Harry Thaw (1871-1947, http://en.wikipedia.org/wiki/Harry_K._Thaw), the privileged, playboy-son of Pittsburgh railroad baron William Thaw. In a murderous, insanely jealous rage, Harry Thaw, in the company of Miss Nesbit, shot White to death at the 1906 premier performance of *Mam'zelle Champagne* in Madison Square Garden. Later, in 1910, perhaps to redeem its murder-tarnished name, the Thaw family contributed Thaw Hall to the University of Pittsburgh (http://upload.wikimedia.org/wikipedia/en/7/7d/ThawPitt.jpg).

All those who knew him will remember that "Jeff" (Professor George Alan Jeffrey, 1915-2000, http://img.cryst.bbk.ac.uk/bca/Obits/GAJ.html), *pater familias* of Pittsburgh crystallographers, greatly relished the tale of Thaw Hall, the home of the Pitt Crystallography Lab and Department that he founded.


Stanford White


Evelyn Nesbit


Harry Thaw


Thaw Hall, Pitt

Saturday, 27th October 2007

Morning Session E

Crystal Structures by Powder XRD and XRD Phasing by Charge Density Flipping

Robert Von Dreele (Argonne, vondreele@anl.gov)
and John Spence (Arizona State U., spence@asu.edu),
Chairmen-Organizers

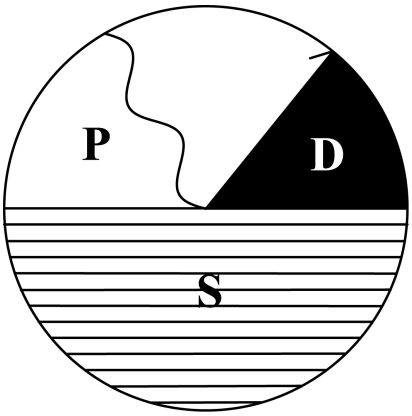
10 00 **E.1.** **Protein Structure Solution via Molecular Replacement**
Jennifer A. Doebbler and Robert B. Von Dreele (APS/ANL)

10:30 **E.2.** **Inorganic and organometallic structures from powder data: success and not-quite success**
Scott T. Misture (Alfred U.)

11:00-11:30 Morning Break

11:30 **E.3.** **Chemical kinetics under extreme conditions using powder diffraction**
Angus P. Wilkinson (Georgia Tech.)

12:00 **E.4.** **The flipping algorithm for powder diffraction. Serial crystallography**
John C.H. Spence (Ariz. State U., LBNL)



LECTURE ABSTRACTS

High Throughput Biomacromolecular Crystallization and Crystal Growth Optimization

Joseph Luft (HWI, luft@hwi.buffalo.edu), Chairman-Organizer

A.1. Screening for new ligands: A key to higher protein crystallization success rate

Abdellah Allali-Hassani, Guillermo Senisterra, Gregory A. Wasney, Patrick Finerty, Jr., Aled M. Edwards, Cheryl Arrowsmith and Masoud Vedadi

Structural Genomics Consortium, University of Toronto, Toronto, Ontario, Canada

Masoud Vedadi mvedadi@uhnres.utoronto.ca

Identification of small molecules that bind to and stabilize proteins can promote their crystallization as well as provide valuable functional information. Identifying optimum buffer conditions which further stabilize aggregating or hard to concentrate proteins also often results in obtaining more soluble proteins that can be further concentrated and screened for crystallization conditions. Availability of a generic screening method which is not dependent on the activity of proteins, and can rapidly identify small molecules that interact with proteins, greatly facilitates analyzing the binding specificity of different members of any family of proteins. We have employed differential static light scattering (DSLS) and differential scanning fluorimetry (DSF) to investigate the thermostability and ligand binding specificity of our protein targets. The presence of identified ligands in many cases (about 10%) resulted in crystallization of hard to crystallize proteins and improved crystal quality thus allowing structure determination. Screening proteins against customized libraries of compounds also generated chemical fingerprints for each protein, and provided the opportunity to compare the small molecule binding specificity of different members of each family.

A.2. Creating and Milking a Macromolecular Crystallization Database: A View from the Trench

M. Thayer[1], A. Lauricella[1], E.H. Snell[1,2], S. Potter[1], J. Wolfley[1], R. Nagel[1], M. Said[1], M.E. Snell[1], M. Rosenblum[1], M. Malkowski[1,2], C.M. Weeks[1,2], T. Veatch[1], E. Cook[1], C. Cumbaa[3], I. Jurisica[3], J.R. Luft[1,2], and G.T. DeTitta[1,2]

(1) Hauptman-Woodward Medical Research Institute and
(2) Department of Structural Biology, SUNY at Buffalo,
700 Ellicott Street, Buffalo New York 14203;
(3) Ontario Cancer Institute, PMH/UHN Toronto Medical Discovery Tower, Division of Signaling Biology, Life Sciences Discovery Centre Room 9-305, 101 College Street Toronto, Ontario M5G 1L7

George DeTitta detitta@hwi.buffalo.edu

Over the course of nearly eight years we have received and analyzed over 9000 proteins for their propensities to crystallize under well defined chemical conditions. The experiments are carried out at high throughput in our labs in Buffalo. Small (~200 nL) aliquots of protein stock solutions (1 – 50 mg/mL, minimally buffered) are added to equally small (~200 nL) aliquots of crystallization cocktails (strongly buffered, containing chemicals recognized as reducing the solubility of proteins) under a protective layer of oil. Crystallizing agents among the 1536 chemically distinct cocktails include 36 inorganic salts, seven polyethyleneglycols of varying molecular weight, eight buffering systems spanning a range of $4 < pH < 10$, and a number of alcohols, polyols, and water-soluble small molecules. The outcomes of each experiment are documented in six digital photomicroscopic tiff images taken over the course of four weeks.

Information relevant to the 14 million crystallization experiments is maintained in a secure data base. It includes somewhat incomplete data concerning the proteins crystallized and exhaustively complete data concerning the crystallization cocktails employed. All of the 84 million images are referenced in the database but are not actually accessed through the database at this point. Plans are underway to make minimally compressed JP2 jpeg images directly available, now that disk storage is so inexpensive. While images are useful they are not simply searchable from a database perspective. They would be most useful if each image was annotated and plans are underway to do this computationally; first extracting important image features, and then constructing a machine learning classifier that will annotate images. Test images for 96 proteins were manually annotated to provide a training set of images for computational image analysis. The majority of crystallization outcomes fell neatly into ten categories. We are constructing a ten-way classifier based on a neural network approach. Feature extraction is currently underway on the World Computing Grid. Once the most relevant (of ~80,000) features are identified, the entire library of outcomes will be processed and the computational categorizations will be imported into the database. With

information about the chemistry and outcomes of 14 million experiments involving 9400 proteins in a unified database we hope to be able to start learning some real chemistry about proteins.

This work was supported by the John R. Oishei Foundation of Western New York, the DOE, Erie County NY, IBM Canada, the World Community Grid, and NIH U54 GM074899. We wish to thank the 800+ structural biologists and structural genomicists who have trusted their proteins to our hands.

A.3. Order from chaos - The design and interpretation of high-throughput crystallization screens to guide optimization

E.H. Snell[1,2], J.R. Luft[1,2], S. Potter[1], A. Wojtaszczyk[1,3], J. Wolfley[1], R. Nagel[1], M. Said, M.E. Snell[1], M.A. Parker[4] and G.T. DeTitta[1,2]

(1) Hauptman-Woodward Medical Research Institute and;
(2) Department of Structural Biology, SUNY at Buffalo; 700 Ellicott Street; Buffalo, New York, 14203;
(3) Canisius College, 2001 Main Street, Buffalo NY 14208; (4) Brown University, Providence, RI 02912.

Edward Snell esnell@hwi.buffalo.edu

High-throughput crystallization screening tests many chemical conditions to locate an initial hit. Conditions are then optimized to produce crystals for diffraction. The Center for High Throughput Structural Biology (CHTSB) provides a high-throughput crystallization screening service available to the outside community. 1536 different experiments are performed with about 400 mL of macromolecule solution. Images are recorded automatically from each experiment over time. The interpretation of the resulting data can be challenging.

The 1536 CHTSB cocktail conditions are divided into two groups. The first, ~1000 conditions, are made up of an incomplete factorial sampling 36 salts, 8 buffers and 5 different PEGs. The remaining conditions are comprised of commercially available screens. Images from the crystallization experiments are sorted into several categories of outcomes. Images can be classified into multiple categories. For the incomplete factorial case, presenting these outcomes in chemical space readily identifies variables that are critical for the crystallization of a particular sample and clearly displays crystallization trends. The chemical space plot provides phase information on the sample that is especially relevant for crystallization. Equally important, it also provides information on the crystallization space that has not been sampled. The commercial screens supplement this information; the grid screens by fine sampling a small area to identify possible signatures of small chemical effects and the other screens providing a coarse sampling that identifies outlier conditions and relates previous results to the information obtained in the chemical space analysis. The combination of an incomplete factorial design, with a graphical representation of the results is powerful. It produces not only potential crystallization hits, but also an appropriate chemical direction for optimizing those hits.

This work is funded by the John R. Oishei foundation, the DOE, Erie County, NY, and through NIH U54 GM074899.

A.4. The Utilization of Detergent Phase Boundary Information for Crystallizing Membrane Proteins

Michael G. Malkowski[1, 2], Mary Koszelak-Rosenblum[1], Adam Krol[2], Joseph R. Luft[1, 2], and George T. DeTitta[1, 2]

[1]The Hauptman-Woodward Medical Research Institute and [2]Department of Structural Biology, SUNY at Buffalo, Buffalo, New York 14203

Michael G. Malkowski malkow@hwi.buffalo.edu

Through the results of various genome-sequencing efforts, it is estimated that 25-30% of all proteins are membrane proteins. Membrane proteins carry out a staggering range of biological functions, including signal transduction, molecular transport, and energy transduction. Many genetic disorders are directly related to membrane proteins and it is estimated that more than 70% of all currently available drugs act via membrane proteins. Thus, elucidating the structures of membrane proteins at atomic resolution is essential to our understanding of disease states as well as a critical component in the rational design of new and more effective drugs.

Crystallographic characterization of a membrane protein requires detergent solubilization of the protein from the lipid bilayer and subsequent purification of a functionally stable, homogeneous protein-detergent complex (PDC). Crystallization of the subsequent PDC requires the manipulation of the dual surface properties of the PDC in the presence of various precipitants. As membrane proteins have been observed to form crystals close to the phase separation boundaries of the detergent used in the crystallization experiment, knowledge of these boundaries under different precipitant conditions can serve as the foundation for the design of rational crystallization screens for membrane proteins[1]. We have generated a significant amount of phase boundary data using different combinations of Polyethylene Glycols (PEG), salts, and detergents. We are utilizing the generated phase boundary curves as a guide to develop detergent specific crystallization screens for membrane proteins, with the ultimate goal of using these tailored cocktails in conjunction with the high-throughput crystallization robots located at the Hauptman-Woodward Institute. A report of our progress to date is presented.

[1]Weiner, M.C. and Snook, C.F. (2001) The development of membrane protein crystallization screens based upon detergent solution properties. *J. Cryst. Growth* **232**, 426-431.

This work is supported by NIH U54 GM074899

A.5. A New Crystallization Plate Design for High Throughput Crystallization and *In Situ* X-ray Evaluation

Robert E. Thorne

Department of Physics, Cornell University, Ithaca, NY 14853-2501.

Robert E. Thorne ret6@cornell.edu
http://www.physics.cornell.edu/profpages/ThorneR.html

We describe a macromolecular crystallization plate compatible with automated drop dispensing robots in which drops are held in place by microfabricated patterns. This plate provides improved control over crystallization kinetics and improved imaging and crystal recognition.

It allows both hanging and sitting drop crystallization, and in situ X-ray inspection with minimal background scatter. Surface micropatterning produces drops with well-defined positions, boundaries and shapes. The drops show excellent stability against vibrations, and arrays of large drops can be rotated 90 degrees to the vertical plane or inverted to the hanging drop configuration with no motion of the drop contact lines. A single micropatterning process strongly pins drops of a wide range of liquids including protein, surfactant and salt solutions.

The drop contact line remains pinned as the drop evaporates, resulting in greatly improved reproducibility of evaporation and crystallization kinetics. Because drop shape is reproducible and well defined, automated image analysis to identify crystals is greatly simplified. Supporting the drops on a patterned, optically and X-ray transparent film allows in situ X-ray examination. This improved crystallization plate is well suited to both high-throughput and manual crystallization experiments, and should provide a very cost-effective alternative to microfluidic crystallization devices.

This research is supported by the NIH (R01 GM65981).

A.6. *In-situ* X-ray inspection of protein crystals in multi-well plates - Latest results

Chris Ceccarelli

Oxford Diffraction, Inc., 2000 Kraft Drive, Suite 1103, Blacksburg, VA 24060

Chris Ceccarelli c/o richard.foster-turner@oxford-diffraction.com

Recently, optical imaging for the identification of diffraction-quality crystals within multi-well crystallisation plates has attracted a great deal of attention. However, the limitation of these systems is that all objects identified as potential crystals must be looped out and screened using traditional X-ray diffraction apparatus.

In order to overcome this problem a novel instrument has been developed, PX Scanner. This automatic system combines an optical and X-ray diffraction imager in a single compact instrument and allows the in-situ X-ray diffraction study of protein crystals in the multi-well plate. PX Scanner is now in use at a number of laboratories around the world and some latest results will be presented including *in-situ* X-ray diffraction studies on sub-40 micron crystals and membrane proteins.

Making X-Rays and Neutrons Work for You

Technical Advances in Biocrystallography with Synchrotron X-rays and Spallation Neutrons

Edward Snell (HWI, esnell@hwi.buffalo.edu), Chairman-Organizer

B.1. Combining Small Angle X-ray Scattering and X-ray Diffraction: Studies of the Anti-HIV Enzyme APOBEC3G and a Small Catalytic RNA Motif

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Proteins and ribonucleic acids often adopt three-dimensional structures that are difficult to analyze solely by crystallography. Here we describe two investigations in which SAXS was used as part of a multidisciplinary approach to relate form to function. In one case, the unknown molecular envelope of the antiviral protein APOBEC3G was restored, which provided a basis to probe subunit organization and regulation of dC-to-dU deaminase activity. In a second case, SAXS was used to discover how a cavity-forming defect in the hairpin ribozyme alters global RNA structure in solution, which is masked by the crystal lattice and appears to be manifested only as a localized conformational change. Summaries of these topics are as follows:

1). Human APOBEC3G (hA3G) is a cytidine deaminase that functions as a potent, post-entry viral restriction factor that targets HIV DNA. However, the Vif protein from HIV counteracts hA3G by recruiting it to an E3 ubquitin ligase complex, ultimately leading to its degradation. In the cell, hA3G activity is regulated by cytoplasmic RNA levels. In the absence of the Vif protein, nascent hA3G is active as a low molecular mass (LMM), RNA depleted deaminase. In contrast, viral RNA transcripts or intermediates from retrotransposable elements interact with hA3G to form large, catalytically inert high molecular mass (HMM) complexes. Thus, elucidation of the molecular determinants that shield hA3G

from Vif, or modulate its ability to bind RNA, represent exciting areas of investigation in the development of antiviral therapeutics.

To discover the basis for hA3G regulation by RNA, we restored molecular envelopes for hA3G in LMM- and HMM-like forms. The results revealed that LMM-like particles have extended shapes that are dissimilar to the globular folds of cytidine deaminases encountered in pyrimidine metabolism. Moreover, the volume distribution of LMM envelopes supported a novel mode of tail-to-tail subunit dimerization. Comparison of the LMM envelope to that of HMM-like particles suggested that dimers of hA3G subunits could organize as 'dimers of dimers' bridged by RNA. This hierarchical assembly model accounts for the propensity of hA3G subunits to form inactive ribonucleoprotein complexes of MDa complexity, and why the RNase H activity of reverse transcriptase is required to restore deaminase activity of hA3G on first strand viral DNA.

2). Ribozymes are naturally occurring RNA enzymes that catalyze biological reactions via strategic positioning of nucleobases. One method to probe functionality is *abasic* substitution, which is hypothesized to generate a cavity that accommodates an exogenous ligand that restores activity. To investigate this concept, structures of the hairpin ribozyme were determined whereby a key catalytic residue at position A38 was removed. In the absence of A38, the global fold remained intact, although two nucleobase conformations were evident at position A-*1*. A minor conformation represented the native fold, and a major conformation revealed a 15 Å movement of A-*1* to fill the abasic void. Both wild type and abasic structures crystallized in space group $P6_122$ with similar unit cell parameters, and nearly identical R_G values (~17.3 Å). However, analysis of R_G values for the respective samples in solution revealed values of 18.4 Å for wild type versus 25.1 Å for abasic molecules. Thus, the SAXS results suggest a destabilization of the compact core in which major and minor base conformations interconvert through an expanded intermediate state incapable of being visualized crystallographically. The results have implications for the use of abasic substitutions to explore nucleobase functionality, as well as the interpretation of rescue activity by exogenous ligands.

B.2. Modulated Crystals of Profilin:Actin

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An unusual crystallography project has been identified that is focused on the structural basis of actin filament formation. Due to the importance of actin filaments in cellular processes, cell motility and metastatic cancer, this topic is of great interest to cell biologists and cancer researchers alike. Scientists need to understand, in molecular detail, the structure(s) of actin in order to understand actin-based biological processes, but to date, no structures of filamentous actin have been determined. Of crystallographic interest is that crystals that are thought to contain actin filaments, or a structural intermediate there of, form modulated crystals; opening an exciting new direction to protein crystallography. It is reassuring that chemical crystallographers, who determine the structures of small molecules, have developed technologies to solve structures from modulated crystals and that these methods can be adapted and developed for the larger molecules visualized by protein crystallography. The talk will summarize recent results on the induction of crystallographic modulation(s) by transferring crystals to conditions known to dissociate profilin and thereby promote actin filament formation within the crystals, modulated diffraction data we have collected from the modulated crystals that contain unusual off-lattice satellite reflections next to the main reflections, and our progress on structure determination of the modulated protein.

B.3. How to Get Diffraction from Almost Nothing. Microcrystals and Microbeams at MacCHESS

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Protein crystallography works because scattered photons from many individual protein molecules in the crystal lattice add together constructively at the detector. Scattered intensity drops dramatically as crystals become smaller, eventually vanishing into the background noise. Even when a copious number of photons are supplied to the sample, radiation damage ultimately limits data collection. Small crystals (< 20 μm in diameter) are not merely an unfortunate accident of non-optimal growth conditions, they sometimes reflect important properties of the underlying protein such as conformational variability, fibril formation, and association with lipids. As biologists probe more challenging systems, structural insights will more frequently hinge upon data obtained from the smallest of samples. How small will we be able to go? I will discuss current efforts at MacCHESS to push the limits of microdiffraction using novel x-ray optics, background scattering reduction techniques, and special sample handling. Beams 18 μm in diameter delivering a flux density of 1.1×10^8 photons/μm^2/sec are routinely available at MacCHESS and fully integrated with ALS-style sample automounting. Several examples of the advantages of microbeam will be illustrated, including our experience with collecting data on 1-2 micron diameter needle-shaped microcrystals.

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B.4. Building a user-friendly beam line

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A user-friendly beam line is one that gives visiting researchers the ability to obtain maximum value from their synchrotron visit. This means that this beam line will let them conduct a variety of experiments as rapidly and easily as possible. The protein crystallography group at SSRL has been working toward this goal in two ways: by building new beam lines optimized for the experiments that users want to do, and by implementing new instrumentation, computers, control systems and software to take maximum advantage of existing stations. This talk will focus on the needs of synchrotron users and how the crystallography facilities at SSRL have evolved to meet these needs. Topics will include the use of advanced control systems to give fixed-wavelength wiggler side-stations MAD and SAD capability, automated fluorescence analysis for SAD and MAD and automated screening with a robotic sample mounting system. In addition to facilitating the efficient use of the SSRL beam lines, these improvements have resulted in more successful experiments. Moreover, this high level of automation has enabled a Remote Access mode of experimentation, released to general users in 2005. Remote Access experiments are carried out from the researchers' home institutions and other remote locations while retaining complete experimental control over even the most challenging systems. During the 2006-2007 experimental run, 75% of SSRL macromolecular crystallography users collected data remotely. Developments at SSRL in automation, expanded remote access capabilities, and the impact on macromolecular crystallography experiments will be presented. Work in progress to expand the scientific capabilities of the beam lines will also be covered.

B.5. Pushing the Limits of Neutron Structural Biology

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Neutron scattering provides a unique non-destructive tool that is able to probe the structure and dynamics of macromolecular complexes and higher order assemblies over a wide range of time and length scales. With the advent of the Spallation Neutron Source (SNS) and the parallel upgrade and development of the 85MW HFIR facility, Oak Ridge National Laboratory (ORNL) is set to become the worlds leading center for the neutron sciences. The field of neutron protein crystallography in particular is likely to benefit enormously from the construction of the Macromolecular Neutron Diffractometer (Mandi) at the SNS, which promises to provide 10-50 fold improvements in performance over the world's current best instruments. A Center for Structural Molecular Biology has been established at ORNL to develop and support the user access and research programs in neutron-based studies of bio-molecular structure and function. The center includes a dedicated Bio-Deuteration Laboratory for isotopic labeling. These facilities offer new opportunities for the characterization of proteins and macromolecular complexes in crystals, in solution and in membrane associated states. We will focus on recent applications in neutron protein crystallography that highlight the new promise and opportunities in the field.

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Biomolecular Dynamics in Crystals

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C.1. Dynamics of Proteins in Crystals

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Protein molecules in crystals retain dynamic behavior not unlike their behavior under physiological conditions. The effect of the lattice contacts can be examined by coarse-grained normal mode methods. The nature of the ensemble of structures can also be examined. X-ray crystallography typically uses a single set of coordinates and B-factors to describe the result of the analysis. Refinement of multiple copies of the entire structure has been previously used in isolated specific cases as alternative means of representing structural flexibility. Interpreting diffuse X-ray scattering patterns from crystals of macromolecules can also provide information about the conformational flexibility in proteins. Of course, the optimal technique for the study of dynamics of proteins in crystals is a time-resolved experiment. Various work on these topics will be presented.

C.2. Protein conformational flexibility in crystallography and in coarse-grained normal mode analysis

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The increase in quality and quantity of X-ray structures of biological macromolecules has provided detailed information about conformational flexibility, in the form of anisotropic displacement parameters (ADPs), as well as from ensembles of different structures. The development of coarse-grained models of macromolecular flexibility of different levels of complexity raises the question of the optimal trade-off between computational efficiency and prediction quality. ADPs from 83 non-redundant ultra-high resolution structures were used to validate predictions of both magnitude and directionality of allowed flexibility from normal mode calculations based on five coarse-grained potentials. Models incorporating chemical information, e.g. CHARMM, have an edge in prediction of direction of motion, while prediction of magnitude is largely invariant at different levels of modeling. Further, we analyze four structures of myoglobin in different crystal forms, and new ensembles of structures of UDP-glucose phosphorylase and gamma-SNAP proteins. The crystal-induced perturbations in the crystallographic ensembles are consistent with predictions of normal modes in vacuo, suggesting that the allowed deformations are robustly encoded in protein structures. Normal mode analysis enables the formulation of mechanistic hypotheses for the functional dynamics of the latter two proteins.

C.3. Crystallographic representation of protein dynamics: Too simple? Too complex? Too confusing?

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Crystallographic representation of protein dynamics - too simple? too complex? too confusing?

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Proteins are dynamic objects. They have been called 'molecular machines', and just as macroscopic machines their function depends on the relative motion of component parts. Many formalisms have been developed to describe or model these motions, and it is natural for crystallographers to want to combine these with the structural information obtained from diffraction data. However, some formalisms are easier than others to incorporate into existing crystallographic methodology.

I will describe how we have approached the use of multi-group TLS models (TLSMD) to describe both large-scale and local dynamics in proteins. Most of our attention has focused on the interpretation of Bragg diffraction data, but similar analysis is possible for experimental observations from NMR or simulations such as molecular dynamics. For diffraction data in particular, it is possible to incorporate the TLSMD model directly into model refinement protocols. This is a very parsimonious protocol compared to alternatives such as multi-copy refinement or coupled dynamic simulation. I will also discuss pitfalls that have become apparent in trying to introduce any of these alternatives to protein crystallographers and to the larger scientific community.

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C.4. Surveying structural flexibility on a proteomic scale

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An area of focus in the lab is analyzing small populations of structures in terms of their detailed 3D-geometry and physical properties. Here, we try to interpret macromolecular motions in terms of packing. We have set up a database of macromolecular motions and coupled it with simulation tools to interpolate between structural conformations; the database also has tools to predict likely motions based on simple models, such as normal modes and localized hinges connecting rigid domains. Part of this project involves devising a system for characterizing motions in a highly standardized fashion. Our motions classification scheme is motivated by the fact that protein interiors are packed exceedingly tightly, and the tight packing can greatly constrains a protein's mobility. We have developed tools for measuring and comparing the packing efficiency at different interfaces (e.g. inter-domain, protein surface, helix-helix, protein vs. RNA) using specialized geometric constructions (e.g., Voronoi polyhedra).

http://molmovdb.org/

The FlexOracle hinge predictor. SC Flores, MB Gerstein (2007). BMC Bioinformatics 8: 215.

Flores, Echols, Milburn, Hespenheide, Keating, Lu, Wells, Yu, Thorpe, Gerstein (2006). Nucleic Acids Res. 34:D296-301.

SC Flores, LJ Lu, J Yang, N Carriero, MB Gerstein (2007). "Hinge Atlas: relating protein sequence to sites of structural flexibility." BMC Bioinformatics 8: 167

http://papers.gersteinlab.org/papers/subject/motions/
http://papers.gersteinlab.org/papers/subject/volumes/

C.5. Time-resolved Macromolecular Crystallography at BioCARS

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In time-resolved crystallographic studies of biomolecules structures of short-lived intermediates are visualized with atomic resolution by taking X-ray snapshots of the molecules along the reaction pathways at room temperature, following the reaction initiation in the crystal. It has been demonstrated that small structural changes and low-occupancy docking sites of small ligands can be detected in difference electron density maps measured with ns and sub-ns time resolution [1-9]. Advancements in the analysis of time-resolved data, such as the application of Singular Value Decomposition (SVD) method, lead to successful determination of structures of pure intermediates from measured mixtures of such states and to elucidation of the reaction mechanism [5-7]. The technique has reached a mature phase. We will present results of the latest ns time-resolved crystallographic studies conducted at the the BioCARS beamline 14-ID at the Advanced Photon Source: allosteric action in real time in a cooperative dimeric hemoglobin [2] and structural relaxation in myoglobin [4].

[1] Key et al., *Biochemistry* 46, 4706 (2007); [2] Knapp et al. *PNAS* 103, 7649 (2006) [3] Bourgeois et al. *PNAS* 103, 4924 (2006); [4] Schmidt et al. *PNAS* 102 11704 (2005); [5] Ihee et al. *PNAS* 102 7145 (2005); [6] Rajagopal et al. *Structure* 13, 55 (2005); [7] Schmidt et al. *PNAS* 101, 4799 (2004); [8] Schotte et al. *Scienc*e 300, 1944 (2003); [9] Šrajer et al. *Biochemistry* 40, 13802 (2001).

Small Angle Scattering Analysis of Biomolecular Structures

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D.1. Biomolecular Signaling and Regulation: A Solution Scattering View

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Beyond the international genome sequencing and structural genomics projects aimed at identifying and determining the structures of the individual biomolecular components of living systems, is the ultimate goal of understanding of how biomolecules interact within complex networks to transmit signals and regulate the functions carried out by biomolecular machines. X-ray and neutron solution scattering with contrast variation have a role to play in filling critical gaps in our understanding of biomolecular complexes and assemblies. In recent years we have seen dramatic improvements in sources, instrumentation, and computational power that increase our ability to interpret scattering data in terms of molecular models, especially when we have high resolution structures of the components. This talk will describe some of our recent solution scattering studies of biomolecular signalling and regulation, including results from a series of studies on kinase regulation, including a bacterial histidine kinase and its inhibitors that regulate sporulation in bacteria. These systems are of interest for biotechnology applications as well as the development of new antibiotics as the histidine kinases are essential for gram negative bacteria and are not found in mammals. I will also describe very recent work on a receptor-ligand complex in nerve synapses whose alteration via mutations can cause autism.

D.2. Protein Solution SAXS and XRD Protein Crystallography

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Recent advances in small angle x-ray scattering (SAXS) technique and analysis have enabled shape prediction of proteins in solution. The SAXS technique is particularly powerful in combination with partial high resolution structures. SAXS can efficiently reveal the spatial organization of protein domains, including domains missing from or disordered in known crystal structures, and establish cofactor or substrate-induced conformational changes. Following a short introduction to SAXS, examples from data collected at SIBYLS, a dual SAXS and protein crystallography synchrotron beamline, will be drawn upon to demonstrate the complimentary use of SAXS with protein crystallography. I will also describe the recent implementation of a sample loading automation tool for true high throughput SAXS data collection. Several examples of the utility of high throughput SAXS will discussed in the context of the DOE/Gtl funded program project MAGGIE (Molecular Assemblies, Genes, and Genomics
Integrated Efficiently).

D.3. The role of inter-subunit crosslinks in the maturation kinetics of HK97 bacteriophage.

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Virus capsids are complex macromolecular machines composed of hundreds of protein subunits. These protective shells assemble first as relatively fragile procapsids that then undergo conformational maturation to form the robust capsid, which is capable of encapsidating the densely packaged viral genome. Accessory proteins bind to and stabilize the capsids of some bacteriophages such as T4 and λ. Others such as HK97 have evolved covalent modification of the capsid lattice as a means of stabilizing the capsid assembly. Here, we demonstrate that intersubunit crosslinks accelerate HK97 capsid maturation by potentiating the transition between immature and mature capsid states. A combination of time-resolved solution small-angle x-ray scattering and crosslinking assays were used to demonstrate that a drastic shift in crosslink pattern coincides with the conversion of a semi-expanded particle intermediate (EI) to the larger Balloon particle form. The structural reorganization occurs as a cooperative, all-or-nothing transition. Progressively severe mutations and alterations to the crosslinking machinery correlate with progressively greater inhibition of the reorganization. We conclude that intersubunit crosslinks bias particle maturation in a forward direction without directly effecting local structural change. A pseudoatomic model of the EI particle form is being built to gain insight into the structural basis for the significant effect of the crosslink formation that may facilitate maturation by spring-loading the capsid at specific hinging vertices.

This research has been conducted in close collaboration with Kelly Lee, Lu Gan, John E. Johnson (Scripps), Crystal Moyer, James Conway, Roger Hedrix, Robert Duda (Univ. Pittsburgh) and Alastair Steven (NIH).

D.4. Solution Structure of Human von Willebrand Factor Studied Using Small Angle Scattering

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Von Willebrand factor (vWF) binding to platelets under high fluid shear is an important step regulating atherothrombosis. We applied light scattering and small-angle neutron and X-ray scattering to study the solution structure of human vWF multimers and protomer. According to our results, these proteins resemble prolate ellipsoids with radius of gyration (Rg) of ~75nm and ~30nm for multimer and protomer, respectively. The vWF multimer was organized into substructural domains at length scales indicative of the entire protein itself (75nm), elements of the protomer quarternary structure (16nm), and individual functional domains (4.5nm). Individual domains of 4.5nm were also observed in the vWF protomer. Amino acids occupy only ~2% of the multimer and protomer volume, compared to 98% for serum albumin and 35% for fibrinogen. vWF treatment with Guanidine.HCl, which increases vWF susceptibility to proteolysis by ADAMTS-13, causes local structural changes at length scales<10nm without altering the protein Rg. Treatment of the multimer but not protomer-vWF with random homobifunctional linker BS3, prior to reduction of inter-monomer disulfide linkages and western blotting reveals a pattern of dimer and trimer units that indicate the presence of stable inter-monomer non-covalent interactions within the multimer. Overall, we demonstrate for the first time that the vWF solution structure is stabilized by non-covalent interactions between different monomer units. Local, and not large scale, changes in multimer conformation are sufficient for ADAMTS-13 mediated proteolysis. Possible functional consequences of non-covalent protein interactions on vWF function during thrombosis will be discussed.

Shankaran, H.; Alexandridis, P.; Neelamegham, S. "Aspects of Hydrodynamic Shear Regulating Shear-Induced Platelet Activation and Self-Association of von Willebrand Factor in Suspension" *Blood* 2003, **101**(7), 2637-2645.

Singh, I.; Shankaran, H.; Beauharnois, M. E.; Xiao, Z.; Alexandridis, P.; Neelamegham, S. "Solution Structure of Human von Willebrand Factor Studied Using Small Angle Neutron Scattering" *J. Biol. Chem.* 2006, **281**(50), 38266-38275.

D.5. Reconstructing static and time-resolved data from SAXS of macromolecules

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Recently, in static SAXS work, presentation of traditional two-dimensional scattering plots has increasingly given way to three dimensional shape reconstructions produced by computer algorithms. We have extended this approach to time-resolved SAXS, reporting on global structural changes of macromolecules. We have taken time-resolved data on the folding of the *Tetrahymena* group I intron. Some of the data was reconstructed in an effort to assess the usefulness of the technique in time-resolved situations. We present the results here.

Crystal Structures by Powder XRD and XRD Phasing by Charge Density Flipping

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E.1. Polycrystalline Protein Structure Solution via Molecular Replacement

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The solution of macromolecular 3-D structures by single-crystal diffraction has become a common and mostly routine process—provided suitable single crystals can be produced. This caveat has lead to difficulties when the proteins form only crystal clusters, highly mosaic single crystals, or micro-crystals. Diffraction of proteins in powder form should broaden the spectrum of candidate proteins, which have historically been overlooked due to unsatisfactory crystal formation. Early work by Von Dreele [1-4] and Margiolaki [5] validated the powder diffraction technique as a tool for macromolecular structure solution.

The challenges faced by solving the powder structure of a protein with a non-ideal search model needs to be addressed in order for this procedure to become widespread. In essence, we need to mimic the techniques of single-crystal diffraction—namely molecular replacement. Often the structure of a related material is known which occurs in a different space group. Molecular replacement provides an algorithm of rotation and translation functions to map a model structure onto the experimentally observed structure factors and space group of the target.

Initial powder studies yielded solutions for molecular replacement of insulin [6] and turkey lysozyme [5], themselves, into alternate space groups. To demonstrate that cross-species molecular replacement is also viable technique for powder diffraction, we present molecular replacement of HEWL using the 60% identical human lysozyme (PDB code: 1LZ1) as the search model. Five data sets taken at different salt concentrations were used in concert for a multi-pattern Pawley refinement. One solution was obtained and further structural refinement was performed on the solution.

Due to the high incidence of overlaps in powder patterns, however, consistent reproduction of the molecular replacement results was not possible with single crystal software. These programs are not equipped to deal with the overlaps present in powder data. This results in failed solutions in Amore, Phaser, and Beast as well as false positive

solutions from Molrep [7]. The search for a consistent approach to the molecular replacement problem for image plate derived powder data will be discussed.

[1] - R. B. Von Dreele, Acta Cryst D **57**, 1836 (2001)
[2] - R. B. Von Dreele. J. Charles W. Carter, R. M. Sweet eds, Methods in Enzymology: Acad Press; (2003)
[3] - R. B. Von Dreele, Acta Cryst D **61**, 22 (2005)
[4] - R. B. Von Dreele, J of Appl Cryst **40**, 133 (2007)
[5] - I. Margiolaki, J. P. Wright, A. N. Fitch, G. C. Fox, R. B. Von Dreele, Acta Cryst D **61**, 423 (2005)
[6] - R. B. Von Dreele, P. W. Stephens, G. D. Smith, R. H. Blessing, Acta Cryst D 56, 1549 (2000)
[7] - S. Bailey, Acta Cryst D 50, 760 (1994)

E.2. Inorganic and organometallic structures from powder data: success and not-quite success

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Routine use of high temperature diffraction in our laboratories often results in observation of new phases, some of which are stable on cooling to ambient conditions. The approach to solve the structures of several Ba- and Ca-containing silicates will be detailed, including high temperature polymorphs and poorly-crystalline specimens with significant microstrain. The use of rigid bodies to correctly establish tricky oxygen positions, even from laboratory X-ray data, will be compared to using individual ions, with and without electrostatic constraints. In addition, the utility of the method to detect cation ordering will be highlighted. An additional example of the use of rigid bodies focuses on silver carboxylates measured using synchrotron radiation. Our struggles with the long-chain carboxylates prompted synthesis of shorter-chain variants that crystallize in more manageable unit cells. Proposed structures will be discussed based upon reasonable Ag-Ag distances and chemical knowledge from related carboxylates.

E.3. Chemical kinetics under extreme conditions using powder diffraction

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Cement slurries for oil well applications can be exposed to pressures in excess of 1000 bar and temperatures > 200 °C during hydration due to the great depth of some wells. We have developed sample environment for the study of cement hydration kinetics using powder diffraction under these conditions. The effect of temperature and high pressure on the hydration of cements will be discussed and possibilities for building sample environment for the simultaneous measurement of mechanical properties and chemistry explored.

E.4. The flipping algorithm for powder diffraction. Serial crystallography

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The powerful charge-flipping algorithm was first proposed in simulations by Ozlanyi *et al.* [*Acta* A**60**, 134 (2004)] and applied to experimental data soon after [Wu *et al., Acta* A**60**, 326 (2004)]. More recently we have extended the method to powder X-ray diffraction [Wu *et al. Nature Materials*, **5**, 647 (2006)] and shown the relationship of this remarkable algorithm to the general class of iterated Fienup projection algorithms in image processing [Wu *et al., Opt Lett.* **29**, 2737 (2004)]. Further applications of the algorithm will be discussed, including femtosecond soft X-ray imaging.

A review will also be given of our serial crystallography project and related activities. This project aims to solve proteins which cannot be crystallized by spraying them across a synchrotron, while using a laser to align them [Starodub *et al*., *J. Chem Phys.* **123**, 244304 (2005); Starodub *et al*., *J. Synch Res*., in press]. Results for photosystem 1 sub-micron crystallites in a Rayleigh droplet beam will be shown, in addition to patterns from droplets doped with gold nanoballs. Recent plans to collect data from membrane proteins in lipid bilayers aligned by magnetic field will also be reviewed.

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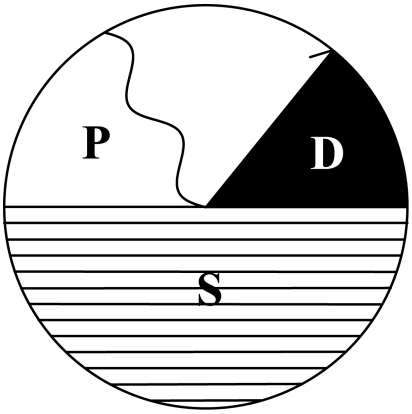

POSTER ABSTRACTS

Posters should be mounted on Thursday morning or afternoon and left on display throughout the conference. Student poster presenters who are candidates for the Chung Soo Yoo Award must be present during the Thursday evening poster session to meet with the poster judges. The Award and its $200 prize will be presented at the Conference Banquet on Friday evening.

P.1. Technology development at the Center for High Throughput Structural Biology (CHTSB).

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The Center for High-Throughput Structural Biology is a consortium comprised of 11 laboratories at six institutions located in upstate New York, California, and Toronto, Ontario. It is a specialized research center created as part of the NIH Protein Structure Initiative to focus on technology developments to overcome the most significant obstacles to structure determination. The center is focusing on several areas; developing yeast (*Saccharomyces cerevisiae*) as a tool for structural biology, the efficient screening, optimization and production of crystals, and technology for crystal handling and remote data collection. The targets include transmembrane proteins and protein-protein complexes that have traditionally been difficult to study by diffraction methods. This work is supported in part by the NIH grant U54 GM074899.

P.2. Glycerol concentrations required for the successful vitrification of cocktail conditions in a high -throughput crystallization screen

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The high-throughput laboratory at the Hauptman-Woodward Medical Research Institute provides a crystallization service to the external community. Macromolecules are screened against 1536 chemical cocktails, 984 an in-house factorial design and 552 comprising commercially available screens. When a hit occurs, several others are usually seen. With a limited amount of sample a decision has to be taken about what conditions and how many to choose for optimization. For macromolecules, X-ray data is largely recorded at cryogenic temperatures requiring the solution to include a cryoprotectant to protect against crystalline ice formation. As a criteria for prioritizing which of the hits to optimize, the minimum glycerol concentrations to successfully vitrify the 984 in-house cocktails were determined. A deliberately worst-case scenario was used with large 0.7-1.0 mm cryoloops. Parallel to this the cryoprotectant concentration as a function of loop size was determined for a small subset of cocktails that required a higher than average glycerol concentration. This allows the global results to be translated to smaller loop sizes. A similar study was carried out on a selection of the commercial grid screens to examine the effect of chemical shifts on the cryoprotectant required. There are a number of cryoprotectants available and many are more suited to the initial cocktail conditions than glycerol. However, glycerol makes a baseline; it can be incorporated before the crystallization step and provides a good starting point for further cryoprotection decisions.

P.3. Interactions of tryptophan, tryptophan peptides and tryptophan alkyl esters at curved membrane interfaces

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Motivated by on-going efforts to understand the mechanism of membrane protein crystallogenesis and transport in the lipidic cubic mesophase, the nature of the interaction between tryptophan and the bilayer/aqueous interface of the cubic phase has been investigated. The association was quantified by partitioning measurements which enabled the free energy of interaction to be determined. Temperature-dependent partitioning was used to parse the association free energy change (DG) into its enthalpic (DH) and entropic (DS) components. As has been observed with tryptophan derivatives interacting with glycerophospholipid bilayers in vesicles, tryptophan partitioning in the cubic phase is enthalpy driven. This is in contrast to partitioning into apolar solvents which exhibits the classic hydrophobic effect whose hallmark is a favorable entropy change. These results with tryptophan are somewhat surprising given the simplicity, homogeneity and curvature of the interface that prevails in the case of the cubic phase under investigation. Nevertheless, the interaction between tryptophan and the mesophase is very slight as revealed by its low partition coefficient. Additional evidence in support of interaction was obtained by electronic absorption and fluorescence spectroscopy and fluorescence quenching. Partitioning proved insensitive to the lipid composition of the membrane, examined by doping with glycerophospholipids. However, the interaction could be manipulated in meaningful ways by the inclusion in the aqueous medium of salt, glycerol or urea. The effects seen with tryptophan were amplified rationally when measurements were repeated using tryptophan alkyl esters and with tryptophan peptides of increasing length. These findings are interpreted in the context of the insertion, folding and function of proteins in membranes.

P.4. Neutron Diffraction and Molecular Dynamics Study of Andrographolide – An Active Diterpenoid Natural Product

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The labdane diterpenoid andrographolide is the main constituent isolated from extracts of the leaves and stems of the annual shrub *Andrographis paniculata* Nees (Acanthaceae), which is in common use in Indian and Chinese traditional medicine. The extracts of the herb have demonstrated anti-inflammatory, antiviral, immunostimulatory, hypoglycemic, hypotensive, and anticancer activities.

We have succeeded in growing unusually large, high-quality, single crystals of andrographolide. The compound was isolated and purified from the dichloromethane extract of the dried herb by silica gel column chromatography using dichloromethane:methanol 95:5 as eluting solvent system. The eluted fractions containing andrographolide were allowed to stand undisturbed for two months, whereupon crystals in the shape of uniform blocks appeared, and slow evaporation of the remaining solvent over a few more weeks enabled the blocks to grow larger, one attaining a length of 2 cm.

Single-crystal neutron diffraction studies were carried out on the SCD instrument at the Intense Pulsed Neutron Source (IPNS), to provide a definitive characterization of the hydrogen-bonding scheme in andrographolide crystals (space group $P2_1$, a = 6.497(1), b = 7.898(1), c = 17.859(3) Å, β = 96.86(1)°, V = 909.8(2) Å^3, T = 20 K). Andrographolide has been examined five times by X-ray crystallography, with some disagreement among these prior studies on the hydrogen bonding pattern. Our results confirm the assignment by Spek et al. (1987) where the OH group directly attached to the decalin ring was found to donate an *intra*molecular hydrogen bond to the O atom of the nearby CH_2OH group. Our results further indicate that the highly deshielded proton (δ 5.00) of the CH(OH) group on the furanone ring is involved in a short C-H···O contact [H···O 2.413(6) Å].

Our neutron diffraction study is complemented by *ab initio* molecular orbital calculations on the isolated andrographolide molecule and molecular dynamics runs *in vacuo* and in water comparing possible alternative *intra*molecular hydrogen bonding arrangements.

Acknowledgement. Work at Argonne was supported by the U. S. Department of Energy, Office of Science, Basic Energy Sciences, under Contract DE-AC02-06CH11357. The isolation and purification of andrographolide study was funded by the Malaysian Ministry of Science, Technology and Innovation (MOSTI) grant no. 5450293.

P.5. Are X-rays damaging to structural biology? A case study with xylose isomerase.

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Structural crystallography is a powerful technique for visualizing a macromolecule at atomic resolution. The resulting pictures are used to improve our understanding of macromolecule function. As more intense X-ray sources become available the amount of detail that can be seen increases. However, X-rays themselves cause detrimental physical effects on the sample through primary and secondary radiation damage. This can be mitigated, but not eliminated through cryocooling. In a case study with xylose isomerase, we demonstrate that high-resolution data can sometimes provide misleading results due to photo-induced structural changes. Crystals of xylose isomerase diffract to very high resolution, 0.87 Å and provide high-quality electron density maps. However, if we collect multiple, identical data sets sequentially with a lower individual X-ray dose, we see the progressive formation of alternative metal sites in the xylose isomerase enzyme and a variation in their occupancy as a function of the cumulative dose. The structure changes progressively during collection of the data sets and ultimately resembles the structure seen in the high-resolution data set, obtained at a corresponding cumulative dose. Some of the features in the high-resolution maps are actually dose-dependent artifacts of the X-rays used to reveal those features. Our picture and therefore understanding of function is altered by the very method we use to obtain it. X-rays can be damaging to structural crystallography, just how damaging we do not yet know.

P.6. Crystal structures and SAXS analysis of *S. aureus* FhuD2 provide insight into the mechanism of iron-siderophore transport

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FhuD2 is a siderophore binding protein from S. aureus that functions in iron acquisition. FhuD2 binds hydroxamate-type siderophores and interacts with the ABC-transporter complex, FhuBG, to initiate ATP binding and hydrolysis with concomitant siderophore transport. For ABC-type import systems, the ligand-induced conformational change in the binding protein is critical for initiating ATPase activity by the integral membrane complex. However, small-angle X-ray scattering studies of FhuD2 indicate that it undergoes only a very small structural change when it binds to various siderophore ligands; therefore, it is not clear how the binding protein is able to stimulate the ATPase activity of FhuBG. To obtain insight into the mechanism of ligand binding by FhuD2 and the nature of the conformational changes produced, we have used reductive methylation and entropy reduction to crystallize and solve the structures of unliganded and Desferal-bound FhuD2. The protein consists of two domains that are connected by an alpha-helical hinge. The Desferal siderophore has three mobile hydroxamate groups, and is able to chelate iron in a number of different ways; however, the Desferal-bound FhuD2 structure indicates that it is selective for only a subset of the possible Desferal structures. In crystals of unliganded FhuD2, the protein is present in several different conformations. These structures, along with SAXS data, indicate that the protein in solution is highly dynamic, populating a variety of conformational states. On this basis, ligand binding does not produce a large change in the average structure of the binding protein, but instead acts to decrease its conformational entropy, which may facilitate a higher affinity interaction with FhuBG such that its ATPase activity is stimulated.

P.7. Substrate specificity in the *E coli* ABC maltose transporter and the structure of a mutant periplasmic sucrose binding protein

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Transporters of the ABC family work on a variety of substrates and have been implicated in the appearance of some multi drug resistant bacterial strains. An understanding of how these transporters recognize novel substrates can aid in the development of drugs against such bacterial strains. To examine the source of substrate specificity in the *Eschericia coli* ABC maltose transporter, $MalFGK_2$, we introduced mutations into wild type *E coli* maltose binding protein (MBP) to create a periplasmic sucrose binding protein (SMBP). SMBP has been shown to adopt a closed conformation when binding maltose or sucrose comparable to wild type MBP in its maltose bound state. This allowed us to determine if $MalFGK_2$ has an integral ability to recognize maltose or depends on the specificity of MBP. SMBP was found to stimulate ATPase activity by $MalFGK_2$, *in vitro*, in the presence of maltose or sucrose. This shows that $MalFGK_2$ does not need to recognize a maltose substrate in order to hydrolyze ATP and may be able to transport sucrose. An elevated Km value for both sucrose and maltose reactions suggests that SMBP has a reduced binding affinity for the transporter. Sucrose liganded SMBP was crystallized and analyzed by X-ray diffraction. We compare the structure of this mutant to the known structure of maltose bound MBP in order to determine the structural origins of the reduced affinity for $MalFGK_2$, reduced affinity for maltose, and increased affinity for sucrose all seen in SMBP.

P.8. Structure and function of the isolated DEAD motor domains of SecA

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SecA is a peripheral ATPase motor that catalyses ATP hydrolysis and mediates the posttranslational translocation of preprotein through SecYEG channel. The SecA N-terminus contains characteristic sequence such as Asp-Glu-Ala-Asp or DEAD which are conserved. It is also called DEAD motor. The N-terminal N68 polypeptide (residues 1-610) consists of the two DEAD motor domains (NBF1 and NBF2), which resemble in RNA helicases, but NBF1 also includes the preprotein binding domain (PPBD). To understand the role of the PPBD in ATP hydrolysis, we created N68-ΔPPBD (deletion of PPBD residues 224-373) and characterized its biochemical properties and structure. The structure was solved via molecular replacement and refined to 3.0 Å resolution. Comparison with the reported crystal structures of SecA and RNA helicases reveals the domain movements that occur in DEAD motor and conformational changes were observed inside the nucleotide binding cleft. It also helped us to understand the regulation of ATP hydrolysis and the mechanism of energy coupling. Along with small-angle X-ray scattering (SAXS) data, the structure, and biochemical properties also suggest a role for the PPBD in interacting with the NBFs to modulate nucleotide binding and release.

P.9. Characterization of the C-terminal domain of a potassium channel from Streptomyces lividans (KcsA)

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KcsA, a potassium channel from *Streptomyces lividans*, is a good model to study the general working mechanism of potassium channels. This channel is pH responsive *in vitro* and its C-terminal domain (CTD) is implicated as a modulator. We investigated the nature of the CTD's involvement in the opening of KcsA by studying the channel's biochemical and electrophysiological properties with/without its CTD. We also investigated the pH dependent aggregation property of a recombinant CTD fragment. Our results show that the CTD destabilizes KcsA in the condition that it is supposed to be open. A detailed mechanism will be presented.

P.10. The Molecular Basis for Aspirin-Triggered Lipoxin Formation by Cyclooxygenase-2

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In contrast to all other NSAIDs, aspirin covalently modifies COX-1 and COX-2 via the acetylation of Ser-530 at the apex of the cyclooxygenase active site, resulting in complete inhibition of prostaglandin (PG) biosynthesis. When aspirin acetylates COX-2, 15R-hydroxyeicosatetraaenoic acid (15R-HETE) is generated from arachidonic acid (AA). 15R-HETE is converted by downstream enzymes into (15R)-epi lipoxin, an aspirin triggered lipid mediator with potent anti-inflammatory actions. We have utilized x-ray crystallographic methods to test the hypothesis that the inversion of stereochemistry at carbon-15 observed in products formed by aspirin-acetylated COX-2 and a S530T mutant of COX-2 is a result of AA binding in an "unconventional" conformation within the cyclooxygenase active site prior to the initiation of catalysis. The crystal structures of aspirin-acetylated and S530T recombinant murine COX-2, both reconstituted with cobalt protoporphyrin-IX and complexed with AA, were determined to 2.0 Å resolution. The overall conformation of AA in both structures is similar. AA is bound in the cyclooxygenase active site such that carbon-13 lies below Tyr-385 for hydrogen abstraction. However, the conformation of the omega end of the AA substrate differs significantly from the productive L-shaped conformation observed in the cyclooxygenase active site of native COX-1. Specifically, the bulky acetyl group and the threonine side chain block access to the hydrophobic groove above Ser-530 causing carbons 14-20 of AA to bend back upon the carboxylate end of the substrate. The conformation of AA observed in these crystal structures provides for a complete mechanistic understanding of how 15R-HETE (and ultimately ATLs) are generated.

Supported by an Arthritis Investigator Award (to M.G.M) from the Arthritis Foundation and by NIH grant GM077176

P.11. Structural Insights Into Endocannabinoid Metabolism by Cyclooxygenase-2

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The endogenous cannabinoids 2-archidonylglycerol (2-AG) and arachidonylethanolamide (AEA, anandamide), as well as the lipoamino acid N-arachidonylglycine (NAGly) are all selectively metabolized by cyclooxygenase-2 (COX-2). Oxygenation of these substrates may represent a unique function for COX-2 in the regulation of the endocannabinoid signaling system. We have purified and crystallized recombinant murine COX-2, reconstituted the apo enzyme with cobalt protoporphyrin-IX, rendering it catalytically inert yet poised for catalysis, and determined the crystal structures of COX-2 in complex with the endocannabinoids 2-AG, AEA & NAGly. The endocannabinoids bind in a novel conformation in the cyclooxygenase active site of COX-2 that differs from the conformation suggested based on mutational and functional analyses. The structures reveal that the delta ends of the endocannabinoid substrates are indeed positioned in the cyclooxygenase site, but not in the COX-2 distinct side pocket as inferred in the literature. The observed conformations are still catalytically competent with carbon-13 of each substrate positioned for hydrogen abstraction below Tyr-385. A full description of the molecular details will be presented, which should assist in elucidating the molecular basis for their oxidative metabolism by COX-2.

This work is supported by NIH grant GM077176 to M.G.M

P.12. Crystal Structure of HoxA9 and Pbx Homeodomains Bound to the Renin PPE *In Vivo* Derived DNA Target

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The Homeobox (Hox) family of proteins plays a key role in the control and the regulation of embryonic development and cell differentiation of all animals possessing bilateral symmetry. However, many Hox proteins may also play a role in post-embryonic transcriptional regulation within several pathways, such as hematopoiesis and many types of cancer. Despite these very crucial roles, relatively few *in vivo* Hox protein DNA targets have been identified. One of the few known examples of a true *in vivo* Hox target is a region found up stream of the mouse *Ren-1*^{c} gene known as the renin proximal promoter element (PPE). Several Hox proteins, including HoxA9 and the Hox cofactor Pbx, have been shown to bind to the renin PPE and upregulate *renin* transcription. Renin is an aspartyl protease which participates in the regulation of blood pressure and electrolyte levels through the conversion of angiotensinogen to angiotensin I, which ultimately results in an increase in blood pressure. Revealing the exact mechanisms of recognition and binding could eventually lead to: a greater understanding of the underlying forces behind this pathway , new treatments for hypertension and an explanation for the lack of discovered *in vivo* binding sites. Here we present the first crystal structure of Hox A9 and cofactor, Pbx, homeodomains bound to an *in vivo* derived DNA sequence similar to that found in the renin PPE. The specific contacts and geometries discovered here were compared to similar structures to draw conclusions on the importance of flanking nucleotides and residues, and specificity of binding.

P.13. Open Reading Frames and Codon Bias in Streptomyces coelicolor and the Evolution of the Genetic Code

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Examination of the complete genome of Streptomyces coelicolor reveals that the antisense strands of 70% of the 7514 genes (5263) contain no stop codons and could in principal be open reading frames (ORFs). Furthermore 2174 genes have a third full length ORF, 228 have a fourth ORF and 56 have a fifth ORF. Examination of the genes in S coelicolor having multiple ORFs revealed a pronounced bias in codon use and a DNA triple distribution that is most severe in the genes having four and five ORFs. When the 170 hypothetical gene products that have four ORFs and at least 100 amino acids are examined, 87% of the coding is from the GC-rich half of the genetic code and 80% of the protein sequences are composed of only 10 amino acids (GPASTDLVER). This population of amino acids is consistent with the probable order of entry of amino acids into proteins in the course of evolution. Only nineteen of these 170 hypothetical gene products are specifically characterized. They are identified as 5 dehydrogenases, 3 kinases, 2 esterases, a permease, a deformylase, 2 ABC transport proteins, a two component regulator, and three ribosomal proteins, [S12, L18 and L33]. Genes in S. coelicolor having four ORFs appear to identify a subset of the codon system that evolved first, a subset of amino acids that make up the composition of the earliest folded proteins and evidence of a possible two letter genetic code that preceded the modern genetic code.

Keywords: Codon Bias, Evolution, Multiple Open Reading Frames

P.14. Structure of acyl adenylate synthetase: a novel enzyme in the "wrong" conformation

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We are studying a family of adenylate-forming enzymes that catalyze a two-step reaction, by transferring a carboxylate to the pantetheine cofactor of Coenzyme A or the adjacent peptidyl carrier protein domain in the non-ribosomal peptide synthetases. Structural and biochemical studies in our lab and others suggests that the enzymes adopt two distinct conformations to catalyze the two half reactions. The hallmark of this domain alternation hypothesis is a 140˚ domain rotation between the two states. A novel subfamily of enzymes known as acyl adenylate synthetases (AAS) has been identified that only undergoes the initial adenylation step of the above-mentioned mechanism and releases a free acyl adenylate as a product. AAS from *Methanosarcina acetivorans* was purified and initial kinetic analysis suggests the production of free acyl adenylate in the presence and absence of coenzyme A. We have crystallized this protein and determined the structure by MAD phasing method. The structure surprisingly reveals the enzyme in the conformation thought to be used for thioester formation. Our structural results will be considered in light of a proposal that fatty acyl-CoA synthetase catalyzes the adenylation reaction using the "thioester forming conformation".

P.15. The Adenylate-forming Enzyme 4-Chlorobenzoate CoA Ligase/Synthetase Performs Two Unique Half-Reactions Using a 140^{o} C-terminal Domain Alternation

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4-Chlorobenzoate CoA Ligase/Synthetase (CBAL) is a member of the adenylate-forming family of enzymes. This family includes the aryl- and acyl-CoA synthetases, adenylation domains of non-ribosomal peptide synthetases (NRPSs), and the firefly luciferase enzymes. The adenylate-forming family of enzymes performs two half-reactions in a ping-pong mechanism. In the first half-reaction, the substrate is reacted with ATP to form an adenylate product. The enzyme then utilizes a 140^{o} C-terminal domain rotation to perform the thioester-forming half-reaction. 4-Chlorobenzoate CoA Ligase/Synthetase has been solved in both the adenylate and thioester-forming conformation using X-ray crystallography. The structure identifies a novel-binding pocket for the CoA nucleotide. A comparison of the original adenylate-forming structure and the new thioester-forming structure will be presented in support of the Domain Alternation hypothesis.

P.16. Synthesis of $AA'(WO_4)_3$ Materials Using the Non-Hydrolytic Sol-Gel Method

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In recent years, there has been an increased interest in negative thermal expansion materials (NTE), which contract upon heating. Materials exhibiting this property have the potential for achieving better control of thermal expansion through the synthesis of composite materials with more desirable expansion coefficients. One family of materials that has been known to show NTE is the tungstate family $A_2(WO_4)_3$, where A can be a variety of trivalent cations.

In the Lind group, we are interested in developing systems where the A site contains two different atoms, leading to materials of the type $AA'(WO_4)_3$ (A= Mg, Zn; A'= Zr, Hf). Previously in the group, the synthesis of these materials was explored via a ball-milling technique, followed by high temperature treatments. $MgZr(WO_4)_3$ and $MgHf(WO_4)_3$ were successfully synthesized. Presented herein are the results of a study aimed at synthesizing these materials using lower temperature routes like the non-hydrolytic sol-gel method (NHSG). Samples were characterized using Powder X-ray Diffraction and Scanning Electron Microscopy.

P.17. Temperature and Pressure Induced Polymorphism of $Y_2Mo_3O_{12}$

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In the past, members of the $A_2M_3O_{12}$ family have been investigated because of their negative thermal expansion (NTE) behavior. NTE materials can be used in composites to alter a material's thermal expansion properties without influencing other properties of the original matrix. As many of these materials undergo temperature or pressure induced phase transitions from a phase that displays NTE (orthorhombic Pbcn) to more dense phases that do not show NTE, it is crucial to test the stability of these compounds prior to use in applications. This presentation focuses on the phase evolution of yttrium molybdate ($Y_2Mo_3O_{12}$) as a function of temperature and pressure. $Y_2Mo_3O_{12}$ was prepared by non-hydrolytic sol-gel chemistry. The phase evolution upon heating was investigated using *in situ* and *ex situ* heat treatments combined with powder X-ray diffraction. This method has led to the isolation of two orthorhombic phases with different atomic connectivity. Yttrium adopts 6- and 7-coordinate sites in the Pbcn and Pba2 structures, respectively. Cocrystallization of both phases was observed in a narrow temperature range, suggesting that crystallization kinetics play a major role in phase formation. It was found that the Pba2 phase is the stable polymorph below 550 °C, and converts to Pbcn at higher temperatures. In addition, high pressure X-ray diffraction studies were completed on this material that revealed pressure induced phase transitions.

P.18. Variable Temperature Neutron and X-Ray Diffraction Studies of the $A^{2+}A'^{4+}W_3O_{12}$ Family

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Variable Temperature Neutron and X-ray Diffraction Studies of the $A^{2+}A'^{4+}W_3O_{12}$ Family

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Negative thermal expansion (NTE) materials have been of interest because of the observed reduction in the overall expansion when they are incorporated into composites. Previous work in the $A^{3+}{}_2W_3O_{12}$ system showed that the thermal expansion is dependent on the identity of the A^{3+} cation. Crystallization studies showed that this family undergoes a transition from a monoclinic to an orthorhombic phase upon heating. NTE has only been observed in the orthorhombic phase. The A site has also been substituted with a mixture of Hf^{4+} and Mg^{2+}.

The systems of interest in this paper involve previously unknown compounds in the $AA'W_3O_{12}$ family ($A=Mg^{2+}$; $A'=Zr^{4+}$, Hf^{4+}). Several possible pathways were explored for the preparation of these materials. The desired phases were obtained by high energy ball-milling and subsequent calcinations of the samples. Crystallization studies as a function of temperature were performed on the samples to establish what phases were preferentially formed. Variable temperature X-ray and neutron diffraction experiments were carried out on $AA'W_3O_{12}$ to determine thermal expansion and phase transition behavior. Both monoclinic and orthorhombic phases were observed. The monoclinic phase could be fitted in $P\,2_1/a$, which is a known structure for $A^{3+}{}_2W_3O_{12}$. In contrast, the orthorhombic phase could not be fitted in Pnca, which is the common phase that displays NTE for $A^{3+}{}_2W_3O_{12}$. From indexing and systematic absences the space group Pnma was established as the highest possible symmetry. There were some inconsistencies between the X-ray and neutron diffraction data for $MgZrW_3O_{12}$, which were addressed by simultaneous Rietveld refinement of the X-ray and neutron data. High pressure experiments were also carried out on both $MgZrW_3O_{12}$ and $MgHfW_3O_{12}$ at the Cornell High Energy Synchrotron Source. The materials each responded differently to the compressions.

P.19. NTE Zirconium Tungstate Materials

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Negative thermal expansion materials contract when they are heated instead of expanding like most solids. Cubic zirconium tungstate exhibits this behavior and is well characterized with a thermal expansion coefficient of -9.0 x 10^{-6}/K from 0.3-1050 K. By incorporating this material into a polymer matrix via an organic linker, it is possible to tailor the thermal expansion coefficient of the composite depending on its percent loading. It is predicted that this will be more effective if the particles of zirconium tungstate are below 100 nm. The small size of the particles will result in better dispersity in the polymer, and the greater surface area to volume ratio will provide a larger area for the polymer to bind, decreasing the chance of the particles separating from the matrix. Cubic zirconium tungstate is prepared by first synthesizing its precursor, zirconium tungstate hydroxide hydrate, and subsequently heat-treating the powder to about 600 ˚C for 30 min. By changing the experimental conditions, in particular, the kinds of acid used, acid concentrations, and the fundamental procedure, some interesting morphology of the precursor has emerged including monodisperse rods with pointed tips, and brush-like and monodisperse agglomerates composed of nanorods. Monodisperse nanocube-like particles were also synthesized of tungsten hydrogen oxide with each particle curiously containing about the same percentage of zirconium defects. Current studies are underway to increase the amount of zirconium in this preparation to yield the right composition without changing the morphology. The synthesis of nearly unagglomerated nanorods was also achieved. The samples were analyzed using x-ray diffraction (XRD) for phase identification and crystallite size estimation was determined using the Scherrer equation. The atoms present in the sample and the particle size and shape were determined by energy dispersive x-ray spectroscopy (EDX), and transmission and scanning electron microscopy (TEM and SEM), respectively.

P.20. Non-Hydrolytic Sol-Gel Route for Preparing Titanium Sulfide

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Sulfide materials, which are employed as catalysts and lubricants, and have some interesting optical and electrical properties, present challenges with respect to their particle size and shape, composition and performance. The non-hydrolytic sol-gel technique was used as a route for preparing different samples of metal sulfides in this research. This technique is used for the low temperature synthesis of titanium sulfides which are of interest as one of the most effective cathode materials in high energy applications and rechargeable batteries. Transition metal halides are expected to react with thioethers to form metal sulfides. *tert*-Butyl sulfide was chosen to react with titanium trichloride and titanium tetrachloride in the presence of either chloroform or acetonitrile to form different structures of TiS_x. Various factors have been studied that affect the final product such as the oxidation state of titanium, metal to sulfur ratio, the type of solvent, heating temperature and time of heating. The obtained products were characterized by several techniques. X-ray diffraction was used to identify the crystalline structure of the prepared samples, and TG/DTA was applied to calculate the sulfur to metal ratio. Electron microscopy was carried out to study the morphology of the formed metal sulfide powders.

P.21. Non-hydrolytic sol-gel synthesis of iron and copper sulfides

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Sulfides have been studied for decades. The first studies were motivated by their occurrence in metallic ores. Then, the discovery of magnetic, electrical and optical properties of sulfides, which are of interest for various industrial applications, led to the development of synthetic routes: Sulfides have been synthesized by classic high temperature techniques, and more recently, by soft low temperature routes. In this work, non-hydrolytic sol-gel techniques were investigated to synthesize copper and iron sulfides. The synthesis consisted of mixing metal chlorides and a covalent sulfur source in a dry solvent such as chloroform or acetonitrile. Because of the relatively oxophilic character of many sulfides, all steps were carried out under controlled atmosphere: The reactants were mixed in a glove box under argon, and sealed ampoules were used to heat the mixtures in an oven. Compared to traditional methods, this route did not involve toxic H_2S gas, and the products were more homogeneous. The products were characterized by powder X-ray diffraction, thermo-gravimetric and differential thermal analysis, and CHN analysis. Troilite (FeS), greigite (Fe_3S_4) and several copper sulfides ranging from Cu_2S to CuS were crystallized at low temperature (130 °C). The influence of the synthesis parameters was investigated to gain control over the reaction and the crystalline phases obtained.

P.22. Twinning in the Adamantine-Like Quaternary Chalcogenide Li_2ZnSnS_4

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Li_2ZnSnS_4 is a diamond-like semiconductor (DLS) material which is of interest as a host structure for the creation of potentially interesting magnetic materials. This compound was predicted to adopt the wurtz-stannite structure with all atoms possessing tetrahedral geometries. The crystal structure determination was initially thought to possess disorder. Upon closer examination, this disorder violated many basic chemical properties. The structure was reevaluated and determined to be a pseudo-merohedral twin. After the reevaluation, the refined structure was chemically reasonable.